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ABSTRACT

Objective: To evaluate the anti-ulcer activity of etanolic extract of leaves of Drynaria quercifolia Linn. Materials and Methods: The etanolic extract of leaves of Drynaria quercifolia was investigated for its anti-ulcer activity against pylorus ligation and ethanol induced ulcer models in experimental rats at doses of 250 and 500 mg/kg body weight p.o. Results: The Aqueous extract of Drynaria quercifolia (250 mg/kg & 500 mg/kg) showed significant (P<0.05) reduction in gastric volume, free acidity and ulcer index as compared to control. Conclusion: This present study indicates that etanolic extract of Drynaria quercifolia have potential anti-ulcer activity in the both models. These results may further suggest that the extract was found to possess antulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.

Key words: Drynaria quercifolia, Pylorus ligation, Ethanol induced ulcer model, ulcer index.

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity 1. Peptic ulcer is a conglomerate of heterogeneous disorders which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid and/or pepsin NSAID ingestion is associated with erosions, petechiae type C gastritis, ulceration interference with ulcer healing, ulcer complications and injury to the small and large intestine 2. In recent years, a powerful association between peptic ulcers and infection of Helicobacter pylori has been adopted. At least 70-90% of patients with gastric ulcers and 80-95 % with duodenal ulcers are infected by H pylori and eradication of this Microorganism seems to be curative for the disease 3. Although a number of antiulcer drugs such as H2 receptor antagonists, proton pump inhibitors and cytoprotectants are available for ulceration all these drugs have side effects and limitations 4. Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects 5. Screening plants for active drugs is still important and might provide a useful source of new anti-ulcer compounds for developing pharmaceutical drugs or alternatively as simple dietary adjuncts to existing therapies6.

Drynaria quercifolia (L.) (Polypodiaceae) is an epiphytic medicinal pteridophyte, distributed widely in the evergreen forests of the Western Ghats of Kerala, locally called ‘Marappannakizhangu’ or ‘Attukalkizhangu’. The rhizome is reported to be used by tribal communities of Tamil Nadu and Kerala to cure various diseases like phthisis, dyspepsia, cough1. The leaves are used for poulicing swellings. The plant is used to treat body ache, headache and with other drugs in rheumatic pain1. The whole plant of Drynaria quercifolia is anthelmintic, pectoral, expectorant and tonic, and is used to treat chest and skin diseases and loss of appetite 7. The rhizome is used against typhoid fever10. It is also used to treat jaundice and as a poultice and antifertility agent11 and antipyretic agent12. The tribals of Kolli hills of Tamil Nadu use the rhizome of the plant as an anti-inflammatory agent 13. The objective of the present study was to screen for anti-ulcer activity of Drynaria quercifolia rhizome etanolic extract.

MATERIALS AND METHODS

Plant material

The rhizomes of Drynaria quercifolia were collected from Tamilnadu and authenticated by Botanical identification was done by Prof.P.Jayaraman, Director, Plant Anatomy Research Centre, Medicinal Plant Research Unit, West Tambaram, Chennai. Reg. No. PARC/2011/484.

Preparation of the plant extract

The rhizomes of Drynaria quercifolia were washed thoroughly with tap water, shade-dried and powdered. The powder (100 g) was extracted with ethanol (1000 ml) for 24 h, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. The yield (w/w) of the crude extract was found to be 4%. The crude ethanolic extract Drynaria quercifolia (DQ) was suspended in 10% Tween-80 to required concentrations and used for the experiments.

Animals

The study was conducted on male and female Wister rats (175 – 200 gm) housed in polypropylene cages under standard conditions of temperature (22 ± 20°C), relative humidity (60 ± 5%) and light (12h light/ dark cycle) were used. They were fed with standard pellet diet and water. The food was withdrawn 18 hours before the experiment but allowed free access of water. To avoid Coprophagy and fighting, the rats were fasted in wire-bottomed cages. All animal experiments were carried out in accordance with the guidelines of CPCSEA.

