

STUDY OF DIURETIC ACTIVITY OF ETHONOLIC EXTRACT OF LEAVES OF *CISSAMPELOS PAREIRA* IN RATS

SURESH BABU SAYANA¹, CHRISTINA², TAMBI MEDABALA³, PRAVEEN S PATIL⁴

¹Tutor, Department of Pharmacology, Raichur Institute of Medical Sciences, Raichur - 584102, Karnataka, India. ²Assistant Professor, Department of Physiology, Koppal Institute of Medical Sciences, Koppal, Karnataka, India. ³Junior Scientific Officer, Department of Physiology, Sports Authority of India, Netaji Subhash National Institute of Sports, Patiala, Punjab, India. ⁴Tutor, Department of Physiology, Raichur Institute of Medical Sciences, Raichur, Karnataka, India. Email: suresh.pharmacology@gmail.com

Received: 16 June 2014, Revised and Accepted: 31 July 2014

ABSTRACT

Objective: The objective was to study the diuretic activity of ethanolic extract of leaves of *Cissampelos pareira* (EELCP) by Lipschitz method in albino rats.

Materials and Methods: Five groups of albino rats were used to study the diuretic activity of EELCP using metabolic cages. Group I serves as normal control received vehicle (carboxymethyl cellulose 2% in normal saline), Group II received furosemide (10 mg/kg, p.o) in the vehicle; other Groups III, IV, and V were treated with low (100 mg/kg), medium (200 mg/kg), and high (400 mg/kg) doses of EELCP in vehicle. Immediately after the extract treatment, all the rats were hydrated with saline (15 ml/kg, p.o) and placed in the metabolic cages (3/cage), specially designed to separate urine and feces, kept at 21°C±0.5°C. A total volume of urine collected was measured at the end of 5th hr. During this period, no food and water were made available to animals. Various parameters like total urine volume and concentration of sodium, potassium, chloride ions in the urine were measured and estimated, respectively.

Results: When compared to vehicle-treated control group, the EELCP at different dose levels (100, 200 and 400 mg/kg) has significantly increased the urine volume and also enhanced the elimination of sodium, potassium, and chloride ions in urine.

Conclusions: Results showed that single dose administration of EELCP as 100, 200, and 400 mg/Kg and standard furosemide (10 mg/kg body weight [b.wt]) has significantly (p<0.001***) increased the urine output along with an increase in concentration of sodium, potassium, and chloride.

Keywords: *Cissampelos pareira*, Diuretic activity, Ethanolic extract, Hydrated rats, Leaves.

INTRODUCTION

Diuretics that enhance the rate of urine flow and sodium excretion are used to maintain the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is helpful in many life-threatening conditions such as congestive cardiac failure (CCF), nephritic syndrome, cirrhosis, renal failure, toxemia of pregnancy, premenstrual tension, and hypertension [1,2]. The presently available diuretics such as thiazides and loop diuretics exhibit several adverse effects like electrolyte imbalance and metabolic alterations [3] etc. Huge number of medicinal plants mentioned in ayurvedic system of medicine are known to possess diuretic properties such as *Achyranthus aspera*, *Boerhavia diffusa*, *Anisochilus carnosus*, *Bixa orellana*, *Costus speciosus*, *Xanthium strumarium*, *Kigella pinnata*, *Bacopa monnieri*, *Barbara vulgaris*, *Abelmoschus esculentus*, *Steganotaemia araliacea*, *Benincasa hispida*, *Morinda citrifolia* (Noni).

Plant description

The *Cissampelos pareira* [4] an extensively spreading, glabrous to soft pubescent, perennial climbing shrub found all over India and is commonly known as Padha and other synonyms are Padvel, Padvali, Aaknadi, Venievel, Poda, and Patha belong to the family of Menispermaceae [4]. In Ayurvedic system of medicine, the leaves and roots are used in the treatment of indolent ulcers (Kirtikar and Basu, 2001) and diarrhea (Amresh *et al.*, 2003). The plant is used in the treatment of urinary tract infections since it is considered as antiseptic (Dandiya and Chopra, 1970). Juice of *C. pareira* is given in migraine, and the plant has a long history of use for inflammation of muscles, snakebite, rheumatism, diarrhea, dysentery, and menstrual problems. *C. pareira* is widely employed in herbal medicine today as a diuretic, tonic as well as to reduce fever and to relieve pain. It is often employed

for menstrual cramps, difficult menstruation, excessive bleeding and uterine hemorrhages, fibroid tumors, pre- and post-natal pain, colic, constipation, poor digestion, and dyspepsia. Hence, midwives in Amazon always carry the *C. pareira* for the above-mentioned ailments (Mukerji and Bhandari, 1959).

