

Diuretic activity of ethanolic extract of whole plant of *Sphaeranthus indicus* linn in albino rats

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ABSTRACT

Background: *Sphaeranthus indicus* Linn (*S. indicus* Linn) is a medicinal plant widely used in Indian traditional system of medicine for treating various ailments. The aim of the study was to evaluate diuretic activity by Lipschitz method in albino rats.

Methods: Thirty albino rats were grouped into five groups and each contained six rats. Group I (control group) received 25ml/kg each of 0.9% sodium chloride orally. Group II (standard) received Hydrochlorothiazide 25 mg/kg orally dissolved in isotonic normal saline. Groups III, IV and V received ethanolic extracts of *S. indicus* Linn in doses 100mg/kg, 200mg/kg and 300mg/kg respectively dissolved in isotonic saline orally. The mice were put in metabolic cages and urine samples were collected for all the groups up to 24 hours after dosing. Urine was analysed for volume, urinary excretion ratio, diuretic activity, sodium and potassium composition electrolytes. Data was analysed using SPSS version 16 for windows.

Results: Single dose administration of *S. indicus* in doses of 100, 200 and 300 mg/Kg as compared to Hydrochlorothiazide (25 mg/kg) have significantly ($p < 0.001$, ANOVA) increased total urine output along with an increase in concentration of sodium and potassium. *S. indicus* Linn 300 mg/Kg produced greater diuretic activity, which is comparable to the effect of standard.

Conclusions: The extract treated rats showed a dose dependent increase in urine volume. Maximum diuretic and kaliuretic activity were observed at the dose of 300 mg/kg of the extract. The present study supports and confirms the basis for folklore use of *S. indicus* Linn as a diuretic agent.

Keywords: Diuretic activity, Ethanolic extract, *Sphaeranthus indicus* Linn, Whole plant

INTRODUCTION

Diuretics are agents that increase the rate of urine flow and adjust the volume and composition of body fluids in several clinical situations such as acute and chronic renal failure, cirrhosis of liver and hypercalciuria. Drug-induced diuresis is useful in many life-threatening disease conditions such as congestive heart failure (CHF),

hypertension, nephritis and pregnancy toxemia.¹ Most diuretic drugs have been associated with numerous adverse effects such as electrolyte imbalance, development of new-onset diabetes, metabolic alterations, activation of the renin-angiotensin and neuroendocrine systems and impairment of sexual function.² In this scenario, the need for novel diuretics such as plant-based substances, which are considered to

be relatively safe and possessing lower potential for adverse effects, is advocated.

India has been endowed with a very rich flora owing to the extreme variations in climate and geographical conditions prevalent in the country. Loss of traditional knowledge is recognized as of great global concern in the present century.³ The need for documentation of traditional knowledge on medicinal plants with scientific studies are necessary to develop more trust and faith towards this potential knowledge. A mechanism with high potential for protection of traditional knowledge is its documentation in databases and registers.⁴ The present study is significant in this direction to capture evidence on diuretic activity of *Sphaeranthus indicus* Linn.

Plant description

Sphaeranthus indicus Linn is a multi-branched aromatic herb 1-2 feet in height, distributed commonly in plains all over India and up to an altitude of 50 feet in hills.⁵ *S. indicus* Linn is known in different names in different Indian languages as Kamadurus, Mudmudiya, Gorakhmundi, Atookamani, Mundi, Buikadam, Gorakhmundi, Adakkamaniyan, Kottakarhai, Bodasaramu.⁶ In Ayurvedic system of medicine, the whole herb is used in insanity, anaemia, piles, asthma, leukoderma, indigestion, bronchitis, spleen diseases, elephantiasis dysentery, vomiting and urinary discharges.⁷ The whole herb is used in Ayurvedic preparations to treat mental disorders and epilepsy.⁸⁻¹⁰ Hot water extract of the herb is used as an anthelmintic, fish poison, diuretic, and as an aphrodisiac.¹¹⁻¹³ Flowers are used in conjunctivitis.¹² The external application of a paste of this herb is useful in treating pruritus and edema, gout, arthritis, filariasis and cervical adenopathy.¹⁴ The plant is traditionally used for diarrhea.¹⁵

