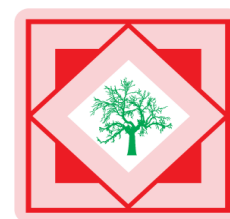




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Studies on diuretic activity of three plants from Menispermaceae family

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ABSTRACT

This study evaluated the comparative diuretic potential of methanolic root extracts of *Cissampelos pareira*, *Cyclea peltata* and *Stephania japonica* (Fam: Menispermaceae) in saline primed normal rats after oral administration. Methanolic root extracts of *Cissampelos pareira*, *Cyclea peltata* and *Stephania japonica* (100 and 200 mg/kg P.O) or the reference drug, furosemide (20 mg/kg P.O) were administered to male Wistar rats (Modified Lipschitz method) and their urine output and electrolyte changes were quantitated at several intervals of time after the dose for 5 h. All the extracts displayed dose-dependent diuretic effects. At 100mg/kg both *Cyclea peltata* and *Stephania japonica* root extracts shown significant increase in urinary out put ($p < 0.001$). However At 200 mg/kg dose all the three extracts significantly increased ($p < 0.001$) urine volume and urinary electrolytes, qualitatively similar to that of furosemide. The methanolic root extract of *Cyclea peltata* has shown highest diuretic activity (Diuretic action – 3.5). These findings collectively indicate that the extracts exhibited significant diuretic activity, providing evidence, at least in part, for their Aurvedic use. Especially root of *Cyclea peltata* has got highest diuretic action among three tested extracts. These results support the use of *C. peltata* roots as "Patha" in Ayurvedic system of medicine for the treatment of urinary related disorders.

Key words: *Cissampelos pareira*, *Cyclea peltata*, Modified Lipschitz method, Patha, *Stephania japonica*.

INTRODUCTION

Diuretics are the drugs used in the treatment of hypertension, congestive cardiac failure, ascites and pulmonary diseases. The presently available diuretics such as thiazides and furosemide exhibit various adverse effects such as electrolyte imbalance, metabolic alterations etc. [1]. Some of oral diuretics are derived from medicinal plants. The Indian Ayurvedic system of medicine is rich in treating renal problems. Hence a vast number of medicinal plants mentioned in Ayurvedic system of medicine are known to possess diuretic properties such as *Abelmoschus esculentus*

(Bhindaka), *Bacopa monnieri* (Brahmi), *Barbarea vulgaris* (Cress), *Boerhavia diffusa* (Punarnava), *Embllica officinalis* (Amla) etc. [2].

The roots of *Cissampelos pareira* L. Var. *hirsuta* (DC.), *Cyclea peltata* (Lam.) J. Hooker and Thoms., and *Stephania japonica* (Thunb.) of Menispermaceae family are known as *Patha*, in Ayurveda, which is used in the treatment of various diseases like stomach pain, fever, skin conditions, cardiac pains etc. [3]. The root extract of *Cissampelos pareira* has been tested for diuretic activity and its moderate diuretic activity has been reported [4]. The roots of *Cyclea peltata* have been tested for their stone formation inhibition activity on ethylene glycol induced nephrolithiasis in rats [5]. The roots of *Stephania japonica* are regarded as substitute for *Cissampelos pareira* and used in the treatment of fever, diarrhoea and urinary diseases [6]. No scientific data is available related to diuretic activity or any other urinary related disorders *Stephania japonica*.

The various literatures suggests that the Ayurvedic drug *Patha* is used as diuretic, and the roots of above said three plants have been used as source of this drug, hence it was decided to evaluate comparative diuretic activity for these three plant materials.

MATERIALS AND METHODS

Plant material

The roots of *Cissampelos pareira* Var. *hirsuta* were collected from dry deciduous forests of Chamundi hills, Mysore. *Cyclea peltata* and *Stephania japonica* roots were collected from evergreen forests of Madikere. The taxonomical identification of the plants were done by Dr. G. Shivamurthy, Department of studies in Botany, University of Mysore, Mysore, and the voucher specimens were deposited at the herbarium (specimen No. KKH-001/2006, KKH-002/2006 and KKH-003/2006).

Preparation of extracts

The roots were washed with water and shade dried. The dried materials were powdered and passed through No. 10 sieve. The powdered plant materials were extracted with methanol as solvent in a soxhlet apparatus. The extracts were concentrated using a rotary flash evaporator (Superfit, Ambala, India) and semisolid mass was obtained (11.6%, 15.8% and 9.1% w/w with respect to the powdered material). The extracts were stored in refrigerator until use and the required quantity was suspended in 1% (w/v) Tween 80 for pharmacological studies.

Experimental animals

The experiment was initiated after approval of Institutional Animal Ethical Committee [(IAEC) (NCP/IAEC/2/06-07)]. Adult male Wistar rats of either sex weighing 250-300 g procured from the animal house of National College of Pharmacy, Shimoga, India and were used for the activity. The animals were maintained under standard conditions as per OECD guidelines (Temperature 25±2°C) with dark and light cycle (14/10 hrs) and fed with standard dry pellets and water *ad libitum* throughout the experiment.

