Antiulcer Activity The Milk Extract Of Ficus Religiosa Bark

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Objective: To evaluate the gastro-protective activities of milk extract barks of Ficus religiosa (F. religiosa) in a different experimental model of gastric ulcer in rat

Method: The milk extract barks of Ficus religiosa was studied in a two dose levels (250 mg/kg and 500 mg/kg, in oral administration) in animals ethanol induced gastric ulcer (0.2 ml, oral) and cold stress induced model. The antiulcer activity was checked by compared the ulcer index in the experimental drug group with the vehicle control and standards drug. Ranitidine drug (50 mg/kg) was used as a standard drug. The parameters taken to evaluated antiulcer activity were Mean ulcer, Gastric pH, gastric juice volume (in ml), and % protection, total acidity, free acidity, was measured.

Results: Administration of Ficus religiosa barks to rats significantly decreases the ulcer index value when compared with the control treated group. Standard drug of Ranitidine (50 mg/kg, oral) also produced a significantly decrease the ulcer index value compared with the control treated group. Phytochemical screening revealed the presence of “alkaloids, steroids, flavonoids, tannins, carbohydrates, and proteins”.

Conclusions: The results were that the barks of the Ficus religiosa possess significantly anti-ulcer activity.

Keywords: Ficus religiosa, Milk Extract, Ranitidine, antiulcer, Ethanol, Cold stress.
INTRODUCTION:
Ulcer is erosion on the skin or on the mucous membrane specified by outward inflamed dead tissues.\textsuperscript{1,2} The word ulcer is derived from Latin word “ulcus” (genitive: ulceris) which stand for sore, wound or an ulcer.\textsuperscript{3}

The erosion are most commonly seen on gastric or duodenal mucosa and are referred to as peptic ulcer.\textsuperscript{4,5} The peptic ulcers are the areas of degradation and necrosis of gastro-intestinal mucosa which is damaged to acid and pepsin secretion.

Peptic ulcers are a rare combination of harmful conditions in the lumen and protective involvement in the gastroduodenal mucosa. The strong gastric corrosive growth by prostaglandin added the two expansions in mucosal opposition only as a lessening in forceful component chiefly corrosive and pepsin.\textsuperscript{6}

The peptic ulcers are two types:

1. **Gastric ulcer:** When the ulcers occur in stomach they are called gastric ulcer.

2. **Duodenum ulcer:** At the point when the ulcer are induce in the duodenum it is called duodenal ulcer.

The duodenal ulcer is the commonest of peptic ulcer with the ratio of 4:1 in duodenum and stomach respectively.\textsuperscript{5,7}

Peptic ulcer can lead to several complications such as obstruction, hemorrhage and perforation.\textsuperscript{5}

Types of peptic ulcer based on site:

**Type I:** These type of gastric ulcer, have ulcer along the lesser curvature of stomach and have normal or decreased gastric acid secretion.

**Type II:** It includes two types of ulcer, one gastric and one duodenal. This type of ulcer has normal or increased gastric acid secretion.

**Type III:** Type-III ulcer ulcers are present on pre-pyloric region of stomach and have normal or increased gastric acid secretion and are known as pre-pylori ulcer.

**Type IV:** Proximal gastro-esophageal ulcer. These type of ulcer is located near the gastro esophageal region with gastric acid secretion rate normal or below normal.

**Type V:** Anywhere.\textsuperscript{1,3}

Common types of ulcers are:

**Esophageal ulcer:** The esophageal ulcers are the ulcers occurring the lower end of esophagus due to acid reflux or gastro esophageal reflux disease (GERD).\textsuperscript{8,9}

**Bleeding ulcer:** bleeding are induce ulcer is untreated peptic ulcer which has not been treated for long time.\textsuperscript{9}

**Refractory ulcer:** The peptic ulcers haven’t healed after three months of treatment are refractory ulcers.

**Stress ulcer:** This type of ulcer consist of group of lesion found in esophagus, stomach, or duodenum.\textsuperscript{9}

**Symptoms of Ulcer:**

- Loss of appetite
- Weight loss
Epigastric tenderness
- melena
- Nausea & vomiting
- Abdominal pain
- Bloating and abdominal fullness
- Chest pain.\(^{10}\)
- Dark or black stools.\(^{11}\)
- Dry tongue
- Weak pulse
- Shortness of breath.\(^{12}\)

