Antiulcer Activity of Methanol Extract of *Erythrina indica* Lam. Leaves in Experimental Animals

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ABSTRACT

Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5–10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. Here, present study was carried out to investigate antiulcer activity of methanol extract of *Erythrina indica* (family: Fabaceae) leaves in pylorus ligated and indomethacin induced ulceration in the albino rats. Preliminary methanol extract of *E. indica* was subjected to the acute oral toxicity study according to the OECD guideline no. 423. Based on which, three dose levels i.e. 125, 250 and 500mg/kg were selected for the further study. In pylorus ligation induced ulcer model, various parameters were studied viz. gastric volume, pH, total acidity free acidity, and ulcer index. Ulcer index and percentage inhibition of ulceration was determined for indomethacin induced ulcer model. Ranitidine at 100mg/kg was used as the standard drug. Pretreatment of methanol extract of *E. indica* leaves showed significant (P<0.01) decrease in the gastric volume, total acidity and free acidity. However, pH of the gastric juice was significantly (P<0.05) increased only at higher dose, 500mg/kg. It showed also significant (P<0.01) decrease in number of ulcers and ulcer score index in pylorus ligation and indomethacin induced ulceration models. The methanol extract of *E. indica* leaves possess significant antiulcer properties in a dose dependent manner. In conclusion the antiulcer properties of the extract may be attributed to the polyphenolic compounds that are present in it.

Keywords: Antiulcer activity, *E. indica*, Indomethacin, Ranitidine.

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries (1). Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors (2). Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage (3). Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors (4–5).

*Erythrina indica* is a middle-sized quick growing tree found in Bengal and many parts of India especially in southern India. It belongs to family Fabaceae commonly known as ‘Mandara’ in Hindi and ‘Indian coral tree’ in English. It grows up to 18 m in height, the leaves are trifoliolate, flowers are borne in dense racemes, coral red and used traditionally for the treatment of liver
trouble, joint pain, dysentery, convulsion, as a diuretic, laxative and an anthelmintic (6–8). Its powdered bark is traditionally used for rheumatism, itching, burning sensation, fever, asthma, and leprosy (9). The leaves are laxative, stomachic, anthelmintic and useful for uropathy, inflammations and gastropathy (10). Aqueous extract of Sri Lankan *Erythrina indica* leaves are reported to exhibit sedative but no analgesic activity (11). It is also known to exhibit antidiuretic activity (12).

Though the leaves of the plant have been used in the treatment of gastrointestinal disorders in the folk medicine, there are no scientific evidences available on its anti-ulcer potential. Hence, the present work was undertaken to investigate the anti-ulcer activity of methanol extract of *E. indica* leaves in experimental animals.

**MATERIALS AND METHODS**

**Plant material**

The leaves of *E. indica* Lam. were collected from the mature plant in and around the city of Mumbai, Maharashtra, India during month of August 2007 and dried under shade. The plant was authenticated by Dr. Ganesh Iyer, Botanist, Ramnarayan Ruia College, Mumbai. A voucher specimen (No. 2007/08/07) has been kept in our laboratory for future reference.

**Preparation of plant extract**

The dried powdered leaves of *E. indica* were defatted using petroleum ether (60–80 °C) and successively extracted with methanol in Soxhlet extractor. Extract was filtered through vacuum filter and the filtrate was concentrated in vacuum evaporator. Dried extract was used for the further study. The yield of the extract was found to be 7.89% w/w.

**Experimental animals**

Albino rats (Sparque Dawley strain) of either sex, weighing 180–200g were procured from Glenmark Pharmaceuticals, Mhape, Navi Mumbai. Swiss albino mice (20–22g) of either sex were purchased from Haffkine Biopharmaceuticals, Parel Mumbai. All the animals were placed in polycarbonate cages in a controlled room temperature 22±1°C and relative humidity of 60–70% in registered animal house (87/1999/CPCSEA). The animals were maintained on standard pellet diet (Amrut brand, Sangli, India) and water *ad libitum*. They were acclimatized to laboratory condition for seven days before commencement of the experiment. Ethical clearance was obtained from Institutional Animal Ethical Committee.

**Acute oral toxicity study**

Acute oral toxicity study of methanol extract of *E. indica* was carried out in Swiss albino mice of both sexes (20–22 g) according to OECD guidelines no 423. Extract at different doses up to 2000mg/kg, p. o. was administered and animals were observed for behavioral changes, any toxicity and mortality up to 48 h (13).

**Assessment of anti-ulcer activity**

**Pyloric ligation induced gastric ulceration**

Albino rats of either sex were divided into five 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. Methanol extract of *E. indica* at 125, 250 and 500mg/kg, p. o. were given to the animals in the treatment group. Ranitidine (100mg/kg) was used as standard. After 1h of drugs treatment, they were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al. (14), avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anaesthetic ether after four hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity. The inner surface of free stomach was examined for gastric lesions.

**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 50ml 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:

\[ n \times 0.01 \times 36.45 \times 1000 \]

Where *n* is volume of NaOH consumed, 36.45 is molecular weight of NaOH, 0.01 is normality of NaOH, 1000 is the factor (to be represented in litre) (15).
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 (15).

