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SCREENING OF ANTI-ULCER ACTIVITY OF METHANOLIC EXTRACT OF *GRACILARIA CORTICATA* J.AG. (RED SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

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ABSTRACT

The effect of methanol extract of *Gracilaria corticata* J.Ag. (Rhodophyceae) collected from Thoothukudi in the south east coast of Tamil Nadu, India was evaluated in aspirin induced ulceration in Wistar albino rats. Anti-ulcer activity was evaluated by measuring ulcer index and percentage of ulcer healing. The methanol extract of 200mg/kg and 400mg/kg of *Gracilaria corticata* J.Ag. showed significant antiulcer activity as evidenced by the data obtained. Among the two concentrations studied, 200mg/kg methanol extract showed more effective compared to 400mg/kg. The present experimental findings suggested that methanol extract of *Gracilaria corticata* J.Ag. can be useful for treating peptic ulcers.

Key Words: Seaweeds, *Gracilaria*, anti-ulcer, methanolic.

INTRODUCTION

The important alternate modern approach to drug discovery is through the medicinal plants. Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. India is a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine [1]. Moreover India is possibly the largest producer of medicinal herbs and is rightly called the botanical garden of the world. India officially recognizes over 3000 plants for the medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine representing about 75% of the medicinal needs of the third world countries (Rajashekaran, 2002). In 1991, 42 new agents were introduced to medical practice of which 16 were natural products or were derived from the natural products. Similarly in 1992, 4 new chemical entities were introduced and among them were natural products or their derivatives [2]. Currently available treatments for peptic ulcers include antacids (systemic and nonsystemic) and drugs which reduce acid secretion such as proton pump inhibitors, anticholinergics, prostaglandin analogues, ulcer protectives and ulcer healing drugs [6]. These drugs have decreased the morbidity rates, but produce many adverse effects including relapse of the disease and are often expensive for the poor [7]. In light of the above, it is pertinent to study natural products from plants as potential anti-ulcer compounds. Due to less side effects compared to synthetic drugs, currently 80 % of the world population depends on plant derived medicine for the first line of primary health care [8]. Marine macro algae or seaweeds have also been proven to be rich sources of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical potential [9]. *Gracilaria corticata* J.Ag. is a red marine macro alga found in abundance on the south east coast of Tamil Nadu, India. Hence, anti-ulcer activity of *Gracilaria corticata* J.Ag. collected from Thoothukudi in the south east coast of Tamil Nadu, India was evaluated in aspirin induced ulceration in Wistar albino rats.

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MATERIALS AND METHODS

Collection of Plant Sample

Gracilaria corticata J.Ag. (Figure 1) is red seaweed belonging to Rhodophyceae member showed much attention in the present study for anti-ulcer activity. *Gracilaria corticata* J.Ag. 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 [10].

Preparation of methanol extract

For the preparation of methanol extract of *Gracilaria corticata* J.Ag., 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. 3g 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 [11].

Experimental Animals

Wistar albino rats (160-200g) and Swiss albino mice 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 excess 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 [12]. 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 [13]. Albino mice (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 [14]

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 green seaweeds 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.* [15] 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 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 number 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:

Ulcer index (UI) was measured by using following formula:

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

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

% Inhibition of Ulceration= Ulcer index (control)-Ulcer index (test)/Ulcer index (control) × 100

RESULTS AND DISCUSSION

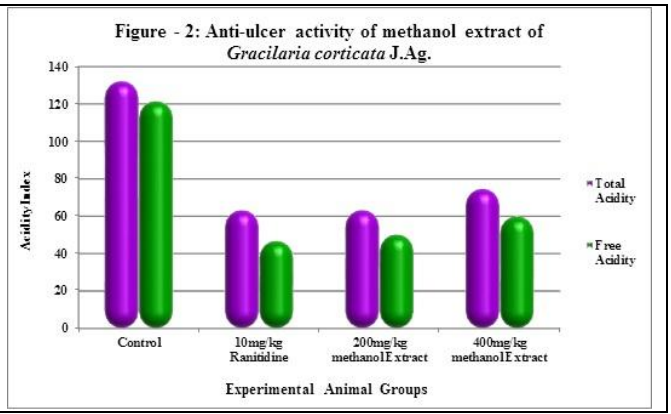
Anti-ulcer activity of *Gracilaria corticata* J.Ag. showed a dose dependent protection against aspirin (500mg/kg body weight) induced ulcers in rats. Maximum protection was seen in the Ranitidine treated group. Even though the methanol extract produced a significant reduction of ulcer index only in the 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.

The effect of methanol extract of *Gracilaria corticata* J.Ag. on aspirin induced ulceration was shown in Table-1 and Figure-2. 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 *Gracilaria corticata* J.Ag. significantly ($P<0.05$) reduced the volume of gastric secretions 5.41 and 5.73ml at the doses of 200 and 400mg/kg respectively. pH of the gastric fluid was significantly ($P<0.05$) elevated up to 5.33 at 200mg/kg and 5.24 at 400mg/kg methanol extract. In addition, total acidity (63.58mEq/l) and free acidity (50.45mEq/l) at 200mg/kg extract and total acidity (74.9mEq/l) and free acidity (60.17mEq/l) at 400mg/kg extract were also reduced significantly ($P<0.05$) in a dose dependant manner. Further it was observed that aspirin induction has caused gastric ulcerations and pre-treatment with the methanol extract of *Gracilaria corticata* J.Ag. has reduced significantly ($P<0.05$) in a dose dependent manner. In this model, the percentage inhibition of ulceration was found to be 65.25 and 53.25 at 200 and 400mg/kg respectively.

Table 1. Anti-ulcer activity of methanol extract of *Gracilaria corticata* J.Ag.

Animal groups	Volume of Gastric juice	pH	Acidity (mEq/l)		Ulcer Index	% of Inhibition of Ulcer
			Free	Total		
Control	4.52±0.2	3.95±0.1	121.7±9.8	132.5±4.5	.....	.....
Ranitidine 10mg/kg	4.70±0.2	4.18±0.2	47.0±1.22	63.50±5.67	74.25±2.9	74.25%
200mg/kg Methanol extract	5.41±0.1	5.33±0.1	50.45±1.14	63.58±2.6	65.25±0.8	65.25%
400mg/kg Methanol extract	5.73±0.1	5.24±0.16	60.17±1.52	74.9±2.14	53.25±1.3	53.25%

Figure 1. Natural Habit of *Gracilaria corticata* J.Ag.



CONCLUSION

The results of the present study suggested that the methanolic extract of *Gracilaria corticata* J.Ag. possesses the potential anti-ulcer activity in both the doses of 200mg/kg and 400mg/kg. Among them 200mg/kg methanolic extract showed the best result as compared with 400mg/kg. Further, chemical analysis on the composition of methanolic extract of *Gracilaria corticata* J.Ag. is necessary to isolate and identify bioactive compounds that may have applications in therapeutic field of anti-ulcer drug.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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