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Original Article

Pharmacology Section

Evaluation of Diuretic Activity of Alcoholic Extract of Roots of Cissampelos Pareira in Albino Rats

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ABSTRACT

Background: In congestive heart failure, nephritis, toxemia of pregnancy, premenstrual tension and hypertension associated with oedema diuretic compounds are much helpful to relieve these conditions.

Aims: To study the diuretic activity of alcoholic extract of roots of *Cissampelos pareira* by Lipschitz method in albino rats.

Methods and Material: Five groups of Albino rats were used to evaluate the diuretic activity of alcoholic extract of roots of *Cissampelos pareira* by using metabolic cages. The group I serves as normal control received vehicle (2% CMC in normal saline), group II with Furosemide (10 mg/Kg, p.o), Groups III, IV and V with low (100 mg/kg), medium (200 mg/kg), and high (400 mg/kg) doses of alcoholic extract of roots of *Cissampelos pareira* respectively. Immediately after the alcoholic extract of roots of *Cissampelos pareira* treatment all the rats were hydrated with saline (15 ml/kg, p.o) and 2 animals placed in each metabolic cage, kept at 21°C±0.5°C. No food and water was made available to animals for 5 hour. The total volume of urine collected with each metabolic cage was measured at the end of 5 hour. Various parameters like total urine volume and concentration of different ions i.e., Sodium, Potassium , Chloride in the urine were measured.

Results: In this model when compared to control group the alcoholic extract of roots of *Cissampelos pareira* treated groups at different dose levels (100,200 and 400 mg/kg) have noted with significant increase in the urine volume and also significantly enhanced the excretion of Sodium, Potassium and Chloride ions in urine.

Conclusion: Results showed that single dose administration of standard Furosemide and alcoholic extract of roots of *Cissampelos pareira* significantly ($p<0.05^{*}$, $p<0.01^{**}$, $p<0.001^{***}$) increased the urine output along with an increase in elimination of Sodium, Potassium, and Chloride ions. Alcoholic extract of roots of *Cissampelos pareira* 400 mg/Kg produced a comparable diuretic activity with standard Furosemide.

Keywords: *C.pareira* , Roots, Alcoholic extract, Hydrated rats, Diuretic activity

INTRODUCTION

Diuretic compounds that stimulate the excretion of water are potentially useful in most of disorders including those exhibiting oedema such as congestive heart failure, nephritis , toxemia of pregnancy, premenstrual tension and hypertension [1]. The presently available diuretics such as thiazides and loop diuretics exhibit various adverse effects such as electrolyte imbalance and metabolic alterations [2] etc. Some of the diuretics are derived from medicinal plants and a vast number of medicinal plants mentioned in ayurvedic system of medicine are known to possess diuretic properties such as *Abelmoschus esculentus, Bacopa monnieri, Barbara vulgaris* and *Cissampelos pareira* .

Plant Description

The *Cissampelos pareira* [3], 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 belongs to the family of Menispermaceae [3]. In Ayurvedic system of medicine, the leaves and roots are used in the treatment of indolent ulcers (Kirtikar and Basu,) and diarrhea (Amresh *et al.*,). The plant is used in the treatment of urinary tract infections since it is considered as antiseptic (Dandiya and Chopra,). Juice of *C. pareira* is given in migraine and the plant has a long history of use for inflammation of muscles, snakebite, rheumatism, diarrhoea, dysentery and menstrual problems. *C. pariera* 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, dysmenorrhoea, 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 [4], antiarthritic [4], cardiotonic [5], anticancer [6], anti-inflammatory [7], antidiarrheal [8], anti-hemorrhagic, antifertility [9], antioxidant, neuroprotective [10], hepatoprotective [11], antioxidant [12], immunomodulatory [12], anti trypanosomal activities. The major constituents of roots of *C.pareira* include [13] Pelosin, O-methylcurine, l-curine Cissamine, Cissampareine, Hyatin, Bebeerine, Cycleanine, Tetrandine and Berberine, Cissampeline, Cissampoline, Dicentrine, Insularine, Pareirine, Hyatinine, Pareirubrine A, Pareirubrine B, Pareitropone, Norimeluteine, Cissampeloflavone, D-Quercitol and Grandirubrine [13]. The roots of *C.pareira* are traditionally used as a diuretic but scientifically not evaluated as a diuretic agent. The main aim of the present study was to evaluate diuretic activity of roots of *C. pareira* in hydrated (Modified Lipschitz test) albino rats.

METHODOLOGY

Collection of the Plant

The roots 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, India.

Preparation of Extract

Roots were thoroughly washed under fresh tap water and shade dried and powdered by using a mechanical grinder. The preparation

of alcoholic extract of roots of *C.pareira* was done by using soxhletation in the Department of Pharmacology, V.L.College of Pharmacy, Raichur. About 200 g of root powder was taken into the soxhlet apparatus and extracted using ethanol (95%). The extraction process was carried out for 18 - 20 h till the appearance of colourless solvent in the side tube. The extract collected was dried by evaporating the solvents on a water bath maintained at <50^{0}C and percentage yield of alcoholic extract was recorded with respect to the total quantity of powder used for the extraction. Phytochemical evaluation for the extract was performed using standard procedures.