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a ×5 magnifier lens to assess the formation of ulcers. The number of ulcers were counted. Ulcer scoring was undertaken according to Vogel et al. (16). The scores were: 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer, 3 = perforation.

Ulcer index was measured by using following formula according to vogel et al. (15).

\[
U_I = \frac{U_N + U_S + U_P}{10}\times 10^{-1}
\]

\[U_I\] = 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 inhibition of ulceration was calculated as below:

% Inhibition of Ulceration = \(\frac{U_I_{\text{control}} - U_I_{\text{test}}}{U_I_{\text{control}}}\) × 100

Indomethacin induced ulceration

Method of C.N. Aguwa, et al (17) was followed with minor modifications for the experiment. Thirty albino rats were taken. They were divided into five groups of six rats each. All the rats were starved for 24 h. After the fasting period, Indomethacin (40 mg/kg, p.o.) was given.

All samples of the plant extract were given 60 min prior to indomethacin as follows:

- Group I: treated with Indomethacin (40mg/kg, p.o.) and was kept as control.
- Group II: treated with Ranitidine (100mg/kg, p.o) and was kept as standard.
- Group III: treated with the methanol extract of E. indica (125mg/kg, p.o).
- Group IV: treated with the methanol extract of E. indica (250mg/kg, p.o).
- Group V: treated with the methanol extract of E. indica (500mg/kg, p.o).

The animals were sacrificed 5h after the treatment. Stomach was cut open in the greater curvature and ulcer scoring was done by using magnifying lens and the ulcer scored according to its severity in comparison with that of standard. Ulcer score, ulcer index and % inhibition of ulceration was calculated as before.

Statistical analysis

The results are expressed as the mean± SD for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnet’s t-test. Results were considered to be statistically significant at P<0.05.

RESULTS

Acute oral toxicity study

Swiss albino mice of both the sexes treated with methanol extract of E. indica did not show any behavioral changes, toxic reaction or mortality. It was found to be safe at the dose of 2000mg/kg. LD50 of the methanol extract E. indica was found to be >2000mg/kg.

Pyloric ligation induced gastric ulceration

Effect of methanol extract of E. indica on pyloric ligation induced ulceration is shown in Table 1 and 2. The pyloric ligation has caused the accumulation of gastric secretions of 11.58±1.27ml with pH 3.82±0.13 in a control group. The total acidity and free acidity of the gastric secretions were found to be 6746.66±874.04 and 5466.66±809.80 meq./l respectively. Pretreatment with the E. indica extract, significantly (P<0.01) reduced the volume of gastric secretions 8.43±0.57, 6.33±0.76 and 6.00±0.43ml at the doses of 125, 250 and 500mg/kg respectively. pH of the gastric fluid was significantly (P<0.05) elevated up to 4.78±0.63 only at higher dose of the extract. In addition, total acidity and free acidity were also reduced significantly (P<0.01) in a dose dependant manner. Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with E. indica extract has reduced them significantly (P<0.01) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 7.89, 22.58 and 55.26 at 125, 250 and 500mg/kg respectively. The gastroprotection offered by the test extract was comparable to that of the standard drug, ranitidine (100mg/kg).

Indomethacin induced ulceration

Indomethacin at dose of 40mg/kg showed superficial, deep ulcers and perforations in the control animals (Table 3). However, animals treated with methanol extract of E. indica at 125, 250 and 500mg/kg doses showed significant (P<0.01) reduction in the number of ulcer and ulcer index (Table 2). It showed 5.82, 52.08 and 53.41%
Table 1: Effect of methanol extract of *E. indica* on gastric content, pH, total and free acidity in pyloric ligation induced ulceration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) p.o.</th>
<th>Gastric content (ml)</th>
<th>pH of gastric content</th>
<th>Total acidity (meq./l)</th>
<th>Free acidity (meq./l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>D/W*</td>
<td>11.58±1.27</td>
<td>3.82±0.13</td>
<td>6147.90±796.46</td>
<td>4981.50±737.93</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>100</td>
<td>6.65±0.93**</td>
<td>5.31±0.58**</td>
<td>1658.47±640.00**</td>
<td>1968.30±543.58**</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>125</td>
<td>8.43±0.57*</td>
<td>3.11±0.47</td>
<td>2855.25±170.44**</td>
<td>1634.17±206.72**</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>250</td>
<td>6.33±0.76**</td>
<td>3.10±0.62</td>
<td>2527.20±263.51**</td>
<td>1318.27±231.00**</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>500</td>
<td>6.00±0.43**</td>
<td>4.78±0.83*</td>
<td>2399.62±518.77**</td>
<td>844.42±231.00**</td>
</tr>
</tbody>
</table>

