ANTI-ULCER ACTIVITY OF *PTEROSPERMUM ACERIFOLIUM* FLOWER

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ABSTRACT

The study was designed to investigate the antiulcer activity of Juice, ethanolic (50%) extract and ethanolic (95%) extract of *P. acerifolium* flower using different models of gastric ulceration in rats. Peptic ulcers were induced by oral administration of aspirin, ethanol and by pyloric ligation. The animals were administered with juice (2 ml/kg, p. o.), ethanolic (50%) extract (400 mg/kg, p. o.) and ethanolic (95%) extract (400 mg/kg, p. o.) before ulcer induction. Omeprazole (20 mg/kg) was used as a reference standard. The antiulcer activity was accessed by determining and comparing the ulcer index in the test group with that of the standard drug treated group. Gastric volume, pH, total acid and free acid were estimated in the pylorus-ligated rats and showed maximum inhibition. The ulcer index in the *P. acerifolium* treated animals was found to be significantly less in all the models compared to standard drug treated cases. The results suggest that *P. acerifolium* possesses significant antiulcer property which could be due to cytoprotective action of the drug or strengthening of gastric with the enhancement of mucosal defence.

Keywords: *Pterospermum acerifolium*, anti-ulcer, pyloric ligation, ulcer index, total acidity, free acidity.

INTRODUCTION

Peptic ulcer disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder [1]. The pathophysiology of PUD involves an imbalance between offensive (acid, pepsin, and H. pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) [2]. An estimated 15,000 deaths occur each year as a consequence of PUD [1]. In India, PUD is common. In the Indian Pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share. Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection [1,2]. Recently, there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most of the studies focus on newer and better drug therapy. These have been made possible largely by the availability of the proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog [3]. However, the clinical evaluation of these drugs showed development of tolerance and incidence of relapses and side effects that make their efficacy arguable. This has been the rationale for the development of new antiulcer drugs, which includes herbal drugs. Indian Medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including PUD [4]. An indigenous drug possessing...
fewer side effects is the major thrust area of the present day research, aiming for a better and safer approach for the management of PUD. *Pterospermum acerifolium* Linn. has a wide application in traditional system of Indian medicine for example, in ayurvedic anticancer treatment flowers are mixed with sugars and applied locally [5]. Flowers and bark, charred and mixed with kamala applied for the treatment of smallpox. Flowers made into paste with rice water used as application for hemicranias [6]. Stem bark of the plant was found to have antimicrobial activity [7]. Isolation of boscialin glucosides from leaves of *P. acerifolium* have been reported [8]. Hepatoprotective effect of ethanolic extract of leaves of *P. acerifolium* was also reported [9]. Chronic effects of *P. acerifolium*on glycemic and lipidemic status of type 2 model diabetic rats was found beneficial [10]. The barks are reported to be used as anti ulcer [11] anti inflammatory analgesic [12] and anti oxidant activity [13]. Flavonoids like keampferol, keampferide, luteolin, steroids and triterpenoids like sitosterol, taraxerol, friedelin, sugars, and fatty acids are present in the plant [14, 15]. The work was aimed at the scientific validation of the ethno-pharmacological claim about the anti-ulcer effect of juice ethanolic (50%) extract, ethanolic (95%) extract of *Pterospermum acerifolium* flower extract on gastric ulceration.

**MATERIALS AND METHODS**

**Chemicals and drugs**

Omeprazole (Dr. Reddy’s Lab, India) and Topfers reagent (Nice Chemicals, India) were used in this study. All other chemicals used in present study were of analytical grade

**Extract preparation**

The flowers of *P. acerifolium* were collected from Cuttack district of Orissa, India in March, 2002. The plant was identified by the Botanical Survey of India, Howrah and a voucher specimen retained in our laboratory for future reference. The collected flowers were air-dried and pulverised using mechanical grinder.

**Preparation and phytochemical study of extracts**

The flowers (500 g) subjected to successive solvent extraction with 50% ethanol and 95% ethanol. A semi-solid extract was obtained after complete elimination of solvent under reduced pressure. The yield of both the extracts was 12.6±0.45% and 16.2±0.32% respectively. The juice of the *P. acerifolium* flower was prepared by compression method. The extracts were stored in desiccators and used for further experiment after suspending in aqueous Tween 80 solution (0.5%). The chemical constituents of the extracts were identified by qualitative chemical tests and further confirmed by thin layer chromatography study for the presence of alkaloids, sterols and flavonoids.

