

Evaluation of Diuretic Activity of Ethanolic Extract of *Gracilaria salicornia* on Mice

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Abstract

The present study was aimed to explore the diuretic activity of the ethanolic extract of *Gracilaria salicornia* (C. Agardh) EY Dawson collected from Mandapam, Ramanathapuram district, Tamil Nadu on albino mice. The dried and powdered *Gracilaria salicornia* was extracted in ethanol to estimate the diuretic activity. The result showed significant diuretic activity in the ethanolic extract of *Gracilaria salicornia* (C. Agardh) EY Dawson with increased the total urinary volume and urinary electrolyte excretion, when compared to control. 400 mg/kg dose of ethanolic extract of *Gracilaria salicornia* showed higher Na⁺, K⁺ and Cl⁻ excretion than the 200 mg/kg dose of ethanolic extract. The study concludes that the ethanolic extract of *Gracilaria salicornia* (C. Agardh) E.Y. Dawson has potential diuretic activity.

Keywords: Diuretic, *Gracilaria salicornia*, Wistar albino mice, electrolytes

Introduction

A Diuretic drug is defined as any compound that raises urination and is also used for the treatment of congestive heart failure, edema, liver and kidney diseases, and hypertension (Angappan *et al.*, 2018). Diuretics increase the urine flow by decreasing Na⁺ re-absorption in the kidney and other epithelial tissues. The class of thiazide, K⁺ sparing, loop such as furosemide and CA inhibitors are commercially available diuretic drugs with various side effects such as dehydration, leading to hypotension, hypokalemia, cough, fever, metabolic alkalosis, excessive weight loss, vomiting, nausea and unusual bleeding (Angappan *et al.*, 2018). Plant-based diuretic drugs are considered a better alternative to commercial diuretic drugs for their consequent side effects. The diuretic effects of plant secondary compounds on humans have been reported for the past centuries (Beaux *et al.*, 1999; Navarro *et al.*, 1994). Traditionally, plants with diuretic effects were administered to the patient orally by direct consumption of leaves or as an infusion of leaves in hot water (Dearing *et al.*, 2001). Marine macro algae are diverse in bioactive compounds with numerous properties, which may be utilized for biotechnological applications (Lomartire *et al.*, 2021). Very few works have explored the diuretic potential of Marine macro algae on diuretic properties (Vasanthi *et al.*, 2003; Shri and Paul, 2014; Paul, 2014). The present study aimed to evaluate the diuretic effect of the ethanolic extract of *Gracilaria salicornia* (C. Agardh) E.Y. Dawson.

Materials and Methods

Preparation of Extract

Gracilaria salicornia (C. Agardh) EY Dawson, belongs to the family Gracilariaceae. It was collected from Manadapam in the Ramanathapuram district, Tamil Nadu. The collected plants were rinsed with marine water to remove debris and epiphytic organisms from the samples. All the epiphytes were completely cleaned off using a soft brush. The plants were again washed with fresh water after being brought to the laboratory and kept in a refrigerator for further analysis (Shri and Paul, 2014). The collected samples were dried in the shade and ground into a fine powder. 30g of powdered samples were extracted with 300ml of ethanol using the Soxhlet apparatus for eight hours. The surplus of ethanol was evaporated and kept in the refrigerator for the diuretic activity (Paul, 2014).

Experimental Animals & Acute Toxicity test

Wistar albino mice (150–200 g), including both males and females, were acquired from Venkateswara Enterprises, Bangalore, Karnataka, India. All the animals were housed for seven days under standard environmental conditions (35±1° C, 45-55% humidity and 12h/12h light/dark cycle). All experiments followed the ethical guidelines set by the International Association for the Study of Pain and were carried out between 10:00 and 17:00 hours (Zimmerman, 1983). The present study had been granted approval by the Institutional Animal Ethical Committee (IAEC) for the research work (Approval No: SBCP/2024-25/CCSEA/IAEC-I(2)/F16/361). An acute oral toxicity study was performed in accordance with OECD guideline 423 (Ecobichon, 1997). Six albino mice of both sexes were randomly selected for the assessment. A total of six albino mice, comprising both sexes, were randomly assigned to undergo the acute toxicity study. The animals were administered orally at the dose level of 5 mg/Kg body weight and kept in observation for 14 days. The experimental animals were administered to higher doses such as 50, 300 and 2000 mg/ kg body weight.

