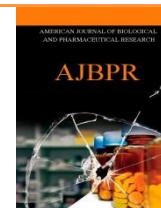




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SCREENING OF DIURETIC ACTIVITY OF METHANOL EXTRACT OF *GRACILARIA CORTICATA* J.AG. (RED SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

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Article Info	ABSTRACT
<p>Received 25/03/2014 Revised 15/04/2014 Accepted 18/05/2014</p> <p>Key words:-Red seaweed, Diuretic, <i>Gracilaria corticata</i>, Furosemide, Electrolyte.</p>	<p>Seaweeds have valuable secondary metabolites that have an important role for the biological activities. In the present study, the screening of diuretic activity of <i>Gracilaria corticata</i> J.Ag. collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu, India was analyzed. Dried powder plant materials were subjected to methanol extract at the dose of 200mg/kg and 400mg/kg body weight. Methanol crude extract showed potential significant diuretic activity in both doses from 1 hour to 4 hour as compared to the standard drug furosemide. The present study has been provided the evidence for the diuretic activity of <i>Gracilaria corticata</i> J.Ag. which could partly contribute to its ethno medical use.</p>

INTRODUCTION

Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health care needs [1]. Diuretics are the drugs that increase the rate of urination. There are several classes of diuretics that increase the excretion of water from the body by acting through different mechanisms. Diuretics are capable of increasing the flow of urine and are useful in the treatment of disease related with the retention of fluids [2].

Seaweeds have been widely used by coastal people for thousands of years owing to the high nutritional values and medicinal purposes [3]. Seaweeds have rich

sources of food, feed, medicines and energy. Moreover, seaweeds are the only sources for industrially important phycocolloids like agar, carrageenan and alginate [4]. Apart from industrial uses, in recent years, polysaccharides of plant origin have emerged as an important class of bioactive natural products [5]. Thus, medical and pharmaceutical industries are also interested since seaweeds have proven to be rich sources of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical potential [6]. Indeed, the complex polysaccharides from red algae especially sulfated galactans and carrageenans defined as polymers of galactose possess broad spectrum therapeutic properties. They are reported to exhibit blood anticoagulant [7-9], immune modulating, antitumor [10], antiviral [11] and antioxidant activities [12-13].

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In fact, red seaweed galactan sulfates are linear polysaccharides with alternating 3-linked β -D-galactopyranose units and 4-linked 3,6-anhydro- α -galactopyranose or α -galactopyranose units [14], having different positions and degrees of sulfation. Other



substituents such as methyl ethers, pyruvic acid, ketals and single stubs of β -D-xylopyranose and/or other monosaccharides are sometimes present [15]. So in this context, the identification of natural and original polysaccharide structures constitute a new field of applications. The review of the scientific literature did not reveal any information on the diuretic studies of *Gracilaria corticata* J.Ag. In the present investigation, an attempt was made to assess the efficacy of *Gracilaria corticata* J.Ag. collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu, India for the diuretic activity in terms of urine output, sodium, potassium and chloride levels in urine in experimental animals using standard methods.

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 diuretic activity. *Gracilaria corticata* J.Ag. were collected from Hare Island, Thoothukudi in the south east coast of Tamil Nadu, India during the month of January 2014. 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 [16].`

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 antipyretic activity [17].

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 $35\pm 1^\circ\text{C}$, 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 [18]. 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 [19]. 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.

Diuretic activity

The method described Wiebelhaus *et al* [20] was employed with modification for the assessment of diuretic activity. Healthy albino rats of either sex (160-200g) were divided into four groups of six animals each. They were fasted 18h prior to the test with free access to water. On the day of the experiment, Group I animals were given 5ml/Kg of body weight normal saline orally and served as control group. Groups II, III and IV were treated with standard drug (Furosemide 5mg/Kg p.o.), methanolic crude extract (200mg/Kg) and methanolic crude extract (400mg/Kg) respectively. Standard drug and crude extracts were administered orally (p.o.). Immediately after dosing, the rats were placed in the metabolic cages with special provision to collect faeces and urine. Animals were kept at room temperature of $35\pm 1^\circ\text{C}$ throughout the experiment.

Urine excreted for the next 4h from 15min was collected and the total 4h urine volume for each rat was compared with the volume of urine produced after the administration of normal saline. The volume of urine excreted during 4h for each animal in the group was expressed as the percent of the liquid (normal saline) administered. This percentage gave a measure of urinary excretion independent of the animal weight. The ratio of urinary excretion in the test group to urinary excretion in the control group was used as a measure of the diuretic action for the given dose of the drug. The diuretic activity of the crude extract was compared to that of the standard drug in the test group [21]. The parameters taken to study were pH, Na⁺, K⁺ and Cl⁻ concentration in urine. Urine



samples were analyzed thereafter for pH by pH meter, Na⁺ and K⁺ concentration by flame photometric method while Cl⁻ concentration will be determined titrimetrically and the results were reported as mean ± SEM.

Statistical analysis

The data were expressed as mean ± SEM. The data of diuretic activity were analyzed by one-way analysis of variance (ANOVA) followed by “Dunnett’s test.” p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Screening of diuretic activity of methanol crude extract of *Gracilaria corticata* J.Ag was studied by determining the effect on albino rats. The methanol extract of *Gracilaria corticata* J.Ag. showed the highest noticeable diuretic activities which was dose dependent on albino rats. Acute toxicity studies showed that the methanolic extracts did not cause any mortality up to 2000 mg/Kg and were considered as safe [22]. The present study examined the diuretic potential of *Gracilaria corticata* J.Ag. The results showed that both the methanol crude extracts (200mg/kg and 400mg/kg) increased urine output up to 4h following its administration which compared to control group (Table 1). The amount of urine collected from Standard Furosemide (group II), 200mg/kg Methanol crude extract (group III) and 400mg/kg Methanol crude extract (group IV) was found to be 6.95ml, 12.3ml and 1.43ml respectively as compared with control (group I) as presented in Table. 1. Diuretic activity of 200mg/kg methanol crude extract was found to be highest in the present study.