Standard drug

Furosemide a high ceiling loop diuretic, in the dose of 20 mg/kg BW [7] was used as the reference drug (Positive control). It was dissolved in water prior to administration.

Biochemical methods

Sodium and potassium levels in the urine were quantitated by flame spectrophotometry (Systronics flame photometer-129) and chlorides by spectrophotometry (Autochem nexgen span).

Toxicity studies

Acute toxicity for the determination of LD₅₀ value was performed with different doses of the extract according to the up and down method using Swiss albino mice of either sex weighing 20 – 25 g. [8].

Diuretic activity

The animals were divided into eight groups of six animals each. Animals were fasted overnight with water *ad libitum* and subjected to pharmacological studies. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 25 ml/kg body weight (BW). The first group served as the control and the second group was treated with an oral dose of 20 mg/kg BW of furosemide. Third and fourth groups were treated with an oral dose of 100 mg/kg and 200 mg/kg BW of methanolic extract of *Cissampelos pareira* respectively. Similarly, fifth and sixth groups were treated with methanolic extract of *Cyclea peltata* while seventh and eighth groups with methanolic extract of *Stephania japonica*. All the animals were placed in individual metabolic cages. Urine was collected and measured at 1, 2, 3, 4 and 5 h after the dose. Sodium and potassium concentration in urine samples was determined at 5th h by standard biochemical methods. The ratio of urinary excretion in the test group to that in the control group was used as a measure of the diuretic action for the given dose of the drug. The diuretic activity was calculated by comparing diuretic action of extract to that of the standard drug [9].

Statistical analysis

Statistical analysis was analyzed using Student's t-test for unpaired data. *p*-Values less than 0.05 were considered statistically significant and less than 0.001 as highly significant. Results are expressed as mean value ± SEM.

RESULTS

Toxicity studies

All the three extracts did not show any kind of toxic effect when administered up to a dose of 2000mg/kg BW.

Diuretic activity

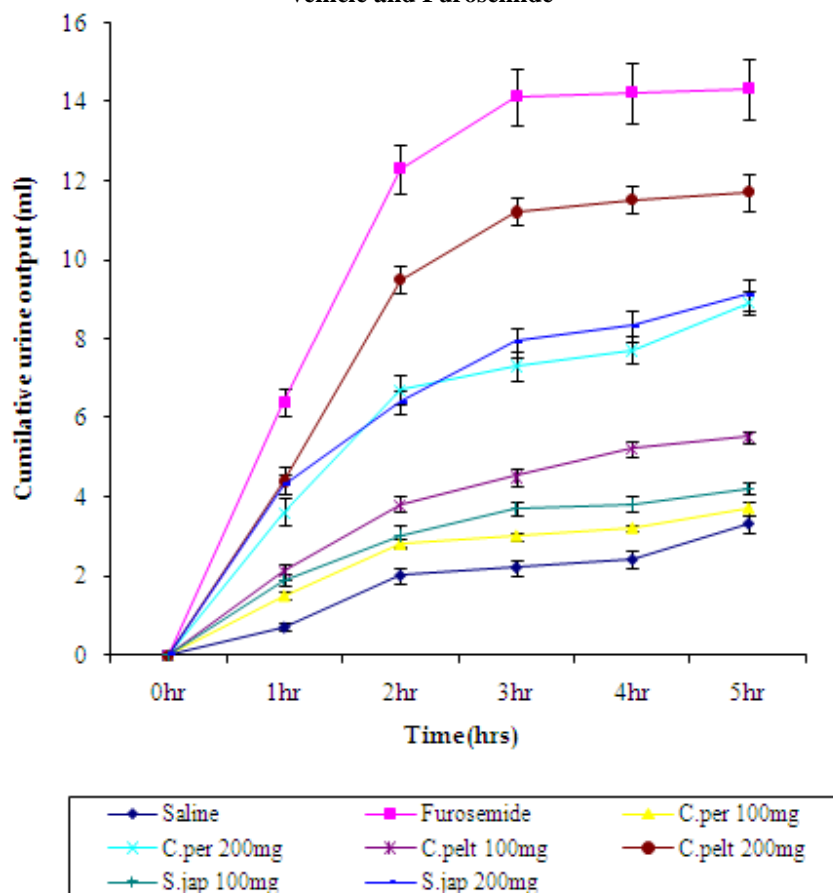
Effect on urine volume

Results are shown in fig. 1. The methanolic extracts of the roots of *Cissampelos pareira* at a dose of 100 mg/kg BW did not show marked diuresis during the 5 h of the test (*Cissampelos pareira* 3.65±0.19mL versus control 3.33±0.23 mL; *P* < 0.05).

