Antiulcer activity of aqueous suspension of *Saraca indica* flower against gastric ulcers in albino rats

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ABSTRACT

The effect of aqueous suspension of *Saraca indica* flower was investigated in albino rats to evaluate the anticulcer activity by using two models, i.e., pyloric ligation and aspirin induced gastric ulcer. The parameters taken to assess antiulcer activity were free and total acidity and ulcer index. The control group received 2 ml of normal saline and varying doses of the *Saraca indica* flower extract were used for the remaining group (10–30 mg kg⁻¹ b.w.). The results indicate that the aqueous flower extract *Saraca indica* significantly (P<0.005) decreases the free and total acidity and ulcer index with respect to control in a dose dependent manner thus justifying the use of *Saraca indica* flower extract as an antiulcer agent in folklore medicine.

Keywords: *Saraca indica*, antiulcer, pyloric ligation, aspirin

INTRODUCTION

A peptic ulcer is a hole in the gut lining of the stomach, duodenum or esophagus. A peptic ulcer of the stomach called a gastric ulcer, of the duodenum, a duodenal ulcer and of the esophagus, an esophageal ulcer. An ulcer occurs when the lining of these organs is corroded by the acidic digestive juices that are secreted by the stomach cells. For many years, excess acid believed to be the major cause of ulcer disease. Accordingly, treatment emphasis was on neutralizing and inhibiting the secretion of stomach acid (Yogendr Bahuguna et al, 2008). Major cause of ulcers is the chronic use of anti-inflammatory medications, commonly referred to as NASAID’s (non-steroidal anti-inflammatory drugs). Cigarette smoking is also an important cause of ulcer formation and ulcer treatment failure (Harsh Mohan, 2002).

According to Akah et al. (1998) reviewed that the plant parts are widely used ethnomedically in Northern Nigeria for the treatment of various gastrointestinal disorders. A pinch of the grounded flowers is taken with water for the treatment of peptic ulcers. *Saraca indica*, a plant belonging to the family Fabaceae, grows as a small evergreen tree native to India. It is popularly called Ashoka. The flowers are orange or orange yellow eventually turning vermillion and very fragrant. Indian materia medica (1982) describes the use of flowers of *Saraca indica* in the treatment of a number of ailments, including internal piles, diabetes, dyspepsia, indigestion, burning sensation, blood disorders, fractures, tumors, bits, anti-inflammatory and skin discoloration. The major constituents of flowers of plants contain saracacin, saracadin, waxy substances, fatty acids, falvinoids and other constituents.

So far no systematic study has been reported for antiulcer properties of *Saraca indica* flower extracts. In the present study effort has been made to establish the scientific validity to the antiulcer property of *Saraca indica* flower extracts using pylorus ligation and aspirin induced ulceration models in albino rats.

MATERIALS AND METHODS

Acute toxicity study

Twenty five albino rats were divided into 5 equal groups. Different doses of *Saraca indica* flower extract ranging from 10 to 100 mg kg⁻¹ b.w. were administered orally to the albino rat each as a single dose. The albino rat were starved for 16 h prior to administration of the extract. The control group received 2 ml normal saline. All rats were observed for general behaviour over a period of 24 h. The mortality rate was recorded daily.

Plant materials and extraction procedure

Fresh flowers of *Saraca indica* were collected from Bharathiar University campus, Coimbatore, India, during Feb. – Apr. 2008 and authenticated at Department of Botany, Bharathiar University, Coimbatore, India by Mr. Thirugnanasambantham. A voucher specimen of the plant was deposited in the Department of Botany herbarium, Bharathiar University, Coimbatore, India.
The clean flowers were sun-dried for 3 days, and pulverized into powder. The powder was successively extracted using Soxhlet extractor. About 600 g of the powdered sample was extracted using distilled water. A condenser and round bottom flask were fitted at the top and bottom of the Soxhlet, respectively. The solvent was poured into the round bottom flask and flask was placed in a water bath. There was a water pump circulator that provided water for cooling the condenser. As the extract was collected in the round bottom flask with the solvent, the water bath heated it and the solvent evaporated and condensed. The solvent was then re-used for more extraction. The cycle continued until the extract obtained from the sample was about 60 g (9.15% yields).