**Animals**

Male wistar strain albino rats (150-250g) were obtained from central animal house of Institute of Pharmacy and Technology Salipur, Cuttack, Odisha, India. The animals were housed under standardized environmental conditions (at normal room temp, with a 12 hour light and dark cycle) and fed with standard pellet chow feed and water ad libitum. Prior to the experiment, the animals were acclimatized to the laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee of I. P. T., Salipur, Cuttack, Odisha, India with registration number 1053/ac/07/CPCSEA. All the experiments were performed as per the CPCSEA guidelines.

**Assessment of Anti-Ulcer Activity**

**Pyloric ligation induced gastric ulceration [16, 17]**

Albino rats of either sex were divided into four groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Animals in the control group received only distilled water. Juice (2 ml/kg, p. o.), ethanolic (50%) extract (400 mg/kg, p. o.), ethanolic (95%) extract (400 mg/kg, p. o.) of *P.
The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted.

Scoring of ulcer will be made as follows:
- Normal colored stomach: 0
- Red coloration: 0.5
- Spot ulcer: 1
- Hemorrhagic streak: 1.5
- Deep Ulcers: 2
- Perforation: 3

Mean ulcer score for each animal will be expressed as ulcer index.

Ulcer index ($U_i$) was measured by using following formula:

$$U_i = U_n + U_s + U_p \times 10^{-1}$$

Where,
- $U_i$: Ulcer Index;
- $U_n$: Average number of ulcers per animal;
- $U_s$: Average number of severity score;
- $U_p$: Percentage of animals with ulcers

Percentage of protection was calculated as

$$\text{Percentage of Protection} = \frac{C - T}{C} \times 100$$

($C =$ ulcer index in control group; $T =$ ulcer index in test group)

Determination of pH

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter.

Determination of total acidity

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as mEq/L by the following formula:

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

Determination of free acidity

Instead of phenolphthalein indicator, the Topfer’s reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

Table 1: Effect of juice (2 ml/kg, p. o.), ethanolic (50%) extract (400 mg/kg, p. o.), ethanolic (95%) extract (400 mg/kg, p. o.) of *P. acerifolium* flower on gastric
content, pH, total and free acidity in pyloric ligation induced ulceration in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer Index</th>
<th>Protection (%)</th>
<th>pH of gastric juice (ml)</th>
<th>Gastric Juice (ml)</th>
<th>Free acidity mEq/ml</th>
<th>Total acidity mEq/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>4.6±0.10</td>
<td>-</td>
<td>2.43±0.06</td>
<td>8.2±0.10</td>
<td>25.37±0.85</td>
<td>46.32±0.72</td>
</tr>
<tr>
<td>Omeprazole (20 mg/kg)</td>
<td>1.3±0.08</td>
<td>71.73</td>
<td>5.36±0.08**</td>
<td>2.98±0.08**</td>
<td>16.32±0.68**</td>
<td>33.36±0.61**</td>
</tr>
<tr>
<td>Juice of P. acerifolium (2 ml/kg)</td>
<td>2.2±0.09</td>
<td>52.17</td>
<td>3.95±0.06**</td>
<td>3.26±0.06*</td>
<td>23.25±0.58*</td>
<td>41.29±0.56</td>
</tr>
<tr>
<td>Ethanolic (50%) extract of P. acerifolium (400 mg/kg)</td>
<td>1.6±0.08</td>
<td>63.04</td>
<td>4.26±0.05**</td>
<td>3.31±0.10**</td>
<td>19.32±0.61**</td>
<td>39.23±0.76**</td>
</tr>
<tr>
<td>Ethanolic (95%) extract of P. acerifolium (400 mg/kg)</td>
<td>1.7±0.09</td>
<td>65.52</td>
<td>4.48±0.06**</td>
<td>3.12±0.09**</td>
<td>19.03±0.69**</td>
<td>38.64±0.56**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six animals in each group.
* P< 0.05 considered statistically significant as compared with control group.
** P< 0.01 considered statistically significant as compared with control group.

