Phytochemical investigation and diuretic activity of *Cyclea peltata* leaf extracts

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INTRODUCTION

*Cyclea peltata* (Lam) Hook. f. Thoms (Fam: Menispermaceae) is a common plant in the west coast of India. The roots of *C. peltata* are being used in Ayurvedic system of medicine under the name Rajapatha for various medicinal properties.¹ ² The leaves of *C. peltata* were being used traditionally as coolant, antidiandurrut, antipyretic and diuretic.³ ⁴ Traditionally leaves of *C. peltata* are being used for the treatment of herpes in some parts of costal Karnataka.⁵ The leaves of *C. peltata* are found to contain alkaloids such as cycleanine, bebeerines, hayatinin, hayatidin and hayatin. Five bisbenzylisoquinoline alkaloids, cycleapeltine, cycleadrine, cycleacuine, cycleanorine and cycleahomine chloride have been isolated from the roots of *C. peltata*.⁶ The protective effect of *C. peltata* leaf on cisplatin-induced nephrotoxicity and oxidative damage has been reported. The results indicated that the post-treatment of *C. peltata* leaf extracts might effectively ameliorate the oxidative stress parameters observed in cisplatin-induced renal toxicity and could be used as a natural antioxidant against cisplatin-induced oxidative stress.⁷

The present study reports the phytochemical investigation and diuretic activity of petroleum ether and ethanolic extracts of *C. peltata* leaves.

MATERIALS AND METHODS

Plant Material

The plant *C. peltata* was collected from local area of Shimoga, Karnataka. The collected material was authenticated by Prof. D. Ruddrappa, Head, Department of Botany, Sahyadri Science College, Shimoga (NCP/COG/002/2008). The leaves were cleaned, air-dried, coarsely powdered, and subjected for Soxhlet-extraction by using petroleum ether and ethanol successively. The extracts were evaporated to dryness by using Rotary-evaporator under reduced pressure at 45°C.

Preliminary Phytochemical Investigation

Both the extracts were subjected to preliminary phytochemical screening for the determination of major chemical groups by standard procedures.
Thin Layer Chromatography
Plant extracts were dissolved in proper solvent and applied to precoated Thin Layer Chromatography (TLC) silica gel plates (silica gel 60 F254, AluGram, Germany). Chromatograms were developed in solvent system containing Toluene: Ethyl acetate: Methanol (4: 0.5: 0.5) and the chromatograms were examined under UV and daylight as well as after derivatization with Anisaldehyde-Sulfuric acid and Dragendorff’s reagent to detect the presence of different phytocconstituents.[9]

Experimental Animals
The Institutional Animals Ethics Committee approved the use of animals for the present study. (Ethical clearance No. NCP/IAEC/CLEAR/P.COL/01/07/2007-08).

Wister albino rats of either sex weighing 200-300 g and mice weighing between 20 and 25 g were used for the experiment work. Animals were maintained under standard conditions husbandry, room temperature 26 ± 2°C, relative humidity of 45-55%, 12 h light/dark cycle, in an animal house approved by the committee for the purpose of control and supervision of experiments on animals. Animals were obtained from the central animal house, national college of pharmacy, Shimoga. The animals were given standard diet supplied by Pranav Agro Industries Ltd., Sangli. The animals had free access of standard diet and water and housed in a poly propylene cages.

Acute Toxicity Study
The acute toxicity of petroleum ether and ethanolic leaf extracts of plant C. peltata was determined using Swiss albino mice. The animals were divided in to three different groups. The group 1 received normal saline (25 mL/kg), served as control. The group 2 and 3 received 3000 mg/kg body weight petroleum ether and ethanolic extracts of leaves of Cyclea peltata. After oral administration of these extracts, the animals were observed continuously for the behavioral changes for the first 2, 4 h and then observed for mortality if any, after 24 h.[9]