*Distilled water, Values in the results are expressed as mean±SD, (n=6).*

*p<0.05

**p<0.01 significantly different in comparison with Negative control (ANOVA followed by Dunnet's t-test).

Table 2: Effect of methanol extract of *E. indica* on gastric lesion in pyloric ligation induced ulceration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) p.o.</th>
<th>Number of ulcers</th>
<th>Ulcer score</th>
<th>% of animals with Ulcers (Incidences %)</th>
<th>Ulcer index</th>
<th>% Inhibition of ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>D/W*</td>
<td>11.13±1.36</td>
<td>2.66±0.51</td>
<td>100</td>
<td>3.8±0.45</td>
<td>——</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>100</td>
<td>0.30±0.05**</td>
<td>0.33±0.51**</td>
<td>33.33</td>
<td>1.14±0.17**</td>
<td>70.75</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>125</td>
<td>3.80±0.75**</td>
<td>2.16±0.75</td>
<td>100</td>
<td>3.5±0.25**</td>
<td>07.89</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>250</td>
<td>3.00±0.52**</td>
<td>1.00±0.63**</td>
<td>88.33</td>
<td>3.1±0.17**</td>
<td>22.58</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>500</td>
<td>0.50±0.54**</td>
<td>0.50±0.54**</td>
<td>50.00</td>
<td>1.7±0.18**</td>
<td>55.26</td>
</tr>
</tbody>
</table>

*Distilled water, Values in the results are expressed as mean±SD, (n=6).*

*p<0.05

**p<0.01 significantly different in comparison with Negative control (ANOVA followed by Dunnet's t-test).

Table 3: Effect of methanol extract of *E. indica* on gastric lesion in indomethacin induced ulceration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) p.o.</th>
<th>Number of ulcers</th>
<th>Ulcer score</th>
<th>% of animals with Ulcers (Incidences %)</th>
<th>Ulcer index</th>
<th>% Inhibition of ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>D/W*</td>
<td>11.20±1.16</td>
<td>2.16±0.75</td>
<td>100</td>
<td>3.77±0.38</td>
<td>——</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>100</td>
<td>1.00±1.26**</td>
<td>0.33±0.51**</td>
<td>33.33</td>
<td>1.15±0.42**</td>
<td>69.30</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>125</td>
<td>4.83±0.75**</td>
<td>0.75±0.75</td>
<td>100</td>
<td>3.5±0.25**</td>
<td>05.82</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>250</td>
<td>3.83±0.98**</td>
<td>0.98±0.54**</td>
<td>83.33</td>
<td>1.81±0.32**</td>
<td>52.08</td>
</tr>
<tr>
<td><em>E. indica</em></td>
<td>500</td>
<td>2.33±0.51**</td>
<td>0.51±0.54**</td>
<td>66.66</td>
<td>1.76±0.17**</td>
<td>53.41</td>
</tr>
</tbody>
</table>

*Distilled water, Values in the results are expressed as mean±SD, (n=6).*

*p<0.05

**p<0.01 significantly different in comparison with Negative control (ANOVA followed by Dunnet's t-test).

ulceration inhibition at the dose of 125, 250 and 500mg/kg respectively whereas ranitidine showed 69.30% ulceration inhibition. Anti-ulcerogenic effect of *E. indica* in indomethacin induced ulcers was comparable to that of ranitidine, 100mg/kg.

**DISCUSSION**

Although in most of the cases the etiology of the ulcers is unknown, it is generally accepted that they are a result of an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defensive mechanisms (18). To regain the balance, different therapeutic agents including plant extracts may be used (19–20). *E. indica* extract is one such herbal drug used in the present study primarily to evaluate the anti-ulcerogenic or ulcer preventive potency in pylorus ligation and indomethacin induced ulcers in rats.

Pylorus ligation induced ulcers are due to autodigestion of the gastric mucosa and breakdown of the gastric mucosal barrier (21). 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 (22).

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 (23). In addition, Brodie (24–25) 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 (20).
The antiulcer property of *E. indica* in pylorus ligation model is evident from its significant reduction in free acidity, total acidity, number of ulcers and ulcer index. *E. indica* treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that *E. indica* can suppress gastric damage induced by aggressive factors.

Ulcer formation induced by NSAID’s like Indomethacin is known to be related with inhibition of cyclooxygenase that prevents prostaglandin biosynthesis, which in turn inhibits the release of mucus, a defensive factor against gastrointestinal damage (26). In addition there is some evidence that NSAID’s may induce ulcer by causing the back diffusion of H+ ion in to mucosal cells (27). *E. indica* showed significant (P<0.01) antiulcer activity in indomethacin induced ulcers in rats. Therefore the gastroprotective effect of the test extract may be due to its ability to inhibit the synthesis of prostaglandins/leukotrienes.

The preliminary phytochemical analysis of *E. indica* extract showed the presence of alkaloids, flavonoids, triterpenoids, carbohydrates and glycosides. It is also reported that, the flavonoids like flavone glycoside (28) and isoflavonoid (Indicaine B and Indicaine C) (29) have been isolated from its root bark. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed (30–32). It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen (33–35). So the antiulcer activity of *E. indica* may be attributed to its flavonoids content. In this study we observed that, the methanol extract of *E. indica* leaves provides significant antiulcer effect against gastric ulcers in rats.

**CONCLUSION**

The results of the present study suggest that the methanol extract of *E. indica* leaves 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.

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**REFERENCES**