Some scientific studies revealed its antinociceptive [5], antiarthritic [5], cardiogenic [6], anticancer [7], anti-inflammatory [8], antidiarrheal [9], antihemorrhagic, antifertility [10], antioxidant, neuroprotective [11], hepatoprotective [12], antioxidant [13], immunomodulatory [13], antitrypanosomal activities. The major constituents of roots and leaves of *C. pareira* include [14] Pelosin, O-methylcurine, l-curine Cissamine, Cissampareine, Hyatin, Bebeerine, Cycleanine, Tetrandine and Beriberine, Cissampeline, Cissampoline, Dicentrine, Insularine, Pareirine, Hyatinine, Pareirubrine A, Pareirubrine B, Pareitropone, Norimeluteine, Cissampeloflavone, D-Quercitol, and Grandirubrine [14]. The leaves of *C. pareira* traditionally used as a diuretic but scientifically not studied as a diuretic agent. The main aim of the present study was to evaluate diuretic activity of ethanolic extract of leaves of *C. pareira* (EELCP) in hydrated (Modified Lipschitz test) albino rats.

MATERIALS AND METHODS

Collection of plant

The leaves of *C. pareira* were obtained from the forest of Tirupati, AP and were identified and authenticated by Dr. Pramod Kumar, Pharmacognocist V. L. College of Pharmacy, Raichur, Karnataka.

Preparation of extract

Leaves were thoroughly washed under fresh tap water and shade-dried and powdered using a mechanical grinder. The preparation of EELCP

was done using soxhlation. About 200 g of leaves powder was taken into the soxhlet apparatus and extracted using (95%) ethanol. The extraction process was carried out for 18-20 hrs till the appearance of the colorless solvent in the side tube. The extract collected was dried by evaporating the solvents on a water bath maintained at 50°C and percentage yield of EELCP was recorded with respect to the total quantity of powder used for the extraction. Then the extract was evaluated for its phytochemicals by following standard procedures [15].

Experimental design

Experimental animals

Albino rats weighing between 140 and 200 g of either sex were used in the study and were obtained from the Central Animal House, V.L. College of Pharmacy, Raichur, Karnataka. The experimental protocol was approved by the Institutional Animal Ethical Committee, and these animals were used to evaluate the diuretic activity of EELCP. The animals were maintained under standard husbandry conditions for an acclimatization period of 15 days before performing the experiments. All rats were housed in metallic cages six in each and temperature maintained at $22\pm 2^{\circ}\text{C}$.

Drugs used

Furosemide 20 mg/ml (Sanofi Aventis, Andheri East, Mumbai).

Acute toxicity study

Determination of LD_{50} : The acute toxicity of EELCP was determined using albino mice of either sex (16-20 g), maintained under standard husbandry conditions [16,17]. The animals were fasted for 3 hr prior to the experiment, and the extract was administered as a single dose and observed for the mortality up to 48 hr study period (short term toxicity). Based on the short term toxicity profile, the next dose of the extract was determined as per OECD guidelines No. 420. The maximum dose tested (2000 mg/kg) for LD_{50} . From the LD_{50} , doses like $1/20^{\text{th}}$, $1/10^{\text{th}}$, and $1/5^{\text{th}}$ were selected and considered as low, medium, and high dose i.e., 100 mg/kg, 200 mg/kg, 400 mg/kg, respectively, to carry out this study.

Experimental model

Lipschitz test

Male albino rats were divided into five groups of six rats in each [18,19]. Group I serves as normal control received vehicle (carboxymethyl cellulose 2% in normal saline 10 ml/kg b.wt), the Group II received Furosemide (10 mg/Kg, p. o) in the vehicle; other Groups III, IV, V were treated with low (100 mg/kg), medium (200 mg/kg), and high (400 mg/kg) doses of EELCP in vehicle and immediately after the extract treatment, all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages (3/cage), specially designed to separate urine and feces and kept at $21^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$. A total volume of urine collected for 5 hr was measured at the end. During this period, no food and water were made available to animals. Various parameters like total urine volume and concentration of sodium, potassium, and chloride in the urine were measured and estimated, respectively.

Estimation of urinary electrolytes

Urine electrolytes (sodium, potassium and chloride) were determined by Ion Selective Electrode method as described by the user instruction

manual of the Biochemical Kits (Roche, Roche Diagnostics Pvt. Ltd, Gurgaon, Haryana).

Statistical analysis

Experimental results were expressed as mean \pm SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Dunnett's 't' test using Graph Pad Prism software.

RESULTS

The EELCP 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 were survived even after 14 days. This indicates that the extract was found to be safe up to a maximum dose level tested (2000 mg/kg). No major behavioral changes were observed during this period of study.

The results obtained with evaluation of diuretic activity of EELCP were shown in Table 1 and Fig. 1. From the result, it can be observed that EELCP has shown a significant diuretic activity by increasing urinary output and increased excretion of sodium, potassium, chloride levels when compared to control. The effect of EELCP was found to be dose dependent, i.e. among the three doses studied, higher dose produced more effect. A comparison was made with the standard diuretic drug furosemide, the diuretic effect observed after treatment with EELCP was found to be significant in terms of urinary output, sodium, potassium, chloride concentrations. Determination of urinary electrolyte concentration revealed that EELCP was effectively increased the urinary electrolyte concentrations for Na^+ , K^+ , Cl^- ions.

