ANTI-ULCER POTENTIAL OF LOBOPHORA VARIEGATA (LAMOUR.) WOMERSLEY EX OLIVIERA (BROWN SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

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Abstract: The anti-ulcer potential of methanolic extract of Lobophora variegata (Lamour.) Womersley ex Oliviera (Phaeophyceae) collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India was studied in aspirin induced ulceration in Wistar albino rats. Anti-ulcer effect was evaluated by measuring ulcer index and percentage of ulcer healing. The methanolic extract of 200mg/kg and 400mg/kg of Lobophora variegata was found significant antiulcer activity as evidenced by the data obtained. Among the two concentrations studied, 200mg/kg methanolic extract showed more effective compared to 400mg/kg. The present experimental findings suggested that methanol extract of Lobophora variegata can be useful for treating peptic ulcers.

Key words: Brown seaweeds, Lobophora variegata, anti-ulcer, methanolic

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INTRODUCTION:
Oceans cover nearly a major portion of earth’s surface and are very rich in terms of biodiversity. It serves as a habitat for nearly 97% of the flora and fauna. The marine biodiversity imparts wide range of services and resources to the humans and besides, play a substantial role in regulation of climate as well as nutrient recycling. Ocean’s diversity is crucial as it produces plant biomass that fulfills the food requirements of all the lives existing in the ocean ranging from simple planktons to large marine mammals. The biodiversity of oceans are enriching, as more and more new species are being discovered and classified systematically. Marine macro algae or seaweeds are an important resource in marine ecosystem and also it is useful to human in various ways. They supply oxygen to the biosphere, are a source of food for fishes, cattle and man. Seaweeds are also used as medicine and fertilizers. Chemically the bioactive metabolites of seaweeds include brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulphur nitrogen heterocyclics, sterols, terpenoids, polysaccharides, peptides and proteins [1]. There are several studies have been undertaken to reveal the medicinal value of seaweeds in different part of the world. Antitumors [2, 3], anticoagulant [4, 5], antifouling [6], antioxidant [7] and antimicrobial activities [8, 9, 10] have been studied on various types of seaweeds. At present there are scanty literatures available on the bioactivity of seaweeds. Therefore, the present study was undertaken in order to examine the anti-ulcer activity of Lobophora variegata (Lamour.) Womersley ex Oliviera from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India using Wistar albino rats.

MATERIALS AND METHODS:
Collection of Plant Sample
Lobophora variegata (Lamour.) Womersley ex Oliviera is brown seaweed belonging to Phaeophyceae member showed much attention in the present study for its anti-ulcer activity. Lobophora variegata were collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu, India. The collected plant samples were rinsed with marine water to remove debris and epiphytes. The entire epiphytes were removed using soft brush. The plants were brought to the laboratory. In the laboratory, the plants were once again washed in freshwater and stored in refrigerator for further analysis [11].

Preparation of methanol extract
For the preparation of methanol extract of Lobophora variegata, the collected plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered sample was packed in Soxhlet apparatus and extracted with methanol for 8h separately. The excess amount of methanol was evaporated and fine methanol crude powder was prepared and stored in the refrigerator for the anti-ulcer studies [12].

Experimental Animals
Wistar albino rats (160-200g) of either sex were procured from Venkateswara Enterprises, Bangalore, Karnataka, India. The selected animals were acclimatized for 7 days under standard husbandry conditions, i.e. room temperature 35±1°C, relative humidity 45-55% and light/dark cycle 12/12h. Animals were provided with standard rodent pellet diet and had free access to water. The composition of diet is 10% protein, 4% Arachis oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conduct between 10.00 and 17.00h and were in accordance with the ethical guidelines of the International Association for Study of Pain [13]. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity test
Acute oral toxicity study was performed as per OECD-423 guidelines [14]. Albino rat (n=6) of either sex selected through random sampling technique was used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract (50% methanolic extract) was administered orally at the dose level of 5 mg/kg body weight by gastric intubation and observed for 14 days. If mortality is observed in 2 out of 3 animals, then the dose administered would be assigned as toxic dose. If mortality is observed in 1 animal, then the same dose would be repeated again to confirm the
toxic dose. If mortality is not observed, the procedure would be repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. According to the results of acute toxicity test, the doses were chosen for experiments.