Aspirin-induced gastric lesions

Aspirin (0.2 g/kg x 3 days) were administered once per day to groups of animals for the number of days specified [18]. Animals of control group received 1% carboxy methyl cellulose (CMC) suspension and test groups received juice (2 ml/kg), ethanolic (50%) extract (400 mg/kg), ethanolic (95%) extract (400 mg/kg) of P. acerifolium flower for 10 days. From day 8 the animals received CMC/ P. acerifolium extracts two hours prior to the administration of aspirin. Overnight fasted animals were sacrificed by cervical dislocation one hour after the last dose of ulcerogen. The stomach was incised along the greater curvature and examined for ulcers.

Table 2: Effect of juice (2 ml/kg, p. o.), ethanolic (50%) extract (400 mg/kg, p. o.), ethanolic (95%) extract (400 mg/kg, p. o.) of P. acerifolium flower on aspirin induced ulceration in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>43.2 ± 1.08</td>
<td>-</td>
</tr>
<tr>
<td>Omeprazole (20 mg/kg)</td>
<td>9.16 ± 0.06**</td>
<td>78.79</td>
</tr>
<tr>
<td>Juice of P. acerifolium (2 ml/kg)</td>
<td>15.23 ± 0.57**</td>
<td>64.74</td>
</tr>
<tr>
<td>Ethanolic (50%) extract of P. acerifolium (400 mg/kg)</td>
<td>13.5 ± 0.46**</td>
<td>68.75</td>
</tr>
<tr>
<td>Ethanolic (95%) extract of P. acerifolium (400 mg/kg)</td>
<td>12.67 ± 0.68**</td>
<td>70.67</td>
</tr>
</tbody>
</table>
Values are mean ± SEM for six animals in each group

**P<0.01 considered statistically significant as compared with control group

**Alcohol-induced gastric lesions**

The rats were fasted for 48 h before the experiment but were allowed free access drinking water up till 2 h before the experiment. Gastric ulcer in *Albino rats* was induced by orogastric incubation of absolute ethanol (5 ml/kg) [19]. Ulcer control group was orally administered with vehicle (carboxymethyl cellulose, CMC, 0.25% w/v, 5 ml/kg). The reference group received oral doses of 20 mg/kg omeprazole in CMC (5 ml/kg) as positive controls. Experimental groups were orally administered with. Juice (2 ml/kg), ethanolic (50%) extract (400 mg), ethanolic (95%) extract (400 mg) of *P. acerifolium* flower in CMC solution (5 ml/kg), respectively. One hour after this pre-treatment; all groups of rats were gavaged with absolute ethanol (5 ml/kg) in order to induce gastric ulcers. The rats were euthanized by cervical dislocation 60 mins later under an over dose of diethyl ether anesthesia and their stomachs were immediately excised. Ulcers found in the gastric mucosa, appeared as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Each specimen of gastric mucosa was thus examined for damage. Lesion severity was determined by measuring ulcer index.

Ulcer index was scored by,

10 = shedding of epithelium
20 = haemorrhage
30 = one or two ulcer
40 = many ulcer
50 = perforated ulcer

Mean ulcer score of each group were calculated, which was designated as the ulcer index and percentage of protection was calculated as

\[
\text{Percentage of Protection} = \frac{C - T}{C} \times 100
\]

(C = ulcer index in control group; T = ulcer index in test group)

**Table 3:** Effect of juice (2 ml/kg, p. o.), ethanolic (50%) extract (400 mg/kg, p. o.), ethanolic (95%) extract (400 mg/kg, p. o.) of *P. acerifolium* flower on ethanol induced ulceration in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>73 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>Omeprazole (20 mg/kg)</td>
<td>19.39 ± 1.08**</td>
<td>73.43</td>
</tr>
<tr>
<td>Juice of <em>P. acerifolium</em> (2 ml/kg)</td>
<td>28.25 ± 0.57**</td>
<td>61.3</td>
</tr>
<tr>
<td>Ethanol (50%) extract of <em>P. acerifolium</em> (400 mg/kg)</td>
<td>24.38 ± 0.46**</td>
<td>66.6</td>
</tr>
<tr>
<td>Ethanol (95%) extract of <em>P. acerifolium</em> (400 mg/kg)</td>
<td>22.19 ± 0.68**</td>
<td>69.6</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six animals in each group

**P<0.01 considered statistically significant as compared with control group
Statistical analysis

Results were expressed as mean ± S.E.M. The statistical evaluation were done by analysis of variance (ANOVA) followed by dunet’s test, P<0.05 was considered to be statistically significant.

