Diuretic Activity of Aqueous Ethanolic Extract of Leaves of Costus speciosus in Normal Wistar Albino Rats.


Department of Pharmacology, Kasturba Medical College, Mangalore, Manipal University, Karnataka, India.

ABSTRACT

Costus speciosus is a medicinal herb with various proven pharmacological actions. Traditionally it has been reported to possess diuretic activities which have not yet been scientifically explored. This study attempts to explore the diuretic activity of aqueous-ethanolic extract of the leaves of Costus speciosus in healthy Wistar albino rats. The study was conducted in saline primed Wistar albino rats (n=6) using frusemide (20 mg/kg per oral) as the reference diuretic drug with two oral doses of aqueous-ethanolic extract of Costus speciosus 200mg/kg and 400mg/kg respectively. Urine volume and electrolytes (Sodium, Potassium and Chloride) excretion was estimated at the end of 24 hours. Data was analyzed by one-way ANOVA followed by Tukey’s test. P < 0.05 was considered as statistically significant. Costus speciosus extract significantly increased the volume of urine (10 ± 3ml/100g/24hr and 14 ±2.5ml/100gm/24hr), increasing the diuretic index to 1.25 and 1.75 for 200mg/kg and 400mg/kg dose ranges respectively (P< 0.05). The test drug, when compared to the control group, showed a significant increase in the excretion of sodium, potassium and chloride excretion. However, the electrolyte secretions were less when compared to frusemide indicating a weaker saluretic action. These findings support the use of Costus speciosus as a diuretic agent with an action similar to that of the loop diuretic, frusemide. Further studies with larger doses and longer duration exploring the exact mechanism of action are warranted.

Keywords: Diuretic, Costus speciosus, saluretic, frusemide, Lipschitz method

*Corresponding author
INTRODUCTION

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[1]. The traditional herbal systems have a rich heritage of use of herbs for their myriad of medicinal properties. However, such claims have to be scientifically validated to prove their clinical efficacy which forms the basis of development of new drugs from plant sources.

Diuretics, either alone or in combination with other drugs, are valuable in the treatment of hypertension, congestive heart failure, ascites & pulmonary edema [2, 3,4, 5,6]. Two widely used diuretics, thiazides and the high ceiling loop diuretic, furosemide, have been associated with a number of adverse effects, such as, electrolyte imbalance, metabolic alterations, development of new-onset diabetes, activation of the renin-angiotensin-neuroendocrine systems and impairment of sexual function [2,5]. Hence the search for newer drugs with lesser adverse effects is ongoing. Herbal drugs with a safety profile proved offer an attractive alternative.

From the innumerable plants being researched since time immemorial, *Costus speciosus* is an important one. This plant of Costaceae (Zingiberaceae) family commonly known as variegated Crepe Ginger is an erect, succulent, perennial herb, up to 2.7 meters in height, arising from a horizontal rhizome, found in tropical region of India and also cultivated for ornamental purposes. Traditionally the rhizomes and roots have been ascribed aphrodisiac, purgative, anthelmintic, febrifuge, expectorant, and appetite stimulant properties. An alkaloid extracted from *Costus speciosus* rhizomes had papaverine-like smooth muscle relaxant and antispasmodic activities in laboratory animals. Rhizomes are given in pneumonia, rheumatism, dropsy and urinary diseases. Leaf infusion or decoction is utilized as a sudorific or in a bath for patients with high fever. Rhizomes exhibit cardiotonic, hydrochloretic, diuretic and CNS depressant activities [7].

Literature survey revealed that previous studies have reported the presence of various pharmacological activities of *Costus speciosus* like antidiabetic, hypolipidemic, anticholinesterase, hepatoprotective, antioxidant, adaptogenic, antibacterial, antifungal, antifertility, estrogenic, antacarolytic, antispasmodic, anti-inflammatory and antipyretic studies [7]. However, no studies have been conducted on the leaf extract of *Costus speciosus* to evaluate its diuretic activity. Hence the present study was conducted to shed more light on the diuretic potential of this indigenous herb.