Experimental Animals

Albino rats weighing between 140-200 g of either sex were used in the study and were obtained from the Central Animal House, V.L.College of Pharmacy, Raichur, Karnataka, India. The experimental protocol was approved by the Institutional Animal Ethical Committee and these animals were used to evaluate the diuretic activity of alcoholic extract of roots of *Cissampelos pareira*. 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 6 in each and temperature maintained at 22±2ºC.

Ethics

The experiment compiled with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethical Committee (IAEC).

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

Acute toxicity study

Determination of LD_{50}: The acute toxicity [14,15] of alcoholic extract of roots of *Cissampelos pareira* was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and the extract was administered as single dose and observed for the mortality up to 48 h 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 1/20th, 1/10th and 1/5th 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 Design

The diuretic activity of alcoholic extract of roots of *Cissampelos pareira* in albino rats was studied by the Lipschitz Test [16-18]. Male Albino rats were divided into 5 groups of 6 rats in each. The group I serves as normal control received vehicle (CMC 2% in normal saline 10 ml/kg b.wt), the group II received Furosemide (10 mg/kg, p.o) in vehicle; other groups III, IV, V were treated with low, medium, and high doses of alcoholic extract of roots of *Cissampelos pareira* in vehicle and immediately after the extract treatment all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages (2 per cage), specially designed to separate urine and faeces and kept at 21°C±0.5°C. The total volume of urine collected for 5 hr was measured at the end. During this period no food and water was 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 ± SEM (n=6). Statistical analysis was performed with one-way-ANOVA followed by Dunnetts t-test.

RESULTS

The alcoholic extract of roots of *Cissampelos pareira* 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 the maximum dose level tested (2000 mg/kg). No major behavioural changes were observed during this period of study.

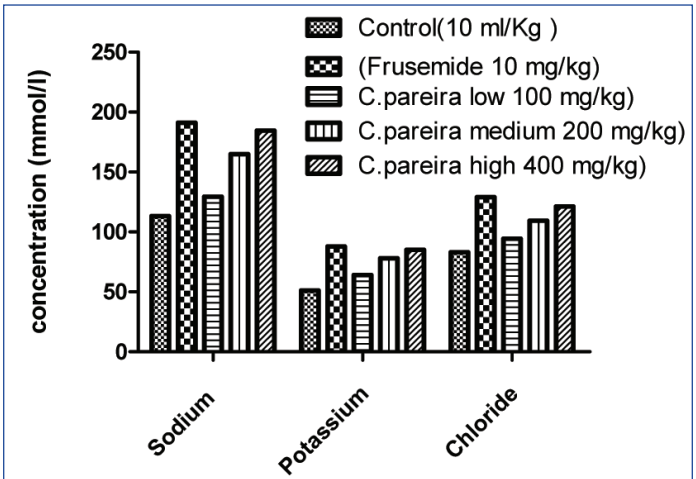
The results obtained with evaluation of diuretic activity of alcoholic extract of roots of *Cissampelos pareira* was shown in [Table/Fig-1-3]. From the result it can be observed that alcoholic extract of roots of *Cissampelos pareira* has shown a significant diuretic activity by increasing urinary output and increased excretion of sodium, potassium, chloride when compared to control. The effect of alcoholic extract of roots of *Cissampelos pareira* 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 alcoholic extract of roots of *Cissampelos pareira* was found to be significant in terms of urinary output, sodium, potassium, chloride concentrations. Determination of urinary electrolyte concentration revealed that alcoholic extract of roots of *Cissampelos pareira* was effective in increasing urinary electrolyte concentrations for all the three ions tested (Na^+, K^+, Cl^-).

DISCUSSION

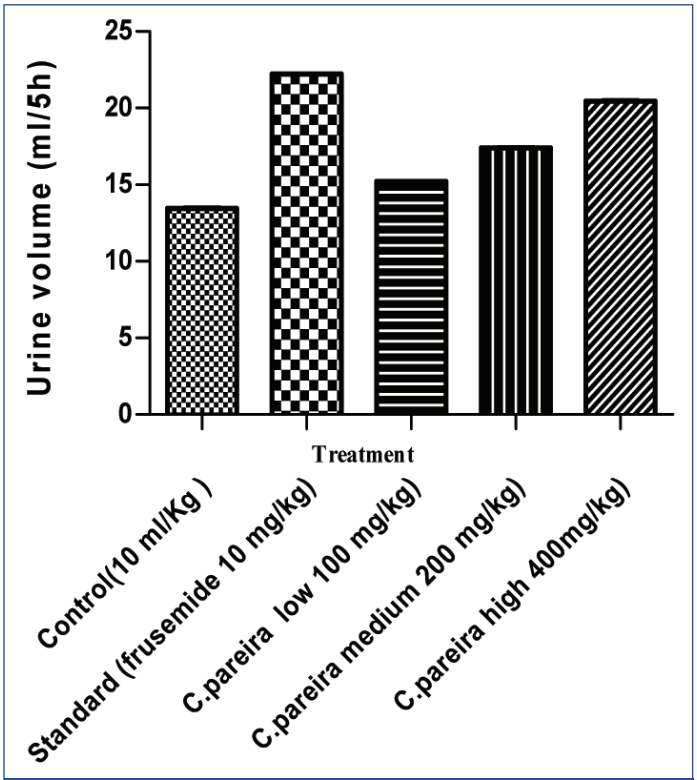
Medicinal plants and botanicals offer a natural safeguard against diseases and are a substantial treatment for certain diseases. Diuretics have proved to be extremely valuable in the treatment of mild to moderate hypertension and also in enhancing the effect of other antihypertensive agents. Diuretics relieve pulmonary congestion and peripheral oedema. These agents are useful in reducing volume over load and relieve orthopnea and paroxysmal nocturnal dyspnoea [19] in CCF and acute left ventricular failure. They decrease plasma volume and subsequently venous return to the heart. This decreases the cardiac work load, oxygen demand and plasma volume and also decreases blood pressure. Thus

