An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

SALURETIC EFFECT OF ALKALOIDAL FRACTION FROM *CYCLEA PELTATA* ROOT IN RATS

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ARTICLE INFO

Key Words

Benzylisoquinoline, Diuretic, Lipschitz test, Menispermaceae, Potassium sparing.

Access this article online

Website: https://www.jgtps.com/

Quick Response Code:

ABSTRACT

The aim of the present study was to evaluate the diuretic and saluretic potential of four fractions of *Cyclea peltata* root extract in rats. The diuretic effect was evaluated using the Lipschitz model in rats. The four fractions petroleum ether fraction (1 mg/kg p.o), methanol fraction (6 mg/kg p.o), dichloromethane fraction (6 mg/kg p.o) and aqueous fraction (60 mg/kg p.o) were administered to the rats. The groups were compared with standard group that received Furosemide (20 mg/kg p.o). The urinary output of each group was recorded every hour for 5 hours and urine and serum and samples were analysed for Na^+, K^+ and Cl^- ions. Alkaloidal rich dichloromethane fraction significantly increased the urine output and excretion of Na^+ and Cl^- ions in the urine and reduced Na^+ levels significantly in serum. Insignificant reduction in K^+ ions was seen in serum. The results provide an evidence for the potentially favourable effects of alkaloidal fraction of *C. peltata* root extract as diuretic and saluretic with a possible potassium sparing property.

INTRODUCTION

Abnormalities often develop in fluid volume and electrolyte composition and can cause conditions that are clinically important. Diuretics belong to a class of drugs which increase the rate of urine flow and help to adjust the volume and composition of fluids in the body by promoting loss of ions and water.[1] Cardiovascular diseases (CVD) represent an increasing cause of mortality globally and developing countries like India are also struggling to manage its impact. Diuretics find use in treatment of Congestive Heart Failure (CHF) and are effective in relieving symptoms, reducing the intracardiac pressure and improving the cardiac performance.[2] The World Health Organization (WHO) has reported that raised blood pressure is estimated to cause around 7.5 million deaths worldwide and accounts for 12.8% of total of all deaths.[3] Diuretics are one of the drugs used as first line treatment in hypertension. These drugs are also often indicated in the treatment of other conditions such as liver cirrhosis, nephritic syndrome, urinary stones and urinary tract infections (UTI). Such wide application has made them the most frequently prescribed

drugs in medicine. However, there are certain limitations in the use of existing diuretics such as electrolyte imbalance, metabolic alterations and hypovolemia.[4] The rising incidence of such disorders associated to electrolyte imbalance and oedema has necessitated the development of effective treatment strategies with minimal systemic adverse effects. The health benefits of herbs and botanicals have increased the popularity of traditional and folklore medicines. There are a number of medicinal plants in the Indian system of medicine, which have been employed as drugs possessing diuretic activity and are considered as mild and nontoxic.[5] *Cyclea peltata* Hook. f. & Thoms (Menispermaceae), commonly known as Patha, is a slender twinning shrub that often climbs tall trees. It is claimed to possess anti-inflammatory and diuretic property.[6] The roots of *C. peltata* are useful in conditions such as painful swellings, cardiac disorders and in the management of urinary stones.[7] Our previous study has shown that the alcoholic extracts of *C. peltata* roots have effective diuretic activity in rats.[8] In view of this information, the present study was undertaken to evaluate 4 fractions of ethanolic extract of *C. peltata* root viz. Petroleum ether fraction, methanol fraction, dichloromethane fraction and aqueous fraction for their diuretic activity in rats.

MATERIALS AND METHODS

Chemicals and Kits: The chemicals and solvents used were of Analytical Grade. The kits for the estimation of Na, K and Cl ions used were from CHEMPAK, Reckon Diagnostics P. Ltd. Furosemide (Lasix®, Sanofi Aventis Pharmaceuticals, India) was purchased from the hospital pharmacy.

Collection and authentication of plant material:

The roots of *C. peltata* were collected in the month of August and September, from the wild along the Chorla Ghats, Karnataka. The plant was authenticated by Taxonomist, Dr. Harsha Hegde (Scientist 'D') at Regional Medical Research Centre, Belagavi. The herbarium was deposited in RMRC with Accession number RMRC-1274.

