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Research Article

Diuretic activity of aqueous extract of roots of *Cissampelos pareira* in albino rats

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ABSTRACT

Background: Diuretic compounds that stimulate the excretion of water with small traceable ions are potentially useful in most of disorders including those exhibiting edema such as congestive heart failure, nephritis, toxemia of pregnancy, premenstrual tension, and hypertension. The aim was to evaluate the diuretic activity of aqueous extract of roots of *Cissampelos pareira* (AQERCP) by Lipschitz method in albino rats.
Methods: Five groups of Albino rats were used to evaluate the diuretic activity of AQERCP by using metabolic cages. The Group I serves as normal control received vehicle (carboxymethyl cellulose 2% in normal saline), the Group II furosemide (10 mg/Kg, p.o) in 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 AQERCP 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 faeces, kept at 21°C±0.5°C.The total volume of urine collected was measured at the end of 5th hr. During this period, no food and water was made available to animals. Various parameters such as total urine volume and concentration of sodium, potassium, chloride ions in the urine were measured and estimated respectively.
Results: In this model, when compared to vehicle treated control group the AQERCP 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.
Conclusion: The results showed that single dose administration of AQERCP as 100, 200 and 400 mg/Kg and standard frusemide (10 mg/kg b.wt) has significantly ($p<0.05$*, $p<0.01$**, $p<0.001$***) increased the urine output along with an increase in concentration of sodium, potassium, and chloride. AQERCP 400 mg/Kg produced a greater diuretic activity, which is comparable to the effect of standard furosemide (10 mg/kg).The present study has supported and justified the basis for folklore use of roots of *C. pareira* as a diuretic agent.

Keywords: *Cissampelos pareira*, Roots, Aqueous extract, Hydrated rats, Diuretic activity

INTRODUCTION

Diuretic drugs that increase 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 useful in many life-threatening disease conditions such as congestive heart failure, 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 various adverse effects such as electrolyte imbalance and metabolic alterations.[3] A vast number of medicinal plants mentioned in ayurvedic system of medicine are known to possess diuretic properties such as *Abelmoschus esculentus*, *Achyranthus aspera*, *Steganotaemia araliacea*, *Boerhavia diffusa*, *Anisochilus carnosus*, *Bixa orellana*, *Costus speciosus*, *Benincasa hispida*, *Morinda citrifolia* (Noni), *Xanthium strumarium*, *Kigella pinnata*, *Bacopa monnieri,*

Barbara vulgaris and *Cissampelos pareira* and some of the diuretics are derived from these medicinal plants.

Plant description[4]

The *C. pareira*, 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.[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. 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, 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] cardiotonic,[6] anticancer,[7] anti-inflammatory,[8] antidiarrheal,[9] anti-hemorrhagic, antifertility,[10] antioxidant, neuroprotective,[11] hepatoprotective,[12] antioxidant,[13] immunomodulatory,[13] anti trypanosomal activities.The major constituents of roots 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 roots of *C. pareira* 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 aqueous extract of roots of *C. pareira* in hydrated (Modified Lipschitz Test) albino rats.

METHODS

Collection of 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.

Preparation of extract

Roots were thoroughly washed under fresh tap water and shade dried and powdered by using a mechanical grinder. The preparation of aqueous extract of roots of *C. pareira* was done by using maceration. About 200 g of root powder was subjected to cold maceration with chloroform water in a conical flask for about 7 days at room temperature. the flask was securely plugged with absorbent cotton and shaken periodically. Later, the material was filtered through a muslin cloth and mark was pressed.The filtered was refiltered through whatman filter paper to get the clear filtrate.The filtrate was concentrated to dry residue in a desiccator over anhydrous sodium sulfate. The resulting extract was weighed and filled into the sample containers. Phytochemical evaluation for the extract was performed using standard procedures.[15]

Experimental design

Experimental animals

Albino rats weighing between 140 and 200 g of either sex was used in the study and were taken 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 aqueous extract of roots of *C. pareira* (AQERCP). 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°C±2°C.

Drugs used

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

Acute toxicity study[16,17]

Determination of LD_{50}

The acute toxicity of AQERCP was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 hr prior to the experiment and the extract was administered as 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 1/20th, 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 model

Lipschitz test[18,19]

Male Albino rats were divided into 5 groups of 6 rats in each. The 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 vehicle; other Groups III, IV, V were treated with low, medium, and high doses of AQERCP 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/cage), specially designed to separate urine and feces 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 such as 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, India).

Statistical analysis

Experimental results were expressed as mean±standard error of the mean (n=6). Statistical analysis was performed with one-way ANOVA followed by Dunnett's t-test.

RESULTS

The AQERCP 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 behavioral changes were observed during this period of study.

