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SKELETAL MUSCLE RELAXANT ACTIVITY OF METHANOLIC EXTRACT OF *GRACILARIA CORTICATA* J.AG. (RED SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

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ABSTRACT

The objective of the present study was to evaluate the skeletal muscle relaxant activity of the methanolic extract of *Gracilaria corticata* J.Ag., an important red seaweed collected from Hare Island, Thoothukudi, Tamil Nadu, India using Rota-rod method test. Experiments were carried out on Wistar albino rat and the animals were randomly allotted to the different control and test groups. The methanol extract was found that a dose up to 2000mg/kg body weight did not show any toxic manifestations or death. The extract was administered orally at a dose of 100 and 200mg/kg. Diazepam in a dose of 5mg/kg was used as a standard. Methanol extract at the dose level of 200 mg/kg body weight showed significant skeletal muscle relaxant activity. On the bases of these results, it can be concluded that *Gracilaria corticata* J.Ag. may be used to develop herbal medicines against the same.

Keywords: Seaweed, Muscle Relaxant, *Gracilaria corticata*, Methanol extract, Wistar rats.

INTRODUCTION

In recent years, there has been an increasing interest worldwide in the use of herbal plants as health supplements or medicines. Systematic studies on the effect of specific medicinal herbs on the immune system are designed to obtain evidence-based scientific knowledge on the appropriate use of traditional medicinal herbs [1]. The potential of marine organisms in producing immense collection of bioactive compounds having antitumor, antiviral, anti-inflammatory, immunosuppressive, neurosuppressive, muscle relaxant, antimalarial, antibiotic and antifouling properties is well appreciated [2]. Being a primitive multicellular organism and because of their sedentary nature, seaweeds are extensively exposed to diverse microbes, organic matters and predators which induces the production of defensive secondary metabolites [3].

The recent trends have seen bioprospecting of seaweeds for diverse potential compounds. Seaweeds are considered as part of a healthy diet, especially in Japan, Korea, China and the Philippines [4, 5]. Seaweeds possess anti-diabetic, anti-oxidant, anti-obesity, anti-hyperlipidaemic and anti-inflammatory activities [6]. Seaweeds contain higher potassium, magnesium and calcium ion concentrations than other foods [5]. Seaweeds may prevent diet-induced cardiovascular disease as an alternative source of dietary fibre [7]. Fibre is the largest component of the seaweed biomass [8,9] and therefore may be present in sufficient amounts when included in the diet to prevent metabolic syndrome associated with obesity, type-2 diabetes and cardiovascular complications [10]. In this context, the present study was carried out to evaluate the muscle relaxant potential of the methanolic extract of *Gracilaria corticata* J.Ag collected from Hare Island, Thoothukudi, Tamil Nadu, India.

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 muscle relaxant activity. *Gracilaria corticata* J.Ag. was collected from Hare Island, Thoothukudi, 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].

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

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. 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 analgesic activity [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 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 [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 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.

Muscle Relaxant Activity

Rotarod:

The rotarod apparatus consists of a metal rod (3cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The rod is in a height of about 50cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller. Wistar albino mice underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20rpm) for 5min, were used for the test [15, 16]. Wistar albino mice were divided into four groups consisting of six animals each. Group I served as control which received saline solution, animals of group II received standard drug Diazepam at a dose of 10mg/kg, while group III and IV received the methanol extract of the selected green seaweeds at the dose of 200mg and 400mg/kg. The animals were placed on the rotating rod and fall off time i.e., when the animal falls from the rotating rod, was recorded which was taken as grip strength.

RESULTS AND DISCUSSION

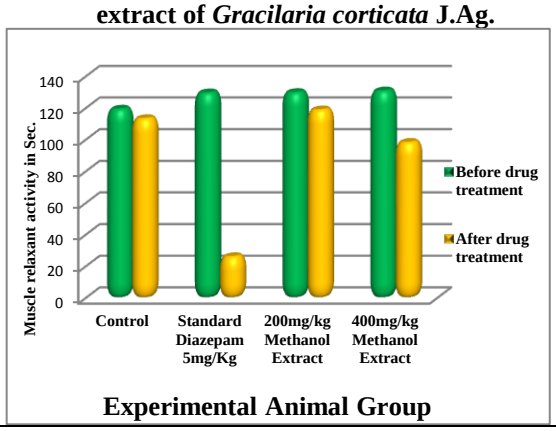
The methanolic extract of the selected red seaweed *Gracilaria corticata* J.Ag was pharmacologically screened for its muscle relaxant study. The result indicated that methanol extract possessed skeletal muscle relaxant activity in experimental mice. The means duration of stay in control was 113.5 seconds after 60min of treatment with distilled water (10ml/kg). The effect in reduction of stay on rotarod was 26.27 seconds by Diazepam at the dose of 5mg/kg which was positive control. The muscle relaxant activity of methanolic extract of *Gracilaria corticata* J.Ag. was shown in Table-1 and Figure-2. The time spent on revolving rod was reduced by 200mg and 400mg/kg methanolic extract of *Gracilaria corticata* J.Ag. in comparison with control group.

The means duration of stay in 200mg/kg methanol extract was 119 seconds after 60 min of treatment. The percentage of the effect in reduction of stay on rotarod was 8.46. The mean duration of stay of the mice in 400mg/kg methanol extract was found to be 98.5 seconds after the treatment of 60 minutes. The percentage of the effect in reduction of stay on rotarod was 24.80. In this test, both 200 and 400mg/kg methanol extract of *Gracilaria corticata* J.Ag reduced the time spent by the mice on revolving rod when compared to control.

Table 1. Muscle relaxant activity of methanol extract of *Gracilaria corticata* J.Ag.

Animal groups	Fall of time (seconds)		% Increase in time
	Before drug	After drug	
Control	119.50±7.92	113.5±6.2	4.93±1.64
5mg/kg Diazepam	129.75±1.47	26.27±3.0	79.74±2.44
200mg/kg Methanol extract	130.00±1.32	119.0±4.8	8.46±2.34
400mg/kg Methanol extract	131.00±2.85	98.50±3.5	24.80±2.96

Figure 2. Muscle relaxant activity of methanol extract of *Gracilaria corticata* J.Ag.



CONCLUSION

The muscle relaxant property can be evaluated using the well recommended animal protocols i.e. rotarod test. In each case, the muscle relaxant effect was checked after 30, 60 and 90 min of the methanol extracts administration. The maximum effect was noticed after 60 min of the extract administration. In all the experimental model, a significant skeletal muscle relaxation was produced by higher dose of the methanol extract (400mg/kg) compared to the lower dose of the extract (200mg/kg) of *Gracilaria corticata* J.Ag. It means that the muscle relaxant property of the crude may be due to the presence of various secondary metabolites.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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