**Etiology and Pathogenesis**
At first the peptic ulcers were accepted to the brought about by the forceful activity of hydrochloric corrosive and pepsin on mucosa.\(^{13}\)

*Table 1: List of Defensive and Aggressive factors*

<table>
<thead>
<tr>
<th>Defensive Factors</th>
<th>Aggressive Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate blood flow.</td>
<td>Reactive oxygen species.</td>
</tr>
<tr>
<td>Secretion of prostaglandin.</td>
<td>Increase secretion of HCL and pepsin.</td>
</tr>
<tr>
<td>Bicarbonate, Mucin.</td>
<td>Inadequate dietary habits.</td>
</tr>
<tr>
<td>Mucus bicarbonate layer.</td>
<td>Free oxygen radicals.</td>
</tr>
<tr>
<td>Cellular regeneration.</td>
<td>Consumption of NSAIDs.</td>
</tr>
<tr>
<td>Mucusal barrier.</td>
<td>Alcohol.</td>
</tr>
<tr>
<td>Surface mucus secretion.</td>
<td>Bile salts.</td>
</tr>
<tr>
<td>Cytokines.</td>
<td>Oxydative stress.</td>
</tr>
<tr>
<td></td>
<td>Stress.</td>
</tr>
<tr>
<td></td>
<td>Neutriphil infiltration.(^{14})</td>
</tr>
</tbody>
</table>

**MATERIAL AND METHOD**

**Collection of plant:**
The barks of *Ficus religiosa* were collected from the local region in the month of September 2019.

The protein gastrin animates the creation of HCl by parietal cells and in patients with *H.pylori* disease the expanded degree of gastrin are delivered that thus expanded creation of corrosive along these lines prompting disintegration of gastric mucosa and in this manner ulceration.\(^{1}\)

Larger part of instances of peptic ulcer are because of *H.pylori* disease, as around 80-90% of patients and duodenal ulcer have *H. pylori* contamination, same goes with 70-90% patients of gastric ulcer.\(^{9}\)

However, the most well-known reason for ulcer stays because of irregularity between the forceful (hostile) variables and cautious elements which are recorded underneath.

**Plant Authentication:** The barks of *Ficus religiosa* were collected from the local region in September 2019. The plant barks material was recognised and authentication by Prof. N.K. Dubey, Botanical deptt. Banaras Hindu University, Varanasi U.P. India (Voucher specimen no. Mora. 2019/1)

**Selection of Standard Drug:** For antiulcer activity Ranitidine was chosen as standard drug.

**Selection of Animals:** Healthy Wister rat of each sex (weighing 250-300gm) were taken in the experiment. All animal experiment was conducted in accordance with CPCSEA guidelines.

**Preparation of the Extraction (Decoction Method):**
10 gm *Ficus religiosa* barks powder was taken in round bottom flask (RBF) with 250 ml of row cow milk. Then heated for 2 hrs up to 70 °C temperature was maintained. Then solution allowed to stands for 45 min. At room temperature. The solution was filter and filtrate solution was allowed centrifuge for 10 min. The solution was withdrawn in Petridis and allowed to hot air oven 40-45°C (till dried material). The dried materials was passing into sieve (120 No.). Then passing material collect and stored.
Experimental animals:
Male albino Wister rat weighing 250-300 gm was used in the experimental study. The experimental animals was maintained under the standard laboratory conditions in an animals house approved by committee for the purpose of control and supervision on experimental on animals (CPCSEA). The under 12 hrs light/dark cycle and control temperature (24±2°C) and feeding with commercial pellet diet and water. All animals was laboratory environmental for at least one week before the commencement of experimental. The experimental protocol was approved by the Institutional Animal Ethical Committee, R K Pharmacy College, Azamgarh, U.P., India.