Scientific studies had revealed its anxiolytic activity, neuroleptic activity, sedative effect, immunomodulatory activity, antioxidant activity, anti-inflammatory activity, analgesic activity and antipyretic activity, mast cell stabilizing action, hepatoprotective activity, antibacterial, antifungal activity, antiviral activity and anti-amoebic activity, bronchodilatory effect, antihyperlipidemic effect, and anticancer activity.¹⁶⁻²⁸ The major constituents of *S. indicus* Linn include methyl chavicol, δ -cadinene, α -ionone, para-methoxycinnamaldehyde, α -terpinene, citral, geraniol, geranyl acetate, β -ionone, oscimene, eugenol, sphaeranthene, sphaeranthol, estragole, and indicusene.^{29,30}

S. indicus Linn is used traditionally used as a diuretic but scientifically was not evaluated as a diuretic agent. The main aim of the present study was to evaluate diuretic activity of whole plant ethanolic extract of *S. indicus* Linn by Modified Lipschitz Test in hydrated albino rats.

METHODS

Fresh whole plant of *S. indicus* Linn were procured from an organic farm and were authenticated by the Pharmacognosy unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram, South Kerala, India.

The whole plants were washed thoroughly with water, shade dried, and powdered coarsely. It was then repeatedly macerated with 95% ethanol in a percolator.³¹ The combined filtrate was subjected to evaporation in order to dry it under reduced pressure at 40-50°C. The resulting crude ethanol extract was stored at 10-15°C. Phytochemical evaluation for the extract was performed using standard procedures.³²

Wistar strain albino rats (100-200 grams) of male sex were used for diuretic study. The animals were housed under standard laboratory conditions in the animal house of Government Medical College, Thiruvananthapuram. The animals were fed standard pellet diet, maintained on a natural light and dark cycle and had free access to water and food. They were acclimatized to laboratory conditions before the study. The experimental protocols were approved by the Institutional Animal Ethics Committee and ethical guidelines were followed throughout the study. All rats were housed in metallic cages 6 in each and temperature maintained at 22°C \pm 2°C. Hydride tablet (Hydrochlorothiazide- 25 mg) of Micro Labs was used as standard for studying diuretic activity.

The acute toxicity of plant *S. indicus* Linn was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 hrs prior to the experiment and the extract was administered as single dose and observed for the mortality up to 48 hours study period (short term toxicity). Based on the short term toxicity profile, the consecutive dose of the extract was determined as per Organisation for Economic Co-operation and Development guidelines No. 420.^{33,34} The maximum dose tested (2000 mg/kg) for LD50. From the LD50, doses like 1/15th, 1/10th and 1/5th were selected and considered as low, medium and high dose i.e. 100 mg/kg, 200 mg/kg, 300 mg/kg respectively to carry out this study.

Diuretic action of *S. indicus* was investigated in male albino rats employing the method of Lipschitz et al.^{35,36} Thirty albino rats were grouped into five groups each containing six. They were deprived of food and water 18 hours prior to the experiment. Control group received 25ml/kg each of 0.9% sodium chloride orally. Standard group was given Hydrochlorothiazide 25 mg/kg orally dissolved in isotonic normal saline. The rats in the other three groups were given the extract dissolved in isotonic saline orally in the doses 100 mg/kg, 200 mg/kg and 300mg/kg each. Metabolic cages made of stainless steel were used to collect the urine. Amount of urine collected at the end of 24 hours was noted. Urinary excretion and diuretic activity were calculated for all groups and

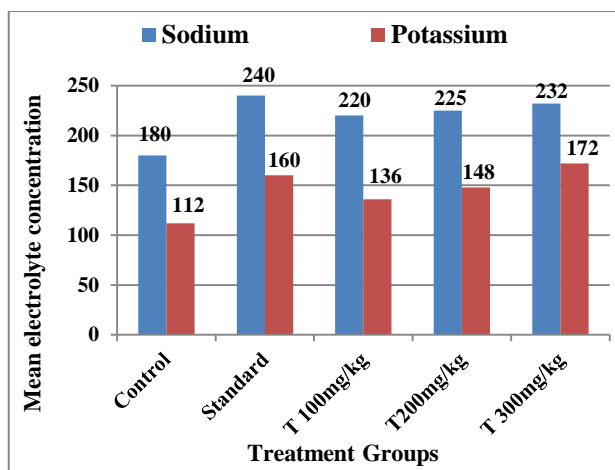
compared. The electrolyte composition (sodium and potassium) of collected urine for different groups were analyzed by Ion Selective Electrode method as described by the user instruction manual of the biochemical kits (Roche, Roche Diagnostics Pvt. Ltd., Gurgaon, Haryana, India) and the results compared statistically.