Preparation of plant extract: The roots were washed under running water and dried under shade. The dried roots were pulverized to coarse powder, and subjected to cold maceration using 70% v/v ethanol for 24 hours. The marc was dried and then subjected to soxhlet extraction using ethanol (95% v/v) as solvent. The macerate and percolate were then combined and concentrated using rotary evaporator (Buchi). The residue obtained was further subjected to fractionation.[9]

Preparation of fractions of plant extract:

The fractionation of ethanolic extract was carried out according to the generic scheme described by Cos et al.[9] with minor modifications (Figure 1). The four fractions obtained were methanol, petroleum ether, dichloromethane and water. The solvent free fractions were obtained using rotary evaporator then packed in air tight containers and stored at 4°C in refrigerator till further use for experimental purpose.

Preliminary Phytochemical screening: The extract and fractions were subjected to phytochemical screening for the presence of various phytochemical constituents.[10]

Animal selection: Healthy male Wistar Albino rats weighing 150-250 g were procured from Shri. Venkateshwara Enterprises, Bangalore. They were housed in clean and transparent polypropylene cage in a group of six per cage and were maintained under 12/12 hr natural light-dark cycle at 25±2°C ambient temperature, 45-55 % relative humidity. They were allowed to acclimatize one week before the experiment. The rats were allowed free access to standard pellet and water *ad libitum.* The study was reviewed and approved by the Institutional Animal Ethics Committee, KLEU's College of Pharmacy, Belagavi (1027/a/07/CPCSEA; 06/09/2014). All the experimental procedures were carried out in accordance with Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Selection of dose: According to Acute Oral Toxicity studies previously reported, LD_{50} of *Cyclea peltata* alcoholic extract was found to be greater than 2000 mg/kg. Significant diuretic activity of the extract was seen at a dose of 200mg/kg. In the present study, doses were calculated based on the extractive value of each fraction which corresponds to 200 mg/Kg of extract. The doses equivalent to 200mg/kg BW of main extract were thus

selected for each fraction: Methanol fraction as 1mg/kg bw, petroleum ether fraction as 6mg/kg bw, dichloromethane fraction as 6mg/kg bw and water fraction as 60mg/kg bw.[8]

Evaluation of diuretic activity: The Lipschitz model was employed for the evaluation of diuretic and saluretic activity in rats.[11] The overnight fasted animals were placed individually in metabolic cages with free access to water for 5 hours during the test period. The animals were divided into the 6 groups consisting of 6 animals each.

Group I; (control); received normal saline 20 ml/kg

Group II; (Furosemide); received Furosemide 20 mg/kg

Group III; received Methanol Fraction 1 mg/kg

Group IV; received Petroleum Ether Fraction 6 mg/kg

Group V; received Dichloromethane Fraction 6 mg/kg

Group VI; received Aqueous Fraction 60 mg/kg.

Collection and analysis of urine and serum: The urine volume was measured every hour for 5 hours and the total volume after 5 hours of study was measured. The urine and serum samples were used to analyse Na^+, K^+ and Cl^- ions concentrations using diagnostic kits. The diuretic action and diuretic activity were calculated by the following equations:

$$Diuretic\ action\ =$$

$$\frac{urine\ output\ of\ test\ group}{urine\ output\ of\ control\ group}$$

$$Diuretic\ activity =$$

$$\frac{diuretic\ action\ of\ test\ group}{diretic\ action\ of\ furosemide\ group}$$

Statistical analysis: All data are expressed as Mean ± SEM. The data were analysed using one way ANOVA followed by Post-hoc test using Graphpad prism software (Version 5.01) The values were considered significant when $p<0.001$.

RESULTS AND DISCUSSION

Phytochemical evaluation of extract and fractions: Phytochemical evaluation of extract and fractions showed presence of Sterols and Tri-Terpenoids, alkaloids, flavonoids, tannins, saponins, waxes and lipids. Phytochemical evaluation of methanol Fraction showed presence of sterols and triterpenoids, petroleum ether fraction showed presence of wax and lipids, dichlormethane fraction showed presence of alkaloids and aqueous fraction showed presence of tannins, flavonoids and saponins.