Table 2 showed the urinary electrolyte content following the administration of the methanol crude extracts. The dose of 200mg/kg methanol crude extract produced a significant increase in Na⁺ (from 76.30meq/L to 132.22meq/L), K⁺ (from 48.54meq/L to 80.67meq/L), Cl⁻ (from 84.22meq/L to 89.86meq/L) excretion and pH showed a small change (from 7.1 to 6.6) compared with the control group (P<0.01). The dose of 400mg/kg methanol crude extract produced a significant increase in the

Na⁺(from 76.30meq/L to 88.43meq/L), K⁺ (from 48.54meq/L to 56.79meq/L) Cl⁻ (from 84.22meq/L to 87.38meq/L) excretion and pH showed decreased state from 7.1 to 6.5 compared with control group (P<0.001).

However, only the 200 mg/kg of the methanol crude extract produced a significant increase in Na⁺ from 128.32meq/L to 132.22meq/L, K⁺ from 78.87meq/L to 80.67meq/L), Cl⁻ from 82.63meq/L to 89.86meq/L) excretion and pH showed a small change from 6.5 to 6.6 compared with the Standard drug Furosemide (5mg/kg p.o.). The dose of 400mg/kg methanol crude extract produced a significant decrease in the Na⁺ (from 128.32meq/L to 88.43meq/L), K⁺ (from 78.87meq/L to 56.79meq/L) excretion followed by increase in Cl⁻ (from 82.63meq/L to 87.38meq/L) excretion and pH remained unchanged 6.5 compared with Standard drug Furosemide (5mg/kg p.o.).

In the present study, methanol crude extract of *Gracilaria corticata* J.Ag. affected urinary electrolyte. Both the methanol extracts (200mg/kg and 400mg/kg) were not accompanied with reduction in urinary K⁺ level. In addition there was no alkalization of urine. These data indicate that they were not acting as potassium sparing diuretics [23, 24]. The methanol crude extract was unlikely to be acting as thiazide diuretics. Thiazide only increase urinary K⁺ level and alter the urinary Na⁺/K⁺ ratio. But in the present study both urinary Na⁺ and K⁺ level were increased without any alteration in Na⁺/K⁺ ratio.

The diuresis induced by methanolic crude extract of *Gracilaria corticata* J.Ag was similar to that of furosemide and accompanied by marked increases in both urinary Na⁺ and K⁺ level. Further the urine was slightly acidified. These characteristics strongly suggested that the methanol crude extract was acting as loop diuretic. Loop diuretics inhibit the Na⁺, K⁺, and Cl⁻ co-transporter system in the thick ascending loop of nephron, thereby increasing natriuresis and kaliuresis and also cause acidification of urine [23, 24].

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

Group	Drugs	Total Amount of Urine collected						Total Volume
		15mts	30mts	1hr	2hr	3hr	4hr	
I	Normal Saline (5mg/kg p.o.)	0ml	0.10ml	1.1ml	2.1ml	2.28ml	2.50ml	2.50ml
II	Standard Furosemide (5mg/kg p.o.)	0.85ml	1.95ml	3.55ml	4.95ml	5.85ml	6.95ml	6.95ml
III	Methanol crude extract (200mg/kg p.o.)	1.2ml	4.7ml	6.9ml	9.2ml	10.8ml	12.3ml	12.3ml
IV	Methanol crude extract (400mg/kg p.o.)	0.00ml	0.35ml	0.60ml	0.74ml	0.99ml	1.43ml	1.43ml



Table 2. Effect of Methanol extract of *Gracilaria corticata* J.Ag.on electrolyte excretion and pH on rat.

Group	Electrolyte Concentration (meq/L)				
	Drugs	Na ⁺	K ⁺	Cl ⁻	pH
I	Normal Saline (5mg/kg p.o.)	76.30 ± 0.48	48.54 ± 1.23	84.22 ± 1.06	7.1 ± 0.2
II	Standard Furosemide (5mg/kg p.o.)	128.32 ± 0.61	78.87 ± 0.93	82.63 ± 0.57	6.5 ± 0.12
III	Methanol crude extract (200mg/kg p.o.)	132.22 ± 1.05	80.67 ± 0.82	89.86 ± 0.96	6.6 ± 0.16
IV	Methanol crude extract (400mg/kg p.o.)	88.43 ± 1.05	56.79 ± 0.82	87.38 ± 0.96	6.5 ± 0.16

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

CONCLUSION

From the above results, it can be concluded that the methanolic crude extract of *Gracilaria corticata* J.Ag. possess significant diuretic activity by increasing the total urine output and increased excretion the total Sodium, Potassium and Chloride levels. However, the activity was dose dependent. At 200mg/kg methanol crude extract was

highly effective than 400mg/kg methanolic crude extract and the standard drug furosemide. Hence, the isolation of active principles will be advantageous to produce novel bioactive constituents from methanol crude extract of *Gracilaria corticata* J.Ag. which may possess more significant for diuretic activity.

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