Animal grouping and treatment schedule for Antiulcer Study-
Male pale cleaned human rodents were chosen for the preliminary models and partitioned into five gatherings of six animals each. Animals were not allowed to take care of or drink for 24 hours preceding the test, however they were permitted unlimited admittance to water. Social event I was utilized as a vehicle screen, accepting just refined water; packs II, III, and IV were utilized as treatment get-togethers, getting the explored segment of F. religiosa (MEFR) at 250 and 500 mg/kg (p.o.) for 7 days (when daily) independently; and bundle V was utilized as a normal social affair, accepting ranitidine 50 mg/kg (p.o.).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Vehicle control (with water)</td>
<td>6</td>
</tr>
<tr>
<td>02</td>
<td>Ulcer induce</td>
<td>6</td>
</tr>
<tr>
<td>03</td>
<td>Standard drug (Ranitidine) 50 mg/kg</td>
<td>6</td>
</tr>
<tr>
<td>04</td>
<td>Milk extract of <em>F. religiosa</em> 250 mg/kg</td>
<td>6</td>
</tr>
<tr>
<td>05</td>
<td>Milk extract of <em>F. religiosa</em> 500 mg/kg</td>
<td>6</td>
</tr>
</tbody>
</table>

*Table 2: Group of experimental animals for anti-ulcer activity*

Pharmacological Screening of plant extraction
Ethanol induced gastric lesions
Animals are divided into five groups. Group I served as the control and received. The experimental animals of the group 2 received absolute ethanol (0.2 mL, oral) alone where as animals of groups 3, 4 & 5 received ranitidine (50 mg/kg) and plant extract at two doses (250 and 500 mg/kg by oral) alone with ethanol. The animals were fasted for 24 h received either plant extract (250 and 500 mg/kg) or control vehicle. After 30 minutes, ulcer activity was induced by oral administration of absolute ethanol (0.2 mL, p.o.). The rat animals were sacrificed after 2 hrs following administration of ethanol. The stomach was removed opened along the greater curvature and ulceration area was calculated. Lesion severity was determined by measuring ulcer index.

Stress induced ulcers (cold water immersion method)
Stress ulcers was induced by stress the experimental animals to swim in the glass cylinder containing water to the height of 35 cm and maintained at 25 °C for 3 h. Animals was fasted for 24 hrs to the experiment and divided into three groups each groups consist of six animals. Group 1 received 1.0 mL/ kg p.o. as control vehicle; Group 2 received 50 mg/kg, oral ranitidine as standard drug; Groups 3 and 4 were respectively collected 250 and 500 mg/kg, oral of *F. religiosa* milk extract. After the drug treatment animals were allowed to swimming in cold water for 4 hrs. After this animals were killed with high dose of anaesthetic chloroform. Each stomach was opened along the greater curvature and examined microscopically for gastric erosions under a dissecting microscope (10X). 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 noted by pH meter and gastric content was 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.16, 17

**Measurement of various parameters**
- The means value were calculated by the subsequent scoring system

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ulcer Score</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>0</td>
<td>Normal coloured stomach</td>
</tr>
<tr>
<td>02</td>
<td>0.5</td>
<td>Red coloured streak</td>
</tr>
<tr>
<td>03</td>
<td>1</td>
<td>Ulcer spot</td>
</tr>
<tr>
<td>04</td>
<td>1.5</td>
<td>Heamarrhagic band</td>
</tr>
<tr>
<td>05</td>
<td>2</td>
<td>Ulcer</td>
</tr>
<tr>
<td>06</td>
<td>3</td>
<td>Perforated Stomach</td>
</tr>
</tbody>
</table>

*Table 3-Measurement of various parameters*

**Estimation of gastric pH**
To decide the gastric volume and pH, the gastric liquid gathered from the stomach of a mouse would be moved into a rotator chamber. The chambers will be centrifuged for 10 minutes at 1000 cycles each moment, and the gastric volume will be controlled by the graduation on the chambers. The supernatant will be gathered, and the pH will be checked utilizing a computerized pH meter.18

**Estimation of total acidity**
One mL gastric juice was weakened with nine mL refined water, at that point filled a 100 mL carafe with a couple of drops of phenolphthalein pointer and titrated with 0.01 N sodium hydroxide until a consistent pink tone was accomplished. The measure of sodium hydroxide (0.01N) retained is accounted for. The supreme causticity is communicated in milliequivalents per liter (mEq/L) in the accompanying formula.

\[
\text{Acidity} = \frac{\text{Vol.of NaOH} \times N \times 100 \text{ mEq/L}}{0.1}
\]

