EVALUATION OF DIURETIC ACTIVITY OF AQUEOUS EXTRACT OF LEAVES OF CENTELLA ASIATICA

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2. ABSTRACT

Aim: The present study was undertaken to investigate diuretic effect of aqueous extracts of the dried leaves of Centella asiatica in normal rats. Centella asiatica (L.) is a perennial herb, faintly aromatic and used traditionally for various medicinal properties from ancient times. Demand for herbal formulation is growing all over the world as they are considered to be safer with fewer side effects than the synthetic ones. Material and methods: The leaves of the plant were washed and dried under the shade, extracted with distilled water. The extracts so obtained dried and concentrated under vacuum using rotary vacuum evaporator. The method of Lipschitz et al. employed for the assessment of diuretic activity by using metabolic cage. The animals randomly divided into four groups- Group 1 (Normal), Group 2 (Standard-furosemide10 mg/kg, p.o.), Group 3 (aqueous extract 250 mg/kg, p.o.) and Group 4 (aqueous extract 500 mg/kg, p.o.) The diuretic effect was assessed by measuring total urine volume and the concentration of sodium, potassium and chloride ions in the urine. Results: The extract significantly increased urine volume and electrolyte concentration in urine when compared with normal group. Conclusion: That aqueous extract of dried leaves of Centella asiatica showed potential diuretic effect and can be used as a diuretic agent. Further studies are warranted to isolate the pure phyto-constituent responsible for diuretic activity.

3. KEYWORDS: Centella asiatica, Aqueous extract, Diuretic, Urine volume and Electrolyte concentration, Furosemide.
4. INTRODUCTION
Herbal drugs are those which are obtained from plant origin and usually contain many biologically active ingredients which are used primarily for treating mild and chronic ailments. The trend towards the utilization of natural plant remedies is growing worldwide which created an enormous need for information about the properties and uses of the medicinal plant. India has an ancient heritage of traditional medicine and hence to exploit and evaluate the potential of the rich heritage of traditional medicine is become essential.\cite{1, 2}

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Diuretics is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxemia. Most diuretic drugs derived from chemical sources are associated with adverse effect on quality of life including impotence, hyperglycemia, ototoxicity, gout and fatigue etc.\cite{1, 3}

Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system. Among the several plants, \textit{Boerhaavia verticillata}, \textit{Urtica dioica}, \textit{Aerva lanata}, \textit{Spergularia purpurea}, \textit{Strychnos potatorum}, \textit{Helichrysum bracteatum}, \textit{Fabiana patagonica}, \textit{Cocculus hirsutus} have shown excellent diuretic activity.\cite{1, 2, 3} \textit{Centella asiatica} L. has been used as a medicinal herb for thousands of years in India, China, Srilanka and Nepal for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, hence primarily known as a "Brain food" in India.\cite{4, 5, 6} \textit{Centella asiatica} (Linn.) Synonym \textit{Hydrocotyle asiatica} Linn. commonly known as Indian Pennywort, belongs to the family Apiaceae (previously known as Umbelliferae).\cite{7, 8}

The present study deals with pharmacological evaluation of aqueous extract of leaves of \textit{Centella asiatica} for diuretic activity. This plant was selected on the basis of its traditional uses and thorough review of previous research work carried out on it.

5. MATERIALS AND METHODS
\textbf{Drugs and chemicals:} Petroleum ether, chloroform (Merck Specialities Pvt. Ltd. Delhi), sodium chloride (A. B. Enterprises, Mumbai), potassium chloride (J N Chemical, Gujarat), silver nitrate (Indian Platinum Pvt. Ltd), potassium chromate (Chmtex Speciality Limited, Kolkata