



EVALUATION OF DIURETIC ACTIVITY OF AQUEOUS AND METHANOL EXTRACTS OF *TINOSPORA SINENSIS* IN RATS

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ABSTRACT

In this present study, the diuretic activity was screened for methanol and aqueous extracts of *Tinospora sinensis* flowers. The animals were grouped into six of six groups. Test extracts were administered to experimental rats orally at doses of 150 and 300 mg/kg p.o suspended in carboxy methyl cellulose. The diuretic effect of the extracts was evaluated by measuring urine volume, sodium and potassium content, conductivity and pH. Urine volume was significantly increased by the two doses of aqueous and methanol extracts in comparison to control group. While the excretion of sodium was also increased by both extracts, potassium excretion was only increased by the aqueous extract at a dose of 300 mg/kg. There was no significant change in the conductivity and pH of urine after administration of the *Tinospora sinensis* extracts. The diuretic effect of the extracts was comparable to that of the reference standard Furosemide (20mg/kg, p.o). The present study provides a quantitative basis for explaining the folkloric use of *Tinospora sinensis* as a diuretic agent.

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]. The study of plant species with diuretic effects is still a fruitful research in search of new diuretics. Diuretics are the drugs that increase the rate of urine flow; clinically useful diuretics also increase the rate of excretion of Na (natriuresis) and an accompanying anion, usually Cl⁻. Most clinical applications of diuretics aim to reduce extracellular fluid volume (edema) by decreasing total body NaCl

content. Although continued administration of diuretic causes a sustained net deficit in total Na⁺, the time course of natriuresis is finite because renal compensatory mechanisms brings Na excretion in line with the Na⁺ intake, a phenomenon known as diuretic braking. Diuretics alter the excretion of other cat ions (e.g. K⁺, H⁺⁺), anions (e.g. Cl⁻, HCO₃⁻ and H₂PO₄) and uric acid. In addition diuretics may alter renal hemodynamic indirectly mediated by local prostaglandins synthesis [2].

Tinospora sinensis (syn: *Tinospora malabarica*) is a plant that grows almost throughout India and other South East Asian countries and belongs to the family Menispermaceae. The stem of this plant has great therapeutic value traditionally in treating debility, dyspepsia, fever, inflammation, syphilis, ulcer, bronchitis, jaundice, urinary disease, skin disease and liver disease and known for its adaptogenic and immunomodulatory properties. The aqueous and alcoholic extracts of this

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species are reported to have many biological potential, such as anti-inflammatory, anti-diabetic, hepato protective, and immunomodulatory, and adaptogenic. Previous phytochemical investigations have discovered that this species contains steroids, flavonoides and alkaloids. In India, cassava is used for the treatment of ringworm, tumor, conjunctivitis, sores and abscesses [3-5].

The literature survey revealed that no scientific study on diuretic activity of flower extract of this plant has been reported. Their fore objective of present study was to evaluate diuretic activity of *Tinospora sinensis* flower extracts.

MATERIAL AND METHODS

Plant material

The *Tinospora sinensis* flowers were collected from medicinal garden of Anurag Pharmacy College. The plant and plant material were identified and authenticated by Department of Pharmacognosy, Anurag Pharmacy College and Voucher herbarium specimens was deposited in the Department of Pharmacognosy of our College. The plant material was dried in sun shade, pulverized, passed through sieve no.40 and stored in air tight container and used for further extraction.

Preparation of extract

The powder was subjected to hot percolation method using Soxhlet apparatus with methanol and aqueous. The extracts was dried and weighed. (Yield 18.10, 20.12% w/w respectively).

Experimental animals

Healthy male albino rats weighing 180-200 g were used for the study. The animals were maintained in polypropylene cages of standard dimensions at a temperature of $37 \pm 1^\circ\text{C}$ and standard 12h : 12h day/night rhythm. The animals were fed with standard rodent pellet diet and water *ad libitum*. Prior to the experiment, the animals were acclimatized to the laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

RESULTS

Table 1. Diuretic activity of *Tinospora sinensis* (urine Volume) in 24 h

Group	Treatment	Urine volume
I	1%CMC	8.2±0.59
II	Furosemide 20mg/kg, p.o	14.6±0.61**
III	Methanolic extract 150 mg/kg, p.o	13.9±0.74**
IV	Methanolic extract 300 mg/kg, p.o	10.2±0.27
V	Aqueous extract 150 mg/kg, p.o	11.9±0.14
VI	Aqueous extract 300mg/kg, p.o	12.3±0.35

Values are Mean \pm SEM, n=6, *p<0.05, **p<0.01.