Where,
- \(N\) = Normality of NaOH solution

**Estimation of free acidity**
The free destructiveness was resolved utilizing Topfer's reagent. Also separable measures of gastric juice were titrated with 0.01N sodium hydroxide before an orange sound was accomplished. It was assessed how much 0.01 N sodium hydroxide was retained. A connected condition for deciding all out acidity was recommended for nothing destructiveness.19, 20

\[
\text{Acidity} = \frac{\text{Vol.of NaOH} \times N \times 100 \text{ mEq/L}}{0.1}
\]

Where,
- \(N\) = Normality of NaOH solution

**Statistical analysis**
Data was expressed as mean ± SEM. Data was calculated by one way ANOVA followed by Dunnett’s multiple comparison control. The significance of difference was accepted at P < 0.01.

**RESULT**
**Phytochemical screening:**
MEFR had flavonoids, alkaloids, tannins, and carbohydrates in its preliminary phytochemical sampling.
Table 4: Preliminary phytochemical screening of Ficus religiosa barks

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Milk extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
</tbody>
</table>

Acute toxicity studies:
MEFR didn't exhibit any sign and indications of apparent poisonousness, with no conduct adjustment or changes, and it didn't cause creature passings inside 72 h. MEFR was protected until the most extreme portion of 4000 mg/kg of body weight.

Ethanol-induced ulcer:
In ethanol-initiated ulcer model, there was critical harm to the gastric mucosal layer in charge gathering, and MEFR-treated gathering showed nearly great anticipation of gastric mucosa in which results are contrasted with standard Ranitidine-treated gathering. Mean ulcer list of MEFR-treated gathering was 0.62±0.09*** which showed better outcomes when contrasted with control bunch which had mean ulcer file 1.24±0.018.
Figure 3: Anti-ulcer activity milk extract obtained from F. religiosa barks
A: The stomach of a rat in the control group has a greater ulcer region.
B: A rat’s stomach after being given ranitidine.
C: Stomach of rats given 250 mg/kg extract.
D: Stomach of rats given 500mg/kg extract.

Table 5: Effect of MEFR on Ethanol-induce ulcer model

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Mean ulcer index±SEM</th>
<th>% Protection</th>
<th>Gastric volume (ml)</th>
<th>Gastric pH</th>
<th>Free acidity (mEq/L)</th>
<th>Total acidity (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>-</td>
<td>1.24±0.018</td>
<td>-</td>
<td>3.90±0.029</td>
<td>3.38±0.096</td>
<td>64.6±2.12</td>
<td>70.1±0.86</td>
</tr>
<tr>
<td>2.</td>
<td>Standard (Ranitidine)</td>
<td>50</td>
<td>0.55±0.018</td>
<td>55.64</td>
<td>2.09±0.031</td>
<td>2.43±0.065</td>
<td>23.3±0.96</td>
<td>27±1.081</td>
</tr>
<tr>
<td>3.</td>
<td>MEFR</td>
<td>250</td>
<td>0.70±0.010</td>
<td>44.54</td>
<td>2.26±0.034</td>
<td>3.14±0.079</td>
<td>50.8±1.06</td>
<td>42.5±1.15</td>
</tr>
<tr>
<td>4.</td>
<td>MEFR</td>
<td>500</td>
<td>0.62±0.009</td>
<td>50</td>
<td>2.22±0.05</td>
<td>3.00±0.037</td>
<td>47.8±1.09</td>
<td>39.1±1.38</td>
</tr>
</tbody>
</table>

Figure 4-Ulcer index of different group.
Value are expressed as (Mean±SEM) (n=6), significant at p < 0.01 as compared with control.
Figure 5 - Average of gastric juice of gastric volume.

Figure 6 - Average of gastric juice of gastric pH.
It is clear that ulcer file of MEFR-treated gathering was not exactly the benchmark group in chilly pressure restriction ulcer model. From a naturally visible photo and histological slides, unmistakably MEFR at a portion of 500 mg/kg showed better gastric mucosal security. Ulcer list of MEFR was 0.59 ± 0.013**, while of standard Ranitidine, was 0.54 ± 0.09*** which was superior to control bunch 1.32 ± 0.015.
Figure 9: Anti-ulcer activity milk extract obtained from *F. relegiosa* barks
A: Stomach of a rat control group rat showing larger ulcer area.
B: Stomach of a rat treated with ranitidine.
C: Stomach of rats treated with extract 250mg/kg.
D: Stomach of rats treated with extract 500mg/kg.