Evaluation of diuretic activity: The standard Furosemide (Group II) showed significant ($p<0.001$), increase in the cumulative urine volume compared to the control group after 5 hours. Among the four fractions, the dichloromethane fraction (Group V) showed highly significant ($p<0.001$) increase in the cumulative urine volume compared to control (Group I) after 5 hours of administration. The diuretic action and diuretic activity of standard Furosemide group was higher compared to the control group. The dichloromethane fraction (Group V) also showed increased diuretic action compared to that of control group. The comparative diuretic activity was also significantly higher than the control (Group I). The results have been displayed in Table 1. The standard Furosemide (Group II) showed significant increase in urine volume after 1 hour of administration of drug. The urine volume significantly increased up to 3 hours of the study period compared to control (Group I). The dichloromethane fraction (Group V) also showed significant increase in urine volume after 1 hour of administration and significantly increased up to 3 hours of study period. The change in the urine volume was observed after every hour and the difference in urine volume between test and control groups were calculated after each hour. Furosemide (Group II) and the dichloromethane fraction (Group V) showed steady increase in diuresis (Figure 2).

Evaluation of Saluretic activity: The concentrations of Na^+, K^+ and Cl^- ions were significantly greater in the urine of Furosemide group (Group II) compared to control (Group I). The dichloromethane fraction (Group V) showed significantly greater Na^+, K^+ and Cl^- ion concentrations in the urine compared to control group. The Na^+/K^+ ratio in case of Furosemide (Group II) and dichloromethane fraction (Group V) groups was significantly

higher ($p<0.05$) and ($p<0.05$) respectively compared to control (Group I). The ratio of dichloromethane fraction (Group V) was significantly higher than the Furosemide group (Group II). The other fractions did not show any significant increase in the Na^+/K^+ ratio. Summary of results has been displayed in Table 2. A significant decrease in the Na^+, K^+ and Cl^- ion concentrations was seen in the serum of the Furosemide group (Group II) compared to control (Group I). The dichloromethane fraction (Group V) showed significant decrease in the Na^+ and Cl^- ion concentration in serum compared to control (Group I). The results have been displayed in Table 3. Several medicinal plants are traditionally being used in case of swellings and as diuretics. In the Indian traditional system of medicine, the root of *C. peltata* has been claimed to have diuretic property and are found to be rich in saponins, alkaloids and flavonoids.[6] Previously, the ethanolic extract of *C. peltata* root extract has shown significant diuretic activity in rats.[7] In the present study, the four fractions of ethanolic extract of *C.peltata* root were evaluated for diuretic activity and the pharmacological response was compared with that produced by furosemide, a widely used diuretic in clinical practice. The renal system that is responsible for the production, storage and elimination of urine out of the body, plays an important role in regulating extracellular fluid volume by adjusting the electrolyte and water excretion. Disturbance in this filtration system may result in edema and electrolyte imbalance giving rise to various conditions.[12] The renal water reabsorption is driven by the active transport of Na^+. Thus, Na^+ and water movements are strongly linked. Any treatment affecting the renal Na^+ transport will also affect the water reabsorption. Diuretics act by increasing the urine volume (water excretion) and a net loss of solutes (i.e. electrolytes) in the urine. Thus they not only promote the loss of water but also cause a net loss of electrolytes, mainly Na^+ ions thereby exerting a saluretic effect.[1,4] In treatment of conditions such as hypertension, maintaining electrolyte balance plays an important role. In the present study, the concentration of electrolytes in urine were analysed and among the fractions, the dichloromethane fraction showed significant increase in Na^+ and Cl^- ions in urine, indicating increased excretion of these ions in the urine and thus increased saluretic effect, similar to that seen in the Furosemide group. However, the excretion of K^+ ions in urine was found to be less than that of Furosemide. The Na^+/K^+ ratio was significantly greater than Furosemide which may indicate a potassium sparing effect.[13] Moreover, the electrolyte concentrations in the serum were significantly reduced by Furosemide, but the dichloromethane fraction significantly reduced only Na^+ and Cl^- ion concentrations in the serum with insignificant reduction in the K^+ ion concentration in serum. This may further suggest a potassium sparing action. The Furosemide group, showed significant increase in the urine volume and showed rapid and considerable diuresis in the first hour of administration. The duration of action was for a period of 3 hours. Among the four fractions, the dichloromethane fraction showed significant increase in urine volume and diuretic activity. The effect was rapid and urine output was stimulated up to 3 hours of the study period, similar to that observed in case of Furosemide group. Based on the pattern of excretion of electrolytes in the urine and the time course of diuresis, the saluretic action of the dichloromethane fraction was found to be similar to Furosemide, which acts on the $Na^+/K^+/2Cl^-$ transporter system in the loop of Henle.[14] However, a potassium sparing action was also observed, suggesting more than one mechanism of action probably due to the presence of different potent phytoconstituents in the fraction. Upon phytochemical screening of the fractions, dichloromethane fraction (Group V) was found to contain alkaloids. *C.peltata* has been reported to contain various bisbenzylisoquinoline alkaloids such as cycleapeltine, cycleadrine, cycleacurine, cycleanorine.[15] Several studies have suggested that Dopamine acting via D-2 receptors may act as an inhibitory modulator of aldosterone secretion and doperminergic receptor agonists may contribute to natriuretic effects.[16] Bisbenzylisoquinoline alkaloids of reticuline-type have been reported to display selectivity towards D2 receptors.[17]