Evaluation of Diuretic Activity
Diuretic activity was evaluated on petroleum ether and ethanolic extracts of leaves of plant C. peltata using Lipschitz et al. method.[10,11] Healthy Wister rats of either sex were divided in to six groups of six animals each. Furosemide (20 mg/kg) was used as standard reference drug. All the drugs were prepared by suspending in 0.5% w/v of Trgacanth mucilage. Before the experiment, the rats were fasted for 18 h with free access to water. On the day of experiment, the animals of group 1 was administered with saline orally (2.5 mL of 0.9% NaCl/100 g body weight).[12] this group served as control. Group 2 was treated with standard drug Furosemide (20 mg/kg) along with saline solution. Group 3 and 4 received petroleum ether extracts of C. peltata 200 mg/kg body weight and 300 mg/kg body weight along with saline solution, respectively. The group 5 and group 6 received orally ethanol extracts of C. peltata 200 mg/kg body weight and 300 mg/kg body weight with saline solution, respectively. Immediately after the treatment, the animals were placed in metabolic cage (1 animal in one metabolic cage) provided with wire mesh bottom and a funnel to collect the urine. Stainless steel sieves are placed in the funnel to retain fecal matter and to allow the urine to pass. The urine was collected in measuring cylinder up to 5 h for all control and drug-treated groups. During this period no food or water was made available to the animals. The volume of urine, electrolytes (Na+, K+, Cl-) were estimated in the urine for assessment of diuretic activity. Na+, K+ estimation was carried out using flame photometry.[13,14] The Cl- ion concentration was estimated by titration with 0.02 N AgNO3 using 5% potassium chromate solution as indicator.[15] The volume of urine was estimated for the assessment of diuretic activity. The diuretic action of tested drug was calculated by using the following formula:

\[
\text{Diuretic action} = \frac{\text{Urinary excretion of test drug}}{\text{Urinary excretion of control}}
\]

\[
\text{Diuretic activity} = \frac{\text{Diuretic action of the test drug}}{\text{Diuretic action of Furosemide}}
\]

Statistical Analysis
The values were expressed as mean ± SEM. The statistical analysis was carried out by one-way analysis of variance (ANOVA) using ezANOVA v. 0.97 software. P-values <0.05 was considered statistically significant.

RESULTS AND DISCUSSION
Preliminary phytochemical screening reveals the presence of phytosterols and alkaloids in petroleum ether extracts. The ethanolic extracts showed the presence of alkaloids, flavonoids, tannins, diterpenes and saponins.

Optimization of TLC Solvent System for the Extract
The extract was dissolved in methanol and subjected for TLC analysis. The solvent system which showed maximum separation of phytochemicals was Toluene: Ethyl acetate: Methanol (4: 0.5: 0.5). About 9 spots in visible light, 10 spots in UV long wave length and 7 spots in UV short wavelength had been detected [Figure 1]. The TLC plate was sprayed with Anisaldehyde-Sulphuric acid reagent and about 9 spots were been detected [Figure 2].

The total alkaloid isolated from the ethanolic leaf extract subjected for TLC analysis. The total alkaloid fraction was dissolved in methanol, and TLC plate was developed and the spots were detected by spraying the TLC plate with Dragendorff’s reagent. The solvent system which showed maximum separation of alkaloids was Cyclohexane: Diethylamine (3: 0.5), which showed 3 spots [Figure 3].
Acute Toxicity Study

Leaf extracts of *C. peltata* were found to be safe orally at the dose of 3000 mg/kg body weight. No mortality was seen at this dose in any of the treated animals after 24 hours. So, the effective dose was chosen 200 and 300 mg/kg body weight.

Evaluation of Diuretic Activity

Treatment with the dose level of 200 and 300 mg/kg body weight of each of petroleum ether and ethanolic extracts of *C. peltata* produced significant diuresis in the treated animals. The ethanolic extract showed significant diuretic activity in dose-dependent manner compared to control rats at 5 h after the dose. Significant diuretic activity was observed with ethanolic extracts of *C. peltata* with both 200 and 300 mg/kg body weight (urine volume of 6.49 and 9.73 mL/kg body weight, respectively) compared to petroleum ether extract (urine volume of 3.65 and 3.93 mL/kg body weight, respectively). Urine output continues to be stimulated throughout the study period, such as cumulative urinary excretion was significantly higher in ethanolic extract compared to petroleum ether extract. The ethanolic extract of *C. peltata* showed more rapid and higher excretion of urine than the petroleum ether extract. The effect became significantly higher at 1 h after the dose administration compared to control rats (*P*<0.01). The diuretic effect remained significantly higher for ethanolic extract treated rats as compared to control rats at all time points, such that the cumulative 5 hour urine excretion for ethanolic extract treated rats was more significant compared to control rats (*P*<0.01). The ethanolic extract of *C. peltata* showed higher excretion of urine at 300 mg/kg body weight dose among all the tested extracts. The results are compiled in [Table 1] and [Figure 4].