**Experimental animal**

Adult albino rats of Wistar strain, weighing between 150 – 250 g were used. They were obtained from the Animal House, Bharathiar University, Coimbatore, India. The animals were housed in separate cages where they were maintained under standard conditions of temperature 27±2°C, relative humidity of 60±5% and 12:12 hour light: dark cycle prior to experimentation for 3 weeks. The animals were fed with standard pellet diet and water *ad libitum.* Experiments were complied with the ruling of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Registration No.: 722/02/a/CPCSEA dt. 04.12.2006) and the study was permitted by the Institutional Ethical Committee (IEC) of the Bharathiar University, Coimbatore, India.

**Pylorus ligation induced gastric ulceration**

Albino rats weighing between 150 – 250 g were selected for pyloric ligation ulcer model as described by Njar et al. (1995). Pyloric ligated ulcer rats were divided into 4 groups, each group consisting of six animals. Animals were fasted for 24 h. One group received normal saline 2 ml/kg b.w., the 2, 3 and 4 group received 10, 20 and 30 mg/kg b.w. respectively of *Saraca indica* flower extract dissolved in distilled water by oral route, 3 min. prior to pyloric ligation. Animals were sacrificed 4 h later by cervical dislocation. The stomach was opened to collect the gastric contents. Scoring of gastric ulceration was done as described by Kunchandy (1985). Ulceration in the stomach was accessed by means of a scoring technique whereby macroscopic examination of the stomach was made using a hand (10x) and ulcers were scored using the method and criteria of Elegba and Bamgbose (1976). Normal gastric mucosa was scored 0, punctuate hemorrhage, pinpoint ulcer was scored 0.5, one or two small hemorrhages ulcer was scored 1.0 while ulcers greater than 3mm in diameter were scored 2.0. Ulcer index and percentage inhibition of ulceration were calculated as earlier described (Njar et al, 1995) thus:

\[
\text{Ulcer index} = \frac{\text{Mean degree of ulceration x percentage group of ulceration}}{100}
\]

\[
\% \text{Inhibition of ulceration} = \frac{\text{Ulcer index in control} - \text{Ulcer index in test}}{\text{Ulcer index in control}} \times 100
\]

The total volume of gastric content was measured. The gastric contents were centrifuged at 100 rpm for 10 min. 1ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01 N NaOH using Topfer’s reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as:

\[
\text{Acidity} = \frac{\text{Vol. of NaOH x Normality x 100}}{0.1}
\]

**Aspirin induced gastric ulceration experiment**

In the aspirin – induced ulcer experiments (Hedge et al, 1994), 4 groups of albino rats (150-250 g), with each group consisting of six animals were used. The first group served as a control group and the 2, 3 and 4 group served as test group. The 2, 3 and 4 group received 10, 20 and 30 mg/kg b.w. respectively of aqueous flower extract of *Saraca indica* dissolved in distilled water by oral route for 8 days. After 8 days of treatment, animals were fasted for 24 h. Ulcer was produced by administration of aqueous suspension of aspirin (a dose of 200 mg/kg b.w. orally) on the day of sacrifice. The animals were sacrificed 4 h later and stomach was opened to calculate the ulcer index by Kunchandy method (1985).

**Statistical analysis**

Data were expressed as mean±SEM. The differences between two or more values were compared using the student’s t-test. The significance of difference was accepted at p<0.005.