Table 1: effect of various fractions of *C. peltata* root extract on cumulative urine volume and diuretic activity

Groups	Urine volume (ml)					Cumulative urine volume after 5hrs (ml)	Diuretic action
	1hr	2hrs	3hrs	4hrs	5hrs		
Control	0.28± 0.07	0.98±0.13	1.33±0.17	1.40±0.14	0.68±0.19	4.68± 0.14	1.00
Furosemide	1.8±0.16 **	3.87±0.18**	6.10±0.37**	3.22±0.53**	1.43±0.34	16.08± 0.63**	3.43
Petroleum ether fraction	0.57±0.06*	0.93±0.07	2.27±0.21*	1.73±0.23	0.82±0.0.20	6.08± 0.30*	1.30
Methanol fraction	0.37±0.042	0.73±0.08	1.30±0.14	1.48±0.16	1.53±0.36	5.32± 0.29	1.14
Dichloro methane fraction	1.55±0.16 **	2.73±0.14**	3.97±0.33**	1.03±0.48	0.38±0.09	9.68± 0.46**	2.07
Aqueous fraction	0.33±0.07	1.07±0.16	1.55±0.20	1.83±0.48	0.90±0.36	5.68± 0.31	1.21

Table 2: Effect of various fractions of *C. peltata* root extract on concentration of electrolytes in urine

Groups	Na^+ (mmol/lt)	K^+ (mmol/lt)	Cl^- (mmol/lt)	Na^+/K^+
Control (20ml/kg p.o)	89.19±3.80	55.53±1.13	67.71±4.51	1.543±0.05
Furosemide (20mg/kg i.p)	137.70±3.68**	85.57±2.40**	94.23±2.37**	1.712±0.02
Petroleum ether fraction (1mg/kg p.o)	104.3±1.22*	64.90±1.86*	69.19±2.83	1.607±0.04
Methanol fraction (6mg/kg p.o)	89.98±7.03	59.74±1.80	65.26±2.23	1.513±0.14
Dichloromethane fraction (6mg/kg p.o)	129.6±2.63**	67.28±2.04**	83.53±2.20**	1.932±0.08
Aqueous fraction (60mg/kg p.o)	80.43±0.66	52.24±2.29	68.73±2.97	1.55±0.07

Table 3: Effect of various fractions of *C. peltata* root extract on concentration of electrolytes in serum

Groups	Na^+ (mmol/lt)	K^+ (mmol/lt)	Cl^- (mmol/lt)
Control (20ml/kg p.o)	139.1±0.80	5.875±0.035	133.0±0.91
Furosemide (20mg/kg i.p)	116.5±0.60**	4.785±0.15**	117.6±1.06**
Petroleum ether fraction (1 mg/kg p.o)	135.9±0.72*	5.69±0.07	128.6±±0.83*
Methanol fraction (6mg/kg p.o)	136.7±1.17	5.675±0.11	132.4±1.02**
Dichloromethane fraction (6mg/kg p.o)	121.6±0.43**	5.798±0.11	126.8±2.10
Aqueous fraction (60mg/kg p.o)	137.8±0.76	5.675±0.084	130.2±1.19