Acute toxicity study

Acute oral toxicity was performed as per OECD – 423 guidelines. Before study the animals were fasted overnight with free access to water. A total of 10 animals were used divided into 2 groups of 5 animals each; Group I served as control (received 1% SCMC solution). Group II received ethanolic extract of Drynaria quercifolia leaf extract with a single oral dose (2000 mg/kg body weight). Animals were observed individually atleast once during first 30 min, after dosing, periodically during first 24 hr (with special attention during first 4 hr) and thereafter once daily for a period of 14 days. Once daily cage side
Pyloric ligation in rats
The animals were divided into 5 groups, each consisting of six rats. Control group were received distilled water orally. Second group of rats were pyloric ligated. Third Group of animals received Ranitidine in the dose of 50 mg/kg as a reference drug for ulcer protective studies. Fourth and fifth groups received aqueous leaf extract of *Drynaria quercifolia* in a dose of 250 and 500 mg/kg respectively. After 45 min of the treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under ether anesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Rats were sacrificed after 4h of surgery and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed 15.

Ethanol induced ulcer model
The ulcer was induced by administering ethanol. All the animals were fasted for 36 hours and then ethanol was administered to induce ulcer. The animals were divided into five groups, each consisting of six rats. The control Group received distilled water, second group received ethanol. Third Group of animals received Ranitidine in the dose of 50 mg/kg as a reference drug. Fourth and fifth groups received aqueous leaf extract of *Drynaria quercifolia* in a dose of 200 and 400 mg/kg respectively. The gastric ulcers were induced in rats by administering absolute ethanol (90%) (1ml/200g). Orally, after 45 min of aqueous extract of *Drynaria quercifolia* and omeprazole treatment. 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 will be scored. A score for the ulcer was study Similar to pyloric ligation induced ulcer model 16.

Scoring of ulcer 17.

<table>
<thead>
<tr>
<th>Normal stomach</th>
<th>-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red coloration</td>
<td>-0.5</td>
</tr>
<tr>
<td>Spot ulcer</td>
<td>-1</td>
</tr>
<tr>
<td>Hemorrhagic streak</td>
<td>-1.5</td>
</tr>
<tr>
<td>Ulcers (&lt;2mm)</td>
<td>-2</td>
</tr>
<tr>
<td>Ulcers (2&lt;4)</td>
<td>-3</td>
</tr>
<tr>
<td>Ulcers (&gt;4mm)</td>
<td>-4</td>
</tr>
</tbody>
</table>

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

\[
\text{% of ulcer protection} = \frac{\text{Control mean ulcer index} \times \text{Test mean ulcer index} \times 100}{\text{Control mean ulcer index}}
\]

**Determination of free acidity:**

\[
\text{Acidity} = \frac{\text{Volume of sodium hydroxide} \times \text{Normality} \times 100\text{mEq/L}}{100g}
\]

**Statistical analysis**

The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Dunnett’s test where P<0.05 was considered statistically significant.

**RESULTS**

**Pyloric ligation induced gastric ulcer** In pyloric ligation induced ulcer model, Oral administration of aqueous extract of *Drynaria quercifolia* in two different doses showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. It was showing protection index of 79.0 % and 83.3 % at the dose of 250 and 500 mg/kg respectively in comparison to control, whereas Ranitidine as reference standard drug was showing protection index of 88.7 % (Table-1).

**Ethanol-induced gastric ulcer**
In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. Extract of *Drynaria quercifolia* has shown significant protection index of 80.2 % and 85.1 % with the dose of 250 and 500 mg/kg respectively in comparison to control, whereas Ranitidine as reference standard drug was showing protection index of 87.9 % (Table-2).