MATERIALS AND METHODS

Experimental animal:

Healthy adult Wistar albino rats of either sex, weighing 150-200 g obtained from our institutional animal house were used for the study. Rodents were housed in clean polypropylene cages, with dust free rice husk as a bedding material; three rats per cage; under
controlled laboratory conditions. (Temperature: 25° ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle as per CPCSEA guidelines). The experimental animals were fed with standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth laboratory animal feed manufactured by Pranav Agro industries Ltd., Sangli) and water ad libitum. The rodents were allowed to acclimatize to these conditions for one week prior to the commencement of the study. The experimental work was approved by the Institutional Animal Ethics committee.

**Drugs:**

Frusemide (Sanofi Aventis Co.) was used as a reference standard diuretic drug.

**Plant extract:**

The leaves of *Costus speciosus* were obtained from in and around the outskirts of Mangalore. The plant materials were dried under shade and reduced to moderately coarse powder and was extracted successively with 50% ethanol and 50% distilled water using soxhlet apparatus. The aqueous-ethanolic extracts were dried in hot air oven (yield 17.04%) and then dissolved in distilled water.

**Evaluation of diuretic activity:**

Each animal was placed in an individual metabolic cage 24h prior to commencement of the study for adaptation. The method of Lipschitz et al.[8,9] was employed for the assessment of diuretic activity. According to this method, the animals deprived of food and water for 18 hours prior to the experiment, were divided into 4 groups (n=6). Group I animals received normal saline (25 ml/kg, p.o.); Group II received the standard diuretic (10 mg/kg, p.o) and Groups III and IV received the test compound, leaf extract of *C. speciosus*,(200mg/kg and 400mg/kg) respectively. The study drug dosages were selected on the basis of other studies conducted in our laboratory previously. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 2.5ml/100g body weight to impose a uniform water and salt load [10]. All the drugs were freshly prepared prior to administration.

Immediately after administration, the animals were placed in metabolic cages (each animal per cage), specially designed to separate urine and faeces, kept at 20°C±0.5°C. The volume of urine collected was measured at the end of 5hrs and 24hrs. During this period, no food and water was made available to the animals. The following parameters were duly noted: body weight before and after test period, total urine volume, and concentration of Na⁺, K⁺ and Cl⁻ in the urine. Na⁺, K⁺, Cl⁻ concentrations were determined by Ion Sensitive Electrode; Roche Hitachi 917automatic analyzer and bicarbonate ion was estimated withBlood gas analyzer: AVLcompact-3.

The diuretic action of test drug was calculated by using the following formula:
Diuretic action = Urinary excretion of test drug group
Urinary excretion in control group

Statistical Analysis:

The results were expressed as mean ± SD. The data was analyzed by one way ANOVA followed by Tukey’s test. A value of \( P < 0.05 \) was considered as statistically significant. Statistical analyses were carried out using the software package SPSS (Version 16.0).

RESULTS

Effect on urine volume:

There was no evidence of dehydration and the animals were found normal at the observed 5hr and 24hr intervals. The reference diuretic frusemide, significantly increased the urine output when compared to control (\( P < 0.01 \)), the diuretic index being 2.25. The test drug at 400 mg/kg dose, showed a statistically significant increase in the urine volume when compared to the control (\( P < 0.05 \)), the diuretic index being 1.75 (Table no.1). However, the increase in urine volume with the test drug was not significant when compared to frusemide.

Effect on urinary electrolyte excretion:

As indicated in table no 2, frusemide significantly increased the electrolyte concentrations with a Na/K ratio of 1.53 when compared to control (saluretic index: 1.31). The test drug, when compared to the control group, showed a significant increase in the excretion of sodium, potassium and chloride at the higher dose used i.e. 400mg/kg (\( P<0.05 \)). Though there was an increase in the electrolytes excretion with the smaller dose (200mg/kg) of test drug, it was statistically insignificant when compared to the control. However, when compared to frusemide, the urinary electrolyte excretion was less than that of frusemide.