The results obtained with evaluation of diuretic activity of AQERCP were shown in Table 1 and Figures 1 and 2. From the result, it can be observed that AQERCP has shown a significant diuretic activity by increasing urinary output and increased excretion of sodium, potassium, chloride levels when compared with control. The effect of AQERCP 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 AQERCP was found to be significant in terms of urinary output, sodium, potassium, and chloride concentrations. Determination of urinary electrolyte concentration revealed that AQERCP was effective in

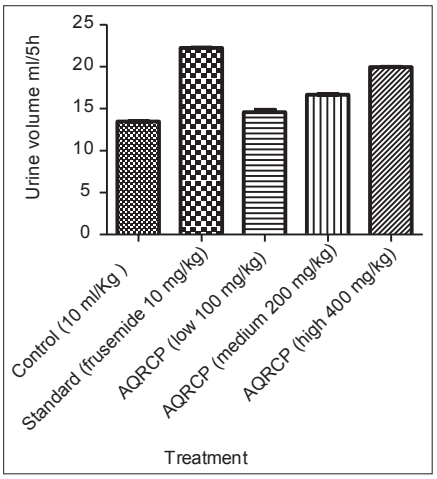

Figure 1: Effect of furosemide and aqueous extract of roots of *Cissampelos pareira* on urinary volume in hydrated rat model in albino rats.

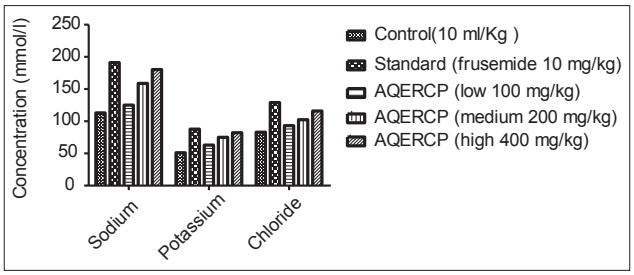

Figure 2: Effect of aqueous extract of roots of *Cissampelos pareira* on urinary sodium, potassium, chloride (mmol/L) ions concentration in hydrated rat model in albino rats.

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

Groups	Total urine volume (ml/kg b.wt/5 hr)	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L
Control (10 ml/Kg b.wt)	13.45±0.02	113.03±2.16	51.09±1.51	82.95±1.42
Standard (furosemide 10 mg/kg b.wt)	22.23±0.01***	191.05±2.09***	87.81±1.60***	129.06±1.67***
AQERCP low (100 mg/kg b.wt)	14.58±0.02***	125.00±2.59***	62.94±1.36***	93.23±2.07***
AQERCP medium (200 mg/kg b.wt)	16.66±0.03***	158.77±2.59***	75.12±2.21***	102.34±1.81***
AQERCP high (400 mg/kg b.wt)	19.95±0.01***	180.22±1.81***	82.23±1.46***	115.86±1.70***

n=6, values expressed as mean±SEM. Significance at p<0.05*, p<0.01**, p<0.001***, Compared with control group (One-way ANOVA followed by Dunnett's t-test). AQERCP: Aqueous extract of roots of *Cissampelos pareira*, SEM: Standard error of the mean

increasing urinary electrolyte concentrations for all the three ions tested (Na^+, K^+, Cl^-).

DISCUSSION

Medicinal plants 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 edema. These agents are useful in reducing volume over load and relieve orthopnea and paroxysmal nocturnal dyspnoea[20] 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 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 prevention of recurrent calculi. The present study revealed that AQERCP 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 methonolic and alcoholic extracts of roots of *C. pareira*.[3,21] In the present work, aqueous extract of roots of *C. pareira* was studied for its diuretic activity. The phytochemical[15] studies reveal that the roots of *C. pareira* contains flavanoids, alkaloids, carbohydrates, sterols, phenolic compounds, tannins, resins. Phytoconstituents like berberine[4] or pelosine are already reported for this diuretic activity. The plant *C. pareira* was also reported with berberine.[13] When tested for diuretic activity, berberine[22] 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[22] induced diuresis is caused by its saluretic effect. Earlier studies reported phytochemical substances such as flavonoids, saponins, organic acids,[2,19] steroids, carbohydrates, tannins, phenolic compounds,[23] terpenoids,[24] alkaloids,[25] glycosides,[26] sterols,[27] sesquiterpenes and aminoacids, carotinoids[28] in different plant extracts. AQERCP 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

The results showed that single dose administration of AQERCP 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. AQERCP 400 mg/Kg produced a greater diuretic activity, which is comparable to that of standard Furosemide (10 mg/kg) Table 1, Figures 1 and 2. The present study supports and justifies the rationale behind the folklore use of roots of *C. pareira* for diuretic activity.

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

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