**Aspirin induced gastric ulceration and experimental design [15]**

Wistar albino rats of either sex were divided into four groups of six animals each. Animals were fasted for 24h before the study, but had free access to water. Animals in the control group received only distilled water. Methanol extract of the selected brown seaweed at 200 and 400mg/kg were given to the animals in the treatment group. Ranitidine (10mg/kg) was used as a standard. After 1h of drugs treatment, they were anaesthetized with the help of anaesthetic ether and the abdomen was opened by a small midline incision. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al. [16] 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 overdose 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 2000rpm for 10min. From the supernatant, aliquots (1ml of each) were taken for the determination of pH, total and free acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity.

**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 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........................ (1.0)
- Hemorrhagic streak............. (1.5)
- Deep Ulcers.................... (2.0)
- Perforation....................... (3.0)

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

\[ UI = UN + US + UP \times 10^{-1} \]

Where,

- UI= Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers

Percentage of inhibition of ulceration was calculated as below

\[ \% \text{ Inhibition of Ulceration} = \frac{\text{Ulcer index (control)} - \text{Ulcer index (test)}}{\text{Ulcer index (control)}} \times 100 \]

**RESULTS AND DISCUSSION:**

Anti-ulcer potential of *Lobophora variegata* showed a dose dependent protection against aspirin (500mg/kg body weight) induced ulcers in rats. Maximum protection was seen in the Ranitidine treated group. Even though the methanol extract produced a significant reduction of ulcer index only in the higher dose treated groups (200 and 400mg/kg body weight), all the test doses produced a decrease in ulcer index as compared to the control. The volume of gastric secretion and total acidity was significantly reduced in all drug treated groups as compared to control. Gastric pH was also found to be increased in all drug treated groups as compared to control, with maximum increase being produced by ranitidine as standard drug.

**Table 1: Anti-ulcer activity of methanol extract of *Lobophora variegata***

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Volume of Gastric juice (ml)</th>
<th>pH</th>
<th>Acidity (mEq/l)</th>
<th>Ulcer Index</th>
<th>% of Inhibition of Ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Free</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.52±0.2</td>
<td>3.95±0.1</td>
<td>121.75±9.8</td>
<td>132.5±4.5</td>
<td>......</td>
</tr>
<tr>
<td>10mg/kg Ranitidine</td>
<td>4.70±0.2</td>
<td>4.18±0.2</td>
<td>47.0±1.22</td>
<td>63.50±5.67</td>
<td>74.25±2.9</td>
</tr>
<tr>
<td>200mg/kg Methanol extract</td>
<td>5.12±0.1</td>
<td>5.17±0.3</td>
<td>82.75±4.7</td>
<td>109.0±5.9</td>
<td>69.42±2.5</td>
</tr>
<tr>
<td>400mg/kg Methanol extract</td>
<td>5.46±0.1</td>
<td>5.01±0.6</td>
<td>117.5±2.9</td>
<td>125.25±3.7</td>
<td>62.55±3.4</td>
</tr>
</tbody>
</table>
The effect of methanol extract of *Lobophora variegata* on aspirin induced ulceration was shown in Table-1. The aspirin induction has caused the accumulation of gastric secretions of 4.52ml with pH 3.95 in the control group. The total acidity and free acidity of the gastric secretions were found to be 132.5 and 121.75mEq/l respectively. Pre-treatment with the methanol extract of *Lobophora variegata* significantly (P<0.05) reduced the volume of gastric secretions 5.12 and 5.46ml at the doses of 200 and 400mg/kg respectively. In addition, total acidity (109mEq/l) and free acidity (82.75mEq/l) at 200mg/kg extract and total acidity (125.25mEq/l) and free acidity (117.5mEq/l) at 400mg/kg extract were also reduced significantly (P<0.05) in a dose dependant manner. Further it was noted that aspirin induction has caused gastric ulcerations and pre-treatment with the methanol extract of *Lobophora variegata* has reduced significantly (P<0.05) in a dose dependent manner. In this model, the percentage inhibition of ulceration was found to be 69.42% and 62.55% at 200 and 400mg/kg respectively.

**CONCLUSION**

The results of the present investigation recommended that the methanolic extract of *Lobophora variegata* possesses the potential anti-ulcer activity in both the doses of 200mg/kg and 400mg/kg. Among the two doses used, 200mg/kg methanolic extract showed the best result as compared with 400mg/kg. Further, chemical analysis on the composition of methanolic extract of *Lobophora variegata* is necessary to isolate and identify bioactive compounds that may have applications in therapeutic field of anti-ulcer drug.

**REFERENCES:**