RESULTS

Pyloric Ligation Induced Gastric Ulceration

Effect of Juice (2 ml/kg), ethanolic (50%) extract (400 mg), ethanolic (95%) extract (400 mg) of *P. acerifolium* flower on pyloric ligation induced ulceration is shown in Table 1. The pyloric ligation has caused the accumulation of gastric secretions of 8.2±0.10 ml with pH 2.43 ± 0.06 in a control group. The total acidity and free acidity of the gastric secretions were found to be 46.32 ± 0.72 and 25.37±0.85 mEq/l respectively. Pretreatment with the Juice (2 ml/kg), ethanolic (50%) extract (400 mg), ethanolic (95%) extract (400 mg) of *P. acerifolium* flower, significantly reduced the volume of gastric secretions 3.26±0.06, 3.31±0.10 and 3.12±.09 respectively. PH of the gastric fluid was significantly elevated up to 4.48±0.06 for the ethanolic (95%) extract of *P. acerifolium* (400 mg/kg). Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with juice (2 ml/kg), ethanolic (50%) extract (400 mg), ethanolic (95%) extract (400 mg) of *P. acerifolium* flower has reduced them significantly. In this model, percentage inhibition of ulceration was found to be 52.17, 63.04 and 65.52 at juice (2 ml/kg), ethanolic (50%) extract (400 mg), ethanolic (95%) extract (400 mg) of *P. acerifolium* flower respectively. The gastroprotection offered by the juice and extract of *P. acerifolium* was comparable to that of the standard drug, omeprazole (20mg).

Aspirin-induced gastric lesions

Juice (2 ml/kg), ethanolic (50%) extract (400 mg), ethanolic (95%) extract (400 mg) of *P. acerifolium* flower was found to possess remarkable ulcer-protective properties (Table 2). The standard drug (Omeprazole) gave 78.79% of ulcer protection.

Ethanol Induced Gastric Ulceration

Ethanol at dose of 0.5 ml/kg showed superficial, deep ulcers and perforations in the control animals (Table 2). However, animals treated with methanol extract of juice (2 ml/kg), ethanolic (50%) extract (400 mg), ethanolic (95%) extract (400 mg) of *P. acerifolium* flower showed significant (P<0.05) reduction in the number of ulcer and ulcer index (Table 3). Anti-ulcerogenic effect of juice (2 ml/kg), ethanolic (50%) extract (400 mg), ethanolic (95%) extract (400 mg) of *P. acerifolium* flower in ethanol induced ulcers was comparable to that of omeprazole (20 mg/kg).

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. To regain the balance, different therapeutic agents including plant extracts may be used [20]. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. Aspirin, phenylbutazone, indomethacin and some non-steroidal anti-inflammatory drugs are also known to cause duodenal and gastric ulceration. Prostaglandin E₂ and I₂ are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant-like phospholipids secretion
in the gastric epithelial cells is also stimulated by the prostaglandin. It is also showed development of gastric ulcers in pyloric ligation model. Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid. [21]. Ethanol is also has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa [22]. 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. It was observed in this study that the \textit{P. acerifolium} significantly reduced the aspirin and ethanol- induced ulcer. This may be due to cytoprotective effect of the extract via antioxidant effects. The extract shows protection against characteristic lesions produced by ethanol administration this antiulcer effect of \textit{P. acerifolium} may be due to both reductions in gastric acid secretion and gastric cytoprotection. The antiulcer property of \textit{P. acerifolium} in pylorus ligation model is evident from its significant reduction in free acidity, total acidity, number of ulcers, ulcer index and increased the pH. It suggests that juice, ethanolic (50\%) extract, ethanolic (95\%) extract of \textit{P. acerifolium} flower can suppress gastric damage induced by aggressive factors. The antiulcer activity of \textit{P. acerifolium} may be attributed to its flavonoids content.

CONCLUSION

The results of the present study suggest that the juice, ethanolic (50\%) extract, ethanolic (95\%) extract of \textit{P. acerifolium} flower may be beneficial in the treatment of gastric lesions. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

REFERENCE


Anti-ulcer activity of *Pterospermum acerifolium* flower