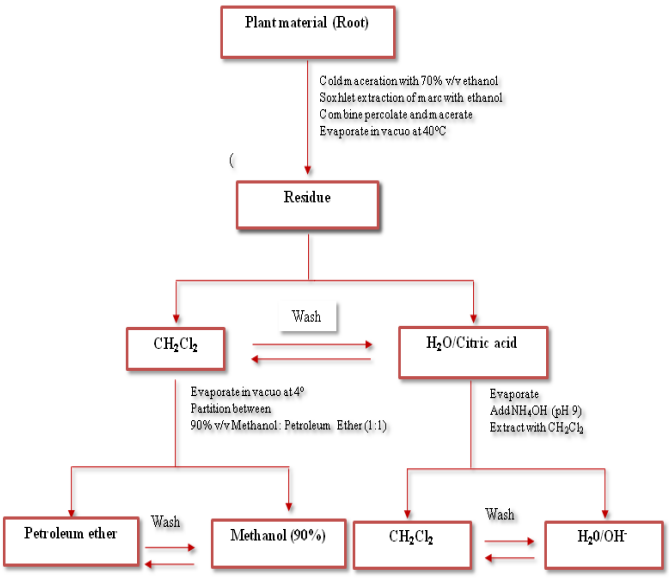

Figure 1: Scheme of extraction and fractionation

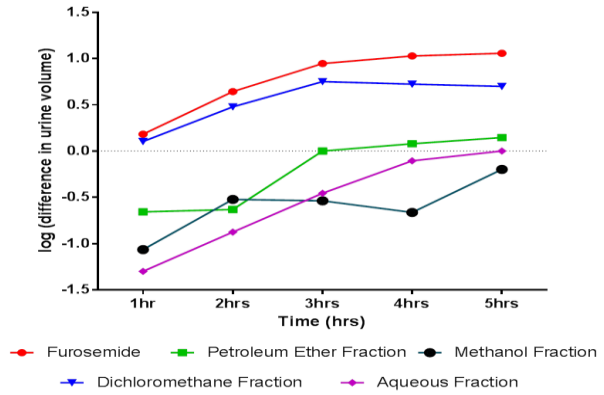

Figure 2: Time course of diuresis of various fractions of *C.peltata* root extract

Table and figure titles and legends:

Table 1: effect of various fractions of *C. peltata* root extract on cumulative urine volume and diuretic activity

For statistical verification the comparison was made between control *vs* fractions, Values are expressed as mean± SEM, n=6, **p* <0.05, ***p* <0.001.

Table 2: Effect of various fractions of *C. peltata* root extract on concentration of electrolytes in urine Na^+ is sodium ion, K^+ is potassium ion, Cl^- is chloride ion. Na^+/K^+ is the sodium-potassium ion ratio. For statistical verification the comparison was made between control *vs* fractions, Values are expressed as mean± SEM, n=6, *P* values: **p* <0.05, ***p* <0.001.

Table 3: Effect of various fractions of *c. peltata* root extract on concentration of electrolytes in serum Na^+ is sodium ion, K^+ is potassium ion, Cl^- is chloride ion. Na^+/K^+ is the sodium-potassium ion ratio. For statistical verification the comparison was made between control *vs* fractions, Values are expressed as mean± SEM, n=6, **p* <0.05, ***p* <0.001.

Figure 1: Scheme of extraction and fractionation

Figure 2: Time course of diuresis of various fractions of *C. peltata* root extract

Benzylisoquinoline type of alkaloids were found to possess potent diuretic activity, exerting effect by binding to Adenosine A1 receptors.[18] The smooth muscles of the urinary bladder have specific properties that make it compliant and help in voiding through muscarinic receptors.[19] Bisbenzylisoquinoline alkaloids are also reported to cause smooth muscle relaxation by interacting with adrenergic and muscarinic receptors.[20] Cycleanine has been reported to cause smooth muscle relaxation by acting as a potent vascular selective Ca-antagonist.[21] Spasmolytic effect of a bisbenzylisoquinoline alkaloid from *C. sympodialis* has been reported in smooth muscles of guinea pigs.[22] Thus, the diuretic and saluretic activity with a notable potassium sparing potential of the alkaloidal fraction observed may be possibly mediated through one or more of the above mentioned mechanisms due to presence of potent alkaloids which need to be further explored. It is well reported that plant extract /fraction(s) are composed with multiple phytoconstituents which can interact with multiple protein molecules via the gene-set enrichment analysis [23,24], network pharmacology [25,26] and molecular docking analysis[27-29]. Further, study can be still investigated for multi-component-multi protein evaluation for the potential lead hit for diuretic activity.

CONCLUSION:

In conclusion, the alkaloidal fraction of ethanolic root extract of *C. peltata* may be beneficial as a saluretic with a potential potassium sparing action that needs to be further evaluated. Further isolation of active phytoconstituents may be carried out to establish its clinical value as diuretics in therapy in conditions associated to electrolyte imbalance and edema. Additionally, few lead hits can be identified for this activity using computational approach.

Acknowledgement : Authors are thankful to the Principal, KLE University College of Pharmacy, Belagavi for providing the necessary facilities to carry out this project. Authors are also thankful to Dr. Harsha V. Hegde (Scientist D), RMRC, ICMR, Belagavi for authenticating the plant material.

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