Diuretic activity

The diuretic activity was evaluated following the procedure reported by Wiebelhaus et al. (1965) with minor modifications. A total of 160–200 g healthy albino mice of both sexes were divided into four groups, each consisting of six animals. They fasted with free access to water before 18 h to the test. On the experiment day, Group I animals served as the control group and were given 5ml/Kg normal saline of body weight orally. Groups II, III and IV were treated orally with 5mg/Kg p.o. of standard drug Furosemide, 200mg/Kg of ethanolic crude extract and 400mg/Kg ethanolic crude extract respectively. After the dose was administered, the rats were housed in metabolic cages with designated sections for collecting feces and urine. Throughout the experiment, animals were kept at a room temperature of $35 \pm 1^\circ\text{C}$. The total urine volume of each rat was recorded for the next 4 hr from 15 min and compared with the total volume of urine produced after the administration of normal saline. The diuretic activity of the crude extract was evaluated against the standard drug in the test group (Mukherjee, 2002). Urinary parameters such as pH, Na^+ , K^+ , and Cl^- concentrations were measured to assess the diuretic effect.

Statistical Analysis

The data were recorded as mean $\pm$ SEM. The diuretic activity data were subjected to one-way ANOVA, followed by Dunnett's post-hoc test for statistical analysis. P-value < 0.05 was considered statistically significant.

Results and Discussion

The volume of urine was measured up to 4 h. Acute toxicity studies showed that the ethanolic crude extract was considered safe and did not cause any mortality up to 2000 mg/kg (OECD, 1996). The total volume of urine excretion data shows that the ethanolic extract and furosemide treated rat group excreted a high volume of urine compared to the untreated control group. The ethanolic extract of *Gracilaria salicornia* (C. Agardh) EY Dawson treated rat groups urine excretion had increased in dose dependant manner (Table-1). The amount of urine collected from standard furosemide (Group-II), 200 mg/kg ethanolic crude extract (Group-III) and 400 mg/kg ethanolic crude extract (Group-IV) was found to be 27.94 ml, 12.07 ml and 19.71 ml respectively. 400 mg/kg ethanolic extract recorded the highest diuretic effect (19.71/4h), when compared to 200 mg/kg ethanolic extract in the present study. Furosemide (5 mg/kg) treated animal group urine volume (27.94 ml/4 h) is very high, when compared to 200 mg/kg and 400 mg/kg ethanolic extracts.

Table-1: Diuretic activity of *Gracilaria salicornia* (C. Agardh) EY Dawson

Group	Drugs	Total amount of urine collected (ml)						Total volume (ml)
		15mts	30mts	1h	2h	3h	4h	
I	Control	0.0	0.22	1.31	1.93	2.52	2.70	8.68
II	5mg/kg Furosemide	1.70	3.51	4.47	5.20	6.12	6.94	27.94
III	200mg/kg extract	0.55	1.56	1.98	2.16	2.78	3.04	12.07
IV	400mg/kg extract	0.78	2.15	3.42	3.83	4.54	4.99	19.71