**RESULT**

The results showed that *Saraca indica* flower possess potent antiulcer properties. In the acute toxicity study carried out a dose of 25 mg/kg given orally caused neither death nor any observable adverse symptoms (Table 1). There was no significant change in daily body weight compared with untreated control during the next 3 weeks. However oral administration of 100 mg/kg of *Saraca indica* flower extract caused 100% mortality in rats. This result indicates that the extract has a sufficient margin of safety; consequently its administration as it is used in folk medicine may not have any immediate deleterious effect.
Table 1: Acute toxicity study of aqueous suspension Saraca indica flower on albino rat

<table>
<thead>
<tr>
<th>Dose (mg/kg b.w.)</th>
<th>Log dose</th>
<th>No. dead</th>
<th>No. alive</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0</td>
<td>2.300</td>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>75.0</td>
<td>2.000</td>
<td>4</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>50.0</td>
<td>1.699</td>
<td>3</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>25.0</td>
<td>1.398</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>12.5</td>
<td>1.097</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

N = 5; * p<0.005 vs. control; values are in Mean ± SEM

The result in table 2 and 3 showed that aqueous flower extract of Saraca indica exhibited a dose dependent gastro-protective effect on pylorus ligation and aspirin-induced ulceration in albino rats. The study on effects of alcoholic flower extract of Saraca indica indicate that the significantly (p<0.005) reduces the pH, free and total acidity, ulcer index of gastric secretion and also has activity against gastric ulcers in albino rats.

Table 2: Effect of aqueous suspension Saraca indica flower on pylorus ligation-induced gastric ulceration in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>Free acidity</th>
<th>Total acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2 ml/kg</td>
<td>2.72±0.06</td>
<td>28.23±0.26</td>
<td>45.67±1.21</td>
</tr>
<tr>
<td>II</td>
<td>Aqueous extract</td>
<td>10 mg/kg</td>
<td>1.45±0.06*</td>
<td>16.95±0.21*</td>
<td>35.22±0.26*</td>
</tr>
<tr>
<td>III</td>
<td>Aqueous extract</td>
<td>20 mg/kg</td>
<td>1.36±0.04*</td>
<td>14.28±0.42*</td>
<td>33.47±0.30*</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous extract</td>
<td>30 mg/kg</td>
<td>1.25±0.04*</td>
<td>11.02±0.40*</td>
<td>29.10±0.18*</td>
</tr>
</tbody>
</table>

N = 6; * p<0.005 vs. control; values are in Mean ± SEM

Table 3: Effect of aqueous suspension Saraca indica flower on aspirin-induced gastric ulceration in albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>% protection from ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2 ml/kg</td>
<td>3.44±0.33</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Aqueous extract</td>
<td>10 mg/kg</td>
<td>1.7±0.12*</td>
<td>50.00</td>
</tr>
<tr>
<td>III</td>
<td>Aqueous extract</td>
<td>20 mg/kg</td>
<td>1.1±0.10*</td>
<td>70.58</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous extract</td>
<td>30 mg/kg</td>
<td>0.7±0.14*</td>
<td>79.41</td>
</tr>
</tbody>
</table>

N = 6; * p<0.005 vs. control; values are in Mean ± SEM

DISCUSSION

According to Robert et al. (1979) reported that the necrotizing agents-induced gastric ulcers, the lesions were characterized by multiple haemorrhage red bands of different sizes along the longitudinal axis of the glandular stomach. This model is extensively used to screen drugs for cytoprotection. This study provided a substantial evidence for anti-ulcer and anti-secretory effects of an aqueous suspension of Saraca indica flower. Saraca indica flower suspension significantly inhibited the ulcerative lesions in all animals treated with necrotizing agents, which was further confirmed by histological findings in which necrosis, inflammatory, dysplastic changes and ulcers were abolished in rats pretreated with Saraca indica flower suspension. The ability of gastric mucosa to resist injury by endogenous secretions (acid, pepsin and bile) and ingested irritants (NSAIDs), can be attributed to a number of factors that have been referred to collectively as mucosal defense (Wallace, 2001). Kinoshita (1995) reported that the gastric mucosal lesions induced by necrotizing agents such strong alcalis are due to depression of the gastric defensive mechanisms. Although aspirin-induced ulcers are not inhibited by anti-secretory agents, they are inhibited by agents that enhance mucosal defensive factors such as prostoglandins (Morimoto et al, 1991). The current results suggest that the anti-ulcerogenic effect of aqueous suspension of Saraca indica flower may be related to its cytoprotective activity.