DISCUSSION

Medicinal plants offer a natural protection against diseases and are a substantial treatment for certain diseases. Diuretics have proved to be extremely useful in the treatment of mild to moderate hypertension and also in enhancing the effect of other antihypertensive agents. Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing volume over load and relieve orthopnea and paroxysmal nocturnal dyspnea CCF and acute left ventricular failure [20]. They decrease plasma volume and subsequently venous return to the heart. This decreases the cardiac work load, oxygen

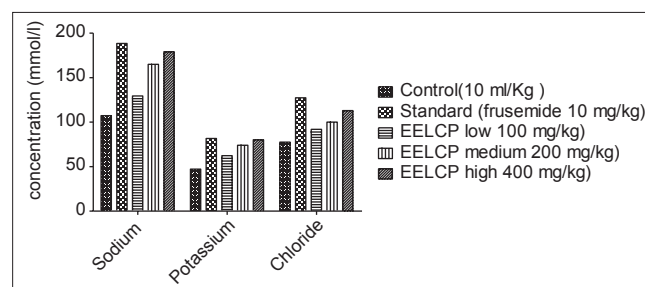


Fig. 1: Effect of ethanolic extract of leaves of *Cissampelos pareira* on urinary sodium, potassium, chloride (mmol/L) ions concentration in hydrated rat model in albino rats

Table 1: Effect of EELCP on urine volume and electrolyte concentration in hydrated rat model (Lipschitz test) in albino rats

S. no	Groups	Total urine Vol (ml/kg BW/5 hrs)	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L
1	Control (10 ml/kg BW)	13.16 \pm 0.59	107.35 \pm 0.73	47.19 \pm 1.54	77.58 \pm 0.85
2	Standard (Frusemide 10 mg/kg BW)	20.26 \pm 0.03***	188.49 \pm 2.45***	81.64 \pm 1.28***	127.41 \pm 1.74***
3	EELCP low (100 mg/kg BW)	12.81 \pm 0.29***	129.40 \pm 2.80***	62.35 \pm 0.06***	91.41 \pm 0.99***
4	EELCP medium (200 mg/kg BW)	14.01 \pm 0.38***	164.99 \pm 2.00***	74.01 \pm 2.77***	100.06 \pm 2.25***
5	EELCP high (400 mg/kg BW)	16.13 \pm 0.92***	179.17 \pm 0.69***	80.23 \pm 0.47***	112.96 \pm 1.05***

n=6, Values expressed as mean \pm SEM. Significance at $p<0.001$ ***. Compared with the control group (one way ANOVA followed by Dunnett's 't'-test). BW: Body weight, EELCP: Ethanolic extract of leaves of *Cissampelos pareira*

demand, and plasma volume and also decreases blood pressure. Thus, diuretics play an important role in hypertensive patients [19]. They are used to induce forced diuresis (forced alkaline diuresis and forced acidic diuresis) in cases of aspirin and morphine poisoning. Diuretics are also useful in the prevention of recurrent calculi. The present study revealed that EELCP significantly increased the urinary out, as well as the elimination of urinary electrolytes in a dose-dependent manner. Earlier Hullatti *et al.*, 2011 and Suresh Babu Sayana *et al.*, 2014 reported diuretic activity with methanolic and alcoholic extracts of roots of *C. pareira* [3,21]. In the present work, EELCP was studied for its diuretic activity. The phytochemical [15] studies reveal that the leaves of *C. pareira* contains flavonoids, alkaloids, carbohydrates, sterols, phenolic compounds. Phytoconstituents like berberine is already reported for this diuretic activity [22]. The plant *C. pareira* was also reported with berberine [13]. When tested for diuretic activity, berberine increased urine excretion in the rats [22]. Increase in the urinary volume was also accompanied by an increase in the Na⁺, K⁺ excretion similar to the standard diuretic hydrochlorothiazide, suggesting that berberine [22] induced diuresis is caused by its saluretic effect. Earlier studies reported phytochemical substances such as flavonoids, saponins, organic acids [19,2], carbohydrates, phenolic compounds, terpenoids [23,24], alkaloids [25], glycosides [26], sterols [27], sesquiterpenes and aminoacids, carotinoids [28], in different plant extracts. EELCP was identified with most of these plant phytochemical substances mentioned above. Hence, it can be reported that the observed diuretic activity is due to these above phytoconstituents.

CONCLUSION

Results showed that single dose administration of EELCP as 100, 200, and 400 mg/Kg and standard Frusemide (10 mg/kg) have increased the urinary output along with an increase in concentration of sodium, potassium, and chloride ions in urine. EELCP 400 mg/Kg produced a greater diuretic activity which is comparable to that of standard Furosemide (10 mg/kg) (Table 1, Fig. 1). The present study supports and justifies the rationale behind the folklore use of leaves of *C. pareira* for diuretic activity.

ACKNOWLEDGMENT

The authors thank all the participants in this study.

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