Table 1: Effect of oral administration of *C. speciosus* on urinary volume excretion

<table>
<thead>
<tr>
<th>Group</th>
<th>Urine volume (ml/100g/24hr)</th>
<th>Diuretic index (24 hr interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8 ±4.5</td>
<td>-</td>
</tr>
<tr>
<td>Frusemide</td>
<td>18± 3.5*</td>
<td>2.25</td>
</tr>
<tr>
<td><em>C. Speciosus</em> (200mg/kg)</td>
<td>10 ± 3</td>
<td>1.25</td>
</tr>
<tr>
<td><em>C. Speciosus</em> (400mg/kg)</td>
<td>14 ±2.5*</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SD; \* \( P <0.05 \) compared with control group (One way ANOVA followed by tukey’s test) \( ^{\dagger} \) Diuretic index = volume of test group/volume of control group
Table 2: Effect of oral administration of *C. speciosus* on urinary electrolyte excretion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na⁺ mmol/L</th>
<th>K⁺ mmol/L</th>
<th>Cl⁻ mmol/L</th>
<th>Saluretic index</th>
<th>Na/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21±5</td>
<td>16±1.3</td>
<td>23±4</td>
<td></td>
<td>1.31</td>
</tr>
<tr>
<td>Frusemide</td>
<td>60±9*</td>
<td>39.1±2.4*</td>
<td>52±8*</td>
<td>2.85</td>
<td>2.44</td>
</tr>
<tr>
<td><em>C. speciosus</em> (200mg/kg)</td>
<td>26±4</td>
<td>20.4±2.3</td>
<td>29±5*</td>
<td>1.23</td>
<td>1.28</td>
</tr>
<tr>
<td><em>C. speciosus</em> (400mg/kg)</td>
<td>34±5*</td>
<td>29.3±2.1*</td>
<td>39±6*</td>
<td>1.61</td>
<td>1.63</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SD; *P <0.05 compared with control group (One way ANOVA followed by Tukey test) †Saluretic index = electrolyte concentration of test group/electrolyte concentration of control group

**DISCUSSION**

The present study revealed that the aqueous-ethanolic extract of the leaves of *C. speciosus* showed significant increase in urinary output and urinary electrolyte concentration when compared to the normal control. When compared to the reference diuretic, frusemide, the saluretic index was lesser in the test groups which could be attributed to a potassium losing effect of the test extract. It also indicates a weaker diuretic effect than that of frusemide. Diuresis has two components: increase in urine (water secretion) and a net loss of solutes (i.e. electrolytes) in the urine [4]. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. The reference drug frusemide, increases urine output and urinary excretion of sodium by inhibiting Na⁺ K⁺2Cl⁻symporter (co-transport system) in the thick ascending loop of Henle [3].

The regulation of sodium, potassium balance is also intimately related to renal control of acid-base balance. The potassium loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended[11].

In the present study, frusemide showed strong diuresis accompanied with high natriuresis, chloruresis, and kaliuresis (P<0.5). Further there was low Na⁺/ K⁺ ratio, as it inhibits Na⁺ K⁺ and Cl⁻ co-transport at the thick ascending loop of Henle. K⁺ excretion was increased perhaps due to high Na⁺ load reaching the distal tube. However, *C. speciosus* extract induced a weaker natriuretic and kaliuretic effect when compared to frusemide; also Na⁺/ K⁺ ratio was less than that of frusemide, indicating the strong kaliuresis or K⁺ losing property of the extract. Treatment with the test extract resulted in elevated levels of potassium in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. The weaker diuretic action could also be due to a smaller dose and hence further studies with higher doses might be required.

The above results raise the possibility of existence of diuretic activity by inhibiting tubular reabsorption of water and sodium ion. It is a good indicator for the efficacy of *C. speciosus* extract as a diuretic, however it might share the hypokalemic effect of frusemide which could be a significant disadvantage.
Phytochemical investigations of *C. speciosus* have revealed the presence of saponins like Diosgenin as the major constituent along with Tigogenin, dioscin and gracillin [7]. High concentrations of phenolic compounds like ferulic acid has been isolated from the leaves of *C. speciosus* [12]. Two active sesquiterpenoids, costunolide and eremanthin, have been isolated from hexane extract of *C. speciosus*. [13] However, the phytoconstituents(s) responsible for its diuretic activity and the role of saponins and polyphenolic compounds in its pharmacological activities need to be investigated to confirm the exact mechanism of action.

**CONCLUSION**

In conclusion, *C. speciosus* leaf extract showed promising diuretic activity in the experimental model studied. Further studies with higher doses and chronic administration to evaluate the mechanism of action and identification of the active ingredients could pave the way for therapeutic use of this ingenious herbal drug.

**REFERENCES**