Experimental results were expressed as mean±standard error of mean. Statistical analysis was performed with one-way ANOVA using SPSS for Windows, Version16.0 (SPSS Inc., Chicago, USA).

RESULTS

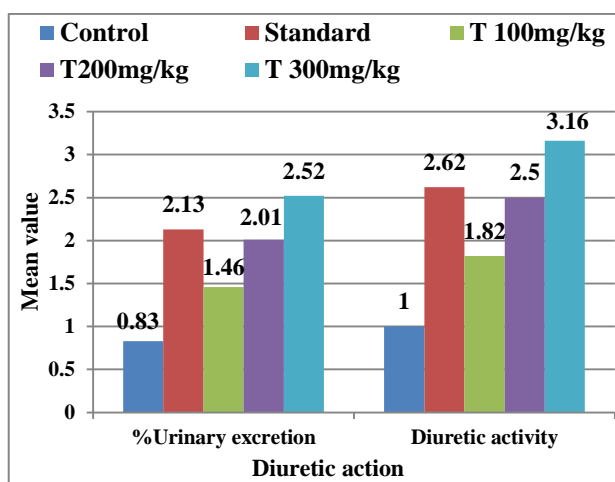
Sphaeranthus indicus Linn was subjected to qualitative phytochemical tests to identify the phytoconstituents and it revealed the presence of carbohydrates, alkaloids, sterols, phenolic compounds, tannins, flavonoids and resins. In acute toxicity study, all the animals survived even after 14 days. This shows that the extract was found to be safe up to the maximum dose level tested (2000 mg/kg). No major behavioural changes were observed during this period of study.

As shown in Table 1, *S. indicus* Linn has showed significant diuretic activity by increasing urinary output. As shown in Table 2 there was increased excretion of sodium, potassium levels when compared with control. The effect of *S. indicus* Linn was found to be dose dependent, i.e., among the three doses studied, higher doses produced more effect as depicted in Figures 1 and 2. A comparison was made with the standard diuretic drug Hydrochlorothiazide. The diuretic effect observed after treatment with *S. indicus* Linn was found to be significant in terms of urinary output, sodium and potassium concentrations. It was found that the extract possessed good kaliuretic action. Sodium excretion was also increased with increasing doses of the extract. Maximum diuretic and kaliuretic activity were observed at the dose of 300 mg/kg of the extract as shown in Figure 1.



Control-Normal Saline, Standard-Hydrochlorothiazide

Figure 1: Diuretic activity (electrolyte concentration) of Ethanolic extracts of *Sphaeranthus indicus*.



Control-Normal Saline, Standard-Hydrochlorothiazide

Figure 2: Effect of ethanolic extract of *Sphaeranthus indicus* Linn on urinary excretion and diuretic activity.

Table 1: Effects of oral Ethanolic extract of *Sphaeranthus indicus* Linn on urine output.

Treatment group	Dose (oral)	Urine output (ml)	%Urine excretion (ml)	Diuretic activity
Control	25ml/kg	12.00±1.51	00.83±0.26	1.00±0.23
Standard	25mg/Kg	32.00±3.09	2.13±0.58	2.62±0.37
Test 1	100mg/kg	22.10±1.83*	1.46±0.51*	1.82±0.52*
Test 2	200mg/kg	30.00±1.81*	2.01±0.33*	2.50±0.31*
Test 3	300mg/kg	38.00±1.70*	2.52±0.39*	3.16±0.12*

Values are Mean±Standard Deviation. The data were analysed by one-way ANOVA.

*p value <0.001, when compared with control and treated groups.

Control-Normal Saline, Standard-Hydrochlorothiazide

DISCUSSION

Modern medicine is making rapid strides day by day. Still it has not been completely successful in the prevention and cure of all human ailments. Many valuable drugs of

today came into the vogue through the study of indigenous remedies. Useful compounds such as digitonins, papain, morphine, codeine exert pharmacological activity. Phytopharmaceuticals are gaining more importance and will open up new vistas in drug development.

Table 2: Effects of oral Ethanolic extract of *Sphaeranthus indicus* on electrolyte excretion.

Treatment Group	Dose(Oral)	Na+ (mEq/l)	K+ (mEq/L)
Control	25ml/kg	180±7.50	112±3.41
Standard	25mg/Kg	240±5.47	160±3.46
Test 1	100mg/kg	220±3.46*	136±2.89*
Test 2	200mg/kg	225±3.74*	148±2.89*
Test 3	300mg/kg	232±6.90*	172±2.36*

Values are Mean±Standard Deviation. The data were analysed by one-way ANOVA.

*p-value <0.001, when compared with control and treated groups.

Control-Normal Saline, Standard-Hydrochlorothiazide

Herbal drugs should be exploited to their zenith so that they are cost effective alternatives or they can provide crude drugs for the final synthesis of active compounds. It is therefore necessary to acquire and preserve the traditional system of medicine by proper documentation and identification of herbal remedies. Though there is a general belief that herbal remedies are totally harmless, safety of herbal drugs should be proven beyond any doubt for human use. There is a revival of interest in herbal medicines because basic molecular and active structures for synthetic fields are provided by rich natural sources. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals coupled with their reduced cost is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs.

S. indicus can be used for the treatment of various disease conditions. Studies done on *S. indicus* justify the therapeutic application of this plant in indigenous system of medicine augmenting its therapeutic value. The diuretic property of ethanolic extract of *S. indicus* was evaluated in the present study. It was proved to be a very safe drug since it did not produce any mortality in animal study when doses up to 2000 mg/kg were given orally.³⁷ Diuretics are drugs which can increase urine output and bring about changes in electrolyte composition of the body. They have their benefit in a good number of clinical conditions. Diuretics relieve pulmonary congestion and peripheral edema, so these agents are useful by reducing the volume overload and offer symptomatic relief of orthopnoea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart. This decreases cardiac work, oxygen demand and plasma volume thus decreasing blood pressure.

The control of plasma sodium is important in the regulation of blood volume and pressure. The control of plasma potassium is required to maintain proper function of cardiac and skeletal muscle.

The present study shows that the ethanolic extract of *S. indicus* has maximum diuresis at 300 mg/kg. The diuretic activity of *S. indicus* in three doses were

considered to be 'good' by Gujral et al.³⁸ Diuretic activity is considered to be good if >1.5, moderate if 1-1.5, little if 0.72-1 and nil if <0.72. On analysing the electrolyte composition of urine, it was seen that it also possess significant kaliuretic property. Both these properties can be utilized in a variety of disease conditions. Diuretics can be used along with massive blood transfusions to prevent volume overload. In this situation both diuretic and kaliuretic properties of *S. indicus* can be utilized since massive blood transfusion can lead to hyperkalemia due to release of potassium from stored red blood cells. It is a promising adjuvant drug along with antihypertensive which increase potassium levels like Angiotensin converting enzyme inhibitors and Angiotensin receptor antagonists in patients not responding when used alone.

In the present study whole plant ethanolic extract showed elevated levels of potassium in urine which may increase the risk of hypokalemia. However, it should be used with caution when other diuretics are also given concurrently since additive hypokalemia can occur. Active phytoprinciples such as flavonoids, saponins and terpenoids are known to be responsible for diuretic activity.^{39,40} The presence of these active principles in *S. indicus* may be responsible for its diuretic activity.

CONCLUSION

The results showed that single dose administration of whole plant ethanolic extract of *S. indicus* Linn in doses of 100,200 and 300 mg/Kg have increased the urinary output along with an increase in concentration of sodium and potassium ions in urine. *S. indicus* Linn 300 mg/Kg produced a greater diuretic activity, which is comparable to that of Hydrochlorothiazide 25 mg/kg. The present study supports and justifies the rationale behind the folklore use of whole plant of *Sphaeranthus indicus* Linn for diuretic activity. However, further systematic photochemical studies are necessary to elucidate the probable structure activity relationship of biomolecules.

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Conflict of interest: None declared

Ethical approval: The experiment complied with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethics Committee (IAEC)

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