Effect on Electrolytes

The urinary level of Na⁺ was significantly increased in all the doses of both ethanolic and petroleum ether extracts of *C. peltata*. Significant increase in K⁺ level in the extracts treated groups as compared to control animals was observed. K⁺ level were higher in ethanolic extract treated groups compared to the petroleum extract treated groups. A dose-dependent increase in the both Na⁺ and K⁺ levels in urine was observed. None of the extracts including standard had shown much changes in Cl⁻ level. Results are given in the [Table 1].

SUMMARY AND CONCLUSIONS

The petroleum ether and ethanolic extract of *Cyclea peltata* were tested for their diuretic activity. From the present investigation we can conclude that, both the petroleum ether and ethanolic leaf extracts of *Cyclea peltata* has shown diuretic action, which is significant with ethanolic extract compared to petroleum ether leaf extract of *Cyclea peltata*. However, the phytoconstituents(s) responsible for this activity need to be investigated. Based on the pattern of excretion of water, Na⁺ and K⁺, it appears that
Table 1: Effect of leaf extracts of *Cyclea peltata* on urine excretion and ionic concentration in rats

| Treatment            | Dose     | Vol. urine (mL/kg b.w) | Diuretic action | Electrolyte concentration (mmol/l) |
|----------------------|----------|------------------------|-----------------|------------------------------------|
|                      |          |                        |                 | Na⁺ | K⁺ | Cl⁻ | Na⁺/K⁺       |
| Saline               | 25 mL/kg | 3.68 ± 0.33             | 1.0             | 88.70 ± 0.95 | 79.05 ± 2.25 | 83.40 ± 2.01 | 1.1          |
| Furosemide           | 20 mg/kg | 15.26 ± 0.58**          | 4.1             | 129.20 ± 2.26** | 89.73 ± 1.79** | 83.75 ± 1.19 | 1.4          |
| CPPE                 | 200 mg/kg| 3.65 ± 0.19             | 1.0             | 91.30 ± 2.65* | 82.21 ± 0.85* | 83.10 ± 2.15 | 1.1          |
|                      | 300 mg/kg| 3.93 ± 0.29             | 1.1             | 94.20 ± 2.28** | 83.48 ± 2.70* | 82.20 ± 2.87 | 1.1          |
| CPEE                 | 200 mg/kg| 6.49 ± 0.56**           | 1.7             | 107.80 ± 3.37** | 85.52 ± 1.98** | 83.55 ± 0.18 | 1.2          |
|                      | 300 mg/kg| 9.73 ± 0.67**           | 2.6             | 121.30 ± 3.39** | 87.60 ± 2.98** | 83.35 ± 1.05 | 1.3          |

Each value represents mean ± S.E.M. of six rats. *P<0.05, **P<0.01. (The statistical analysis was carried out by one way analysis of variance using ezANOVA v. 0.97 software.) CPPE: Cyclea peltata Petroleum ether extract; CPPE: Cyclea peltata ethanolic extract.

Figure 4: Time course of diuresis in rats with leaf extracts of *Cyclea peltata*, vehicle and furosemide

the active principle present in these two extracts having a Furosemide like activity. Some of the plants with potent diuretic and hypotensive activity were found contain benzyl isoquinoline type of the alkaloids.[16] Certain flavonoids were found to exert their diuretic activity by binding with Adenosine A1 Receptor associated with the diuretic action.[17] The diuretic activity of studied plant may be through any of these possible mechanisms since it is rich in alkaloids and flavonoids. The precise site, molecule and cellular mechanisms of the extract remain to be elucidated. The present study supports the traditional use of the plant *C. peltata* for its diuretic activity. Hence, there is a further scope in detail phytochemical investigation and activity guided isolation of active constituents from the plant *C. peltata*. The parameters such as phytochemical evaluation and the TLC system will help in further standardization of *C. peltata* in future.

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