Gastric ulceration is a major limitation to the use of non-steroidal anti-inflammatory drugs (NSAIDs) (Wallace, 2000). NSAIDs can cause damage to the gastric mucosa via several mechanisms, including their topical irritant effect on the epithelium, impairment of the mucosal barrier function, suppression of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow and interference with the repair of superficial injury. The presence of acid in the lumen of stomach also contributes to the pathogenesis of NSAIDs-induced ulcers and bleeding by impairing the restitution process, interfering with haemostasis and inactivating several growth factors that are important in mucosal defence and repair (Toma, 2005; Whittle, 2003). In the present study, aspirin-induced gastric lesions were extensively prevented by aqueous suspension of Saraca indica flower.

Sulphhydryl compounds have been significantly implicated in the maintenance of gastric integrity, particularly when reactive oxygen species are involved in the pathophysiology of tissue damage (Blandizzi et al, 2005). Since Saraca indica flower suspension significantly enhances gastric tissue NP-SH concentration, it is conceivable that it is endowed with antioxidant properties accounting for its gastroprotective action. Hence, it may be presumed that the replenishing potential of sulphhydryl levels might play an important role in the gastroprotective activity of aqueous suspension of Saraca indica flower-treated rats. Furthermore, the ability of anise suspension to protect against ulcers in NSAID-induced gastric damage may be due to the enhanced synthesis of mucus, bicarbonates and prostaglandins, as well as reduced acid output. Consequently these activities can promote the inhibition of basal gastric acid secretion as observed in our pylorus-ligated shay rat model (Kimura et al, 2001; Loguercio et al, 1991). On the other hand, it is also important to note that NSAIDs can increase gastric acid secretion, through prostaglandin inhibitory effects on parietal cells (Ligumsky et al, 1983; Soll, 1986).

In the present study, Saraca indica flower aqueous suspension treatment significantly reduced basal gastric acid volume, titratable acidity and completely inhibited ulcer formation in rats. However, to date it is still controversial about relationship between the acid output and the genesis of acute gastric mucosal lesions (AGML). Our results support this correlation as Saraca indica flower suspension significantly reduced basal gastric secretion and prevented the occurrence of AGML in pylorus-ligated rats and thus, supporting the hypothesis of “no acid no ulcer” (Melé et al, 2006). It has been postulated that histamine may be involved in the formation of pylorus-ligated ulcers and play a mediating role in the gastric secretion stimulated by gastrin, vagal stimulation and cholinergic agents (Blandizzi et al, 1993). The correlation between gastric mucus and acid
secretions in our experiments, clearly demonstrated that the gastric protective activity observed may be associated with correction or normalization of the altered balance between erosive action of acid and gastric mucosal defence. Gastric wall mucus is thought to play an important role as a defensive factor against gastric mucosal damage (Marhuenda et al., 1993). The determined gastric wall mucus is used as an indicator for gastric wall mucus secretion (Mersereau and Hinchey, 1982). In the present investigation, *Saraca indica* flower suspension caused a significant enhancement of aspirin-induced gastric wall mucus depletion in rats, which further confirms the ability of aqueous suspension to prevent the effects of damaging agents. These findings indicate that *Saraca indica* flower suspension preserves gastric mucus secretion and strengthens gastric mucosa defense factors in experimental rats.

In conclusion, *Saraca indica* flower suspension exhibits an anti-ulcer potential activity through at least one or more possible mechanisms including inhibition of basal gastric secretion, stimulation of mucus secretion and endogenous gastric mucosal prostaglandin synthesis.

REFERENCES


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