**DISCUSSION**

The etiology of peptic ulcer is unknown in most of the cases, it is generally accepted that gastric ulcer results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism 18. Different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis 19. The prostaglandins can provide gastric cytoprotection in rats against strong necrotizing irritants without reducing gastric acid secretion 20. The causes of gastric ulcer by pyloric ligation are believed to be due to stress induced increase in gastric hydrochloro-

### Table: 1 Effect of aqueous leaf extract of *Drynaria quercifolia* on various parameters in pyloric ligation induced Gastric ulcers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>pH of Gastric Juice</th>
<th>Gastric juice</th>
<th>Free acidity</th>
<th>Total acidity</th>
<th>Ulcer Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal (Distilled water)</td>
<td>6.42±0.2</td>
<td>4.6±0.2</td>
<td>421±0.2</td>
<td>63.4±0.2</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Control (Pyloric ligation)</td>
<td>2.42±0.3</td>
<td>8.7±0.2</td>
<td>93.2±0.4</td>
<td>116.3±0.3</td>
<td>18.6±0.6</td>
</tr>
<tr>
<td>III</td>
<td>Ranitidine (50 mg/kg)</td>
<td>5.67±0.2*</td>
<td>2.8±0.2*</td>
<td>18.6±0.3*</td>
<td>32.6±0.4*</td>
<td>21.4±0.3*</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous extract of <em>Drynaria quercifolia</em> (250 mg/kg)</td>
<td>4.6±0.2</td>
<td>3.2±1.2</td>
<td>43.6±0.2</td>
<td>68.9±0.4</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>V</td>
<td>Aqueous extract of <em>Drynaria quercifolia</em> (500mg/kg)</td>
<td>5.32±0.4*</td>
<td>3.1±0.4*</td>
<td>21.3±0.3*</td>
<td>44.8±0.4*</td>
<td>3.1±0.4*</td>
</tr>
</tbody>
</table>

**Values are mean ± SEM (n =6). Statistical significance was determined by ANOVA, followed by Dunnett’s “t” test (n=6). **P<0.001, **P<0.01, *P<0.05 when compared against control a P < .001, when compared against standard**

### Table: 2 Effect of aqueous leaf extract of *Drynaria quercifolia* on various parameters in Ethanol induced Gastric ulcers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>pH of gastric juice</th>
<th>Ulcer Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal (Distilled water)</td>
<td>6.27±0.2</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Control (Ethanol induced)</td>
<td>2.86±0.6</td>
<td>18.2±0.3</td>
</tr>
<tr>
<td>III</td>
<td>Ranitidine (50 mg/kg)</td>
<td>5.92±0.4*</td>
<td>2.24±0.4*</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous extract of <em>Drynaria quercifolia</em> (250 mg/kg)</td>
<td>-4.82±0.3</td>
<td>3.6±0.3*</td>
</tr>
<tr>
<td>V</td>
<td>Aqueous extract of <em>Drynaria quercifolia</em> (500mg/kg)</td>
<td>5.46±0.4*</td>
<td>2.7±0.2*</td>
</tr>
</tbody>
</table>

**Values are mean ± SEM (n =6). Statistical significance was determined by ANOVA, followed by Dunnett’s “t” test (n=6). **P<0.001, **P<0.01, *P<0.05 when compared against control a P < .001, when compared against standard**
In the present study aqueous extract of Drynaria quercifolia showed protection against gastric lesions in the experimental rats. Aqueous extract of Drynaria quercifolia reduced the gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory mechanism involved in the extract for their antiulcerogenic activity. Ulcer index parameter was used for the evaluation of antiulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity.

The protection of aqueous leaf extract of Drynaria quercifolia against characteristic lesions may be due to Scopadulcic acid B (SA-B), a novel diterpenoid, is the main ingredient of the biologically active compounds of Drynaria quercifolia and its debenzoyl derivative, diacetyl scopadol (DAS), has been shown to inhibit gastric H+, K(+)-ATPase. These results support the ethnomedical uses of Drynaria quercifolia in the treatment of ulcer. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

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REFERENCES


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