S. No.	Groups	Total Urine Vol (ml/kg b.wt/5 h)	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L
1	Control (10 ml/Kg b. wt)	13.45±0.02	113.03 ± 2.16	51.09 ± 1.51	82.95 ± 1.42
2	Standard (Frusemide 10 mg/kg b.wt)	22.23±0.01***	191.05±2.09***	87.81±1.60***	129.06±1.67***
3	Alcoholic extract of roots of *C.pareira* Low (100 mg/kg b.wt)	15.20±0.02***	129.40±2.80***	64.13±1.82***	94.42 ± 1.73***
4	Alcoholic extract of roots of *C.pareira* Medium (200 mg/kg b.wt)	17.41±0.02***	164.99±2.00***	77.93±2.67***	109.44±1.20***
5	Alcoholic extract of roots of *C.pareira* High (400 mg/kg b.wt)	20.46±0.02***	184.53±2.32***	85.11±1.79***	121.39±2.00***

[Table/Fig-1]: Effect of alcoholic extract of roots of *Cissampelos pareira* on urine volume and electrolyte concentration in hydrated rat model in albino rats
Values expressed as mean ± S.E.M.,n=6, Significance at p<0.05*, p<0.01**, p<0.001***, Compared with control group (One Way ANOVA followed by Dunnetts `t' test).



[Table/Fig-2]: Effect of alcoholic extract of roots of c. pareira on urinary sodium, pot assium, chloride (mmol/l) ions concentration in hydrated rat model in albino rats



[Table/Fig-3]: Effect of frusemide and alcoholic extract of roots of c. pareira on urine volume in hydrated rat model in albino rats

diuretics play an important role in hypertensive patients [17]. They are used to induce forced diuresis (forced alkaline dieresis and forced acidic diuresis) in cases of aspirin and morphine poisoning. Diuretics are also useful in prevention of recurrent calculi. The present study revealed that alcoholic extract of roots of *Cissampelos pareira* significantly increased the urinary out put, as well as the elimination of urinary electrolytes in a dose dependant manner. Earlier Hullatti et al., 2011 reported diuretic activity with methonolic extract of roots of *C.pareira* [2]. In the present work alcoholic extract of *C.pareira* was studied for its diuretic activity. The phytochemical [20] studies reveals that the roots of *C.pareira* contains flavanoids, alkaloids, carbohydrates, sterols, phenolic compounds, tannins, resins. phytoconstituents like berberine [12] or pelosine are already reported for this diuretic activity. The plant *C.pareira* was also reported with berberine [12]. When tested for diuretic activity, berberine[21] increased urine excretion in the rats. Increase in the urinary volume was also accompanied by an increase in the Na^{+},K^{+} excretion similar to the standard diuretic hydrochlorthiazide, suggesting that berberine [21] induced diuresis is caused by its saluretic effect. Earlier studies reported phytochemical substances like flavonoids, saponins, organic acids [1,17], steroids, carbohydrates, tannins, phenolic compounds, terpenoids [22], alkaloids [23], glycosides [24], sterols [25], sesquiterpenes & aminoacids, carotinoids [26] in different plant extracts. Alcoholic extract of roots of *Cissampelos pareira* 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 alcoholic extract of roots of *Cissampelos pareira* as 100,200 and 400 mg/Kg and standard Furosemide (10 mg/kg) have increased the urinary output along with an increase in concentration of Sodium, Potassium and Chloride ions in urine. Alcoholic extract of roots of *Cissampelos pareira* mg/Kg produced a greater diuretic activity which is comparable to that of standard Furosemide (10 mg/kg). In traditional medicine the plant is used for its diuretic activity. Ours scientific study come up with identification of so many phytoconstituents reported earlier for this diuretic effect in our alcoholic extract of roots of *Cissampelos pareira*. Thus our study supports and justifies the rationale behind the folklore use of roots of *C.pareira* for its diuretic activity.

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