Table 6: - Effect of MEFR on Cold stress-restraint ulcer

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Means ulcer index±SEM</th>
<th>% Protection</th>
<th>Gastric volume (ml)</th>
<th>Gastric Ph (mEq/L)</th>
<th>Free acidity (mEq/L)</th>
<th>Total acidity (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>-</td>
<td>1.33±0.015</td>
<td>-</td>
<td>3.3±0.032</td>
<td>2.1±0.019</td>
<td>69.66±1.32</td>
<td>65.5±1.63</td>
</tr>
<tr>
<td>2.</td>
<td>Standard (Ranitidine)</td>
<td>50</td>
<td>0.54±0.009</td>
<td>59.09</td>
<td>1.9±0.027</td>
<td>4.9±0.013</td>
<td>33.33±1.85</td>
<td>23.3±1.47</td>
</tr>
<tr>
<td>3.</td>
<td>MEFR</td>
<td>250</td>
<td>0.66±0.014</td>
<td>50</td>
<td>2.5±0.025</td>
<td>3.2±0.012</td>
<td>44.16±1.06</td>
<td>51.5±1.22</td>
</tr>
<tr>
<td>4.</td>
<td>MEFR</td>
<td>500</td>
<td>0.59±0.013</td>
<td>53.03</td>
<td>2.2±0.030</td>
<td>4.1±0.013</td>
<td>40.33±1.28</td>
<td>44.6±0.02</td>
</tr>
</tbody>
</table>
Figure 10- Ulcer index of different group.

Value are expressed as (Mean±SEM) (n=6), significant at p < 0.01 as compared with control.

Figure 11- Average of gastric juice of gastric pH.
Figure 12- Average of gastric juice of gastric volume.

Figure 13- Average of gastric juice of free acidity.

Figure 14- Average of gastric juice of total acidity.
DISCUSSION
The present study discussion that *Ficus religiosa* exhibits both gastroprotective and ulcer healing properties. In ethanol and cold restraint stress induced gastric ulcer model, the milk extract of *Ficus religiosa* reduced the gastric 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, anticholinergic and H₂ antagonist are used, most of these drugs produce several adverse reactions such as gynecostasia, hematopoietic change, acute interstitial nephritis, thrombocytopenia, anaphylaxis reaction, nephrotoxicity and hepatotoxicity. There for medicinal plants with lesser side effects are better alternatives for the treatment of gastric ulcer.

Oral administration is absolute ethanol in rat lead to strong vasoconstriction of is accompanied by rapid and vigorous arteriolar dilation and this combination of microvascular events induces damage in mucosal capillaries. The protective effect of the extract of *Ficus religiosa* against the gastric damage may be due to their action against 5-lipoxygenase pathway. The cytoprotectives action probably stimulates the prostaglandin synthesis, which in turn protects the gastric mucosa. The result of the present study showed that *Ficus religiosa* barks of milk extract possesses anti-ulcer activity, as evidence by its significant inhibition in the formation of ulcer induce by stress induce model.

Although the mechanism of ulcer prevention by this extract is not clear, phytochemicals such as flavonoids present in the extract might play important role. In our earlier study milk extract of similar results were obtained and we have reported. 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 antiulcer 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.

CONCLUSION
Peptic ulcer is the widespread disorder of gastro intestinal tract with recurrent relapses and several complications. Also, the allopathic drugs used in its treatment are associated with adverse effects causing further damage to human health. As a result of this researchers are focusing on herbal plants having therapeutic effects. These herbal plants are rich in several phyto-chemicals such as alkaloids, tannins, flavonoids, phenols, saponins etc; isolation and use of these compounds provides health benefits. Therefore, the medicinal plants having anti-ulcer potential were discussed here which are not only safe but are also relatively cheap.

ACKNOWLEDGMENT
The authors would like to thanks Dr. Abhay Pratap Yadav for granting permission to use the institute facilities to carry out my research work. Our sincerely thanks to my guide Mrs. Bhavana Yadav for her guidance and support throughout the work.

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How To Cite This Article:

Source of Support: Nil
Conflict of Interest: None declared

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