Table 1 Effect of methanolic root extracts of *C. pareira*, *C. peltata* and *S. japonica* on urine excretion and ionic concentration in rats

Treatment	Dose	Cumulative Vol. of urine (ml/kg BW)	Diuretic action	Electrolyte concentration (mmol/l)			
				Na ⁺	K ⁺	Cl ⁻	Na ⁺ /K ⁺
Saline	25 ml/kg	3.33±0.23	1.0	85.45±1.13	55.23±0.83	111.50±1.64	1.55±0.04
Furosemide	20mg/kg	14.26±0.78**	4.3	132.68±0.40**	89.83±1.17**	134.50±1.64**	1.48±0.02
<i>C. pareira</i>	100mg/kg	3.65±0.19*	1.1	87.52±0.57	54.50±2.07	124.00±1.41**	1.61±0.06
	200mg/kg	8.93±0.29**	2.7	135.62±0.49**	84.50±1.87**	130.50±2.17**	1.60±0.04
<i>C. peltata</i>	100mg/kg	5.47±0.16**	1.6	86.07±0.38	52.50±1.87	134.67±2.66**	1.64±0.05
	200mg/kg	11.73±0.47**	3.5	138.15±0.41**	89.93±1.47**	139.17±1.20**	1.50±0.03
<i>S. japonica</i>	100mg/kg	4.17±0.15**	1.3	85.12±0.26	53.34±1.21	140.34±1.37**	1.59±0.04
	200mg/kg	9.13±0.40**	2.7	136.45±0.35**	89.50±1.87**	132.50±1.05**	1.50±0.04

Values are mean ± S.E.M., n = 6, * P < 0.05, ** P < 0.001. In parenthesis the cumulative urine volume compared with control

Fig.1. Time course of diuresis in rats with methanolic root extracts of *C. Pareira*, *C. peltata*, *S. japonica*, vehicle and Furosemide

The *Cyclea peltata* and *Stephania japonica* root extracts significantly increased urinary output to that of the control (*Cyclea peltata* 5.47±0.16 mL and *Stephania japonica* 4.17±0.15 mL versus control 3.33±0.23 mL; P < 0.001) but the effect was much less than that of furosemide

(14.26±0.78 mL versus control 3.33±0.23 mL). However, all the extracts of *Cissampelos pareira*, *Cyclea peltata* and *Stephania japonica* at the dose of 200 mg/kg BW significantly increased the urinary output. Urine output continued to be stimulated throughout the study period, such that the cumulative urinary excretion was significantly higher compared to the control (*Cissampelos pareira* - 5.47±0.29 mL, *Cyclea peltata*- 11.73±0.39 mL and *Stephania japonica*- 9.13±0.86 mL versus control 3.33±0.23 mL at 5 h after dose; $P < 0.01$) (Table 1.).

Effect on urinary electrolyte excretion

The effect of single doses of furosemide (20 mg/kg BW) and the methanolic root extracts of *Cissampelos pareira*, *Cyclea peltata* and *Stephania japonica* (100 and 200 mg/kg BW) on electrolyte (Na^+ and K^+) excretion in the 5 h urine is presented in Table 1. All the three plant root extracts enhanced the excretion of the electrolytes ($P < 0.01$) which was almost similar to that of the furosemide. The Na^+ / K^+ excretion ratio was almost stable (1.48 to 1.64) in all the groups.

DISCUSSION

The aim of this study was to investigate the comparative diuretic activity of methanolic root extracts of *Cissampelos pareira*, *Cyclea peltata* and *Stephania japonica*, As these three plants are used as sources of “*Patha*” an important Ayurvedic drug which is used in treatment of urinary related and heart related disorders [10].

Our current study examined the diuretic potential of *Cissampelos pareira*, *Cyclea peltata* and *Stephania japonica*, root extracts in rats. Oral route was chosen to meet the way used by people in traditional medicine. The diuretic action induced by the methanolic plant extracts was also investigated and compared with a standard reference drug, furosemide and control group (distilled water). The acute treatment of rats by the plant extracts showed a significant diuretic activity in a dose-dependent manner. The results showed that the highest dose of *Cyclea peltata* possesses a strong diuretic activity when given orally at single dose (Fig. 1). The extract of *Cyclea peltata* caused a significant increase in urine output from the first hour as observed with clinically used loop diuretic, while *Cissampelos pareira*, and *Stephania japonica*, extracts showed comparatively moderate diuretic activity. In comparison with these results, a single dose of furosemide induced a rapid and significant diuresis within 1 h of administration. The duration of action with furosemide is brief (less than 4 h when oral administration was performed and 3 h in case of intravenous administration) [11]. All the three plants demonstrated significant increase in urinary excretion of Na^+ and K^+ with the dose of 200mg /kg used in our experiments. These features suggest that the plant extract is acting in a similar way as furosemide, which increases urinary output and urinary excretion of sodium by inhibiting $\text{Na}^+/\text{K}^+/\text{Cl}^-$ transporter system in the thick ascending loop of Henley [12].

CONCLUSION

Out of three extracts, methanolic extract of *Cyclea peltata* at a dose of 200mg/kg BW has shown the highest diuretic activity. Since all the three plants are known to contain Bisbenzylisoquinoline type of alkaloids [13] the possible constituents responsible for the diuretic activity may be these alkaloids, but however the exact mechanism of action and active principle need to be investigated. This finding supports the Ayurvedic use of these plants as diuretic

specially roots of *Cyclea peltata*. Further studies are in progress to evaluate the diuretic activity of alkaloidal fractions of these plants.

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