The effect of single doses of furosemide and the ethanolic extracts of *Gracilaria salicornia* on electrolyte (Na^+ , K^+ and Cl^-) excretion in the 4 h urine is presented in Table-2. Both doses (200 mg/kg and 400 mg/kg) of ethanolic extract of *Gracilaria salicornia* enhanced the excretion of urine output and electrolytes compared to the control. A 400 mg/kg dose of ethanolic extract of *Gracilaria salicornia* resulted in greater excretion of Na^+ , K^+ , and Cl^- compared to the 200 mg/kg dose. The dose of 400 mg/kg ethanol crude extract produced a significant increase in the Na^+ (from $69.13 \pm 0.56\text{meq/L}$ to $95.81 \pm 2.19\text{meq/L}$), K^+ (from 45.19 ± 1.18 to $68.81 \pm 1.17\text{meq/L}$), Cl^- (from 80.89 ± 0.95 to $85.45 \pm 1.98\text{meq/L}$) excretion and the pH value was decreased from 7.1 to 6.8 compared with control group. The standard furosemide (5 mg/kg) showed the highest urinary electrolyte excretion with Na^+ ($121.87 \pm 1.89\text{meq/L}$), K^+ ($77.15 \pm 1.65\text{meq/L}$), Cl^- ($91.94 \pm 1.34\text{meq/L}$) with the pH value 6.8 ± 0.09 .

Table-2: Effect of ethanolic extract of *Gracilaria salicornia* (C. Agardh) EY Dawson on electrolyte excretion and pH on mice.

Drugs	Electrolyte concentration (meq/L)			
	Na^+	K^+	Cl^-	pH
Control	69.13 ± 0.56	45.19 ± 1.18	80.89 ± 0.95	7.1 ± 0.11
5mg/kg Furosemide	121.87 ± 1.89	77.15 ± 1.65	91.94 ± 1.34	6.8 ± 0.09
200mg/kg Ethanolic extract	82.29 ± 1.86	54.99 ± 1.19	82.56 ± 2.23	6.9 ± 0.19
400mg/kg Ethanolic extract	95.81 ± 2.19	68.81 ± 1.17	85.45 ± 1.98	6.8 ± 0.17

The standard drug furosemide increases the urine output and electrolytes excretion by inhibiting $\text{Na}^+/\text{K}^+2\text{Cl}^-$ symporter (co-transporter system) in the ascending limb of the loop Henley and cause acidification of urine while thiazide diuretics

enhance the excretion of Na^+ and Cl^- by inhibiting the Na^+/Cl^- symporter in the distal convoluted tubule, competing with Cl^- at its binding site (Jackson, 1996). In the present study, ethanolic extracts of *Gracilaria salicornia* (200mg/kg and 400mg/kg) enhanced the urinary and electrolytes excretion in a dose-dependent manner. However, furosemide, the reference drug, resulted in the maximum urinary output and electrolyte excretion. Both the ethanolic extracts of *Gracilaria salicornia* were not exhibited reduction in urinary K^+ level and alkalization of urine indicated that they were not acting as potassium sparing diuretics (Rang *et al.*, 2003). Thiazide diuretics work by increasing urinary K^+ level and altering the urinary Na^+/K^+ ratio. But the present study showed increased Na^+ , K^+ excretion with an altered Na^+/K^+ ratio. Based on urinary output, electrolyte excretion, Na^+/K^+ ratios, and pH changes, the ethanolic extract of *Gracilaria salicornia* exhibits a thiazide-like diuretic profile. Diuretic activity of the *Gracilaria salicornia* may be due to the presence of phenolics, alkaloids and terpenes (Rang *et al.*, 2003). The diuretic activity observed in this study may be attributed to initial vasodilation or the stimulation of regional blood flow, or alternatively, to the inhibition of tubular reabsorption of water and anions (Hailu and Engidawork, 2014).

Conclusion

The ethanolic extract of *Gracilaria salicornia* (C. Agardh) EY Dawson possess notable diuretic efficacy by increasing the total urinary volume and urinary electrolyte excretion when compared to control. 400 mg/kg doses of ethanolic extract of *Gracilaria salicornia* showed higher Na^+ , K^+ and Cl^- excretion than the 200 mg/kg doses of ethanolic extract. The development of plant-based drugs is essential due to the side effects associated with synthetic compounds. From the study, it is concluded that the ethanolic extract of *Gracilaria salicornia* (C. Agardh) EY Dawson can be used for diuretic activity.

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