Drug Treatment

The extracts (suspended in 1% carboxy methyl cellulose) at the dose levels of 150, 300 mg/Kg body wt, p.o. was administered once daily for three consecutive days. Furosemide (10, 20 mg/Kg; p.o.) was used as standard for diuretic activity. Control group of animals (n=6) received suspension of 1% CMC in distilled water (10 ml/Kg)

Experimental design

The animals were divided into 6 groups of 6 rats each as follows; Group I: received only 1% CMC, Group II: received Furosemide 20 mg/kg, Group III: received pet.ether extract 150 mg/kg body weight p.o, Group IV: received pet.ether 300 mg/kg body weight p.o, Group V: received ethanol extract 150 mg/kg body weight p.o, Group VI: received ethanol extract 300 mg/kg body weight p.o,

Diuretic activity

Rats were fasted overnight and treated with vehicle, Furosemide and test extracts as stated above along with normal saline (50 ml/kg). The rats were placed in metabolic cages and the urine samples were collected for 24h, measured using a standard measuring cylinder. The amount of urine (in ml) collected for 24 h was compared (tabulated in table 2) [6-8].

Natriuretic activity

Estimation of Sodium and Potassium content of the urine samples of all groups of animals were done by using a laboratory model flame photometer. The ratio of Na+/K+ is calculated for Natriuretic activity. A value greater than 2.0 indicates a favourable Natriuretic effect. Ratio greater than 10.0 indicates a potassium sparing effect.

Statistical analysis

The result s were expressed as mean \pm S.E.M. Statistical comparisons were made by means of Dunnet's 't' test and p values smaller than 0.05 was considered as significant.



Table 2. Natriuretic activity of *Tinospora sinensis*

Group	Treatment	Na+	K+	Cl-	(Na+/K+)
I	1% CMC	79.32 ± 1.324	52.93 ± 2.132	94.34 ± 2.371	2.4985
II	Furosemide 20mg/kg, p.o	139.2 ± 2.342	88.37 ± 1.543	172.2 ± 2.521**	1.5751
III	Methanolic extract 150 mg/kg, p.o	132.4 ± 1.322**	89.13 ± 0.2821**	171.5 ± 1.947**	1.4854
IV	Methanolic extract 300 mg/kg, p.o	125.2 ± 0.5126**	71.20 ± 0.5133**	146.2 ± 1.637**	3.7584
V	Aqueous extract 150 mg/kg, p.o	117.1 ± 2.042**	66.60 ± 0.6141*	128.3 ± 1.868**	2.7542
	Aqueous extract 300 mg/kg, p.o	125.7 ± 0.923**	73.60 ± 0.5213**	152.6 ± 2.218**	4.7072

Values are Mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001, NS -not significant

Table -1 show the urine volume collected in 24 hr for all the groups. It is evident that test extract treated groups excreted more urine than the control groups. The extract at 300 mg/kg exhibited comparable effect with reference drug furosemide 20 mg/kg and the results was statistically significant. Table-2 shows the sodium and potassium content of the urine for all groups. The amount of Sodium excreted was increased for Furosemide treated group; statistically significant rise in Na⁺ excretion was also noticed for ethanol extract treated groups. The potassium content excreted in the urine was statistically insignificant for all the groups. The Natriuretic effect was calculated by employing the formula Na⁺ / K⁺. It was found that the extract treated groups possess favourable Natriuretic effect. The present study showed that the ethanol extract of *Tinospora sinensis* significantly increases the urine output and excretion of urinary sodium and had no effect on the urinary potassium excretion.

DISCUSSION

Diuretics have two separate connotations; increase urinary phrase and net loss of solute (i.e. electrolyte) and water (i.e. saluretic). These two processes are involved in the suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream and a consequence;

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promote the formation of urine. An attempt to extrapolate the diuretic action of plant extract from rats to man using the activity of furosemide in an organism as a guideline has been reported [9-11].

The results clearly shows that the ethanol extracts at doses of 150 and 300 mg/ kg produced significant dose dependent increase in urinary excretion and urinary sodium loss but no effect on urinary potassium loss with respect to control and standard drug treated groups. The data demonstrates that the extract has diuretic effect and natriuretic effect but no potassium sparing effect and is as potent as furosemide. This indicates the use of ethanolic extract as a diuretic agent based on a sound mechanistic background. Also the excretion of potassium ions was similar to the untreated group, which rule out the possibility of hypokalemia and associated ototoxicity.

CONCLUSION

From the above result s, it is concluded that *Tinospora sinensis* used by tribal's traditionally showed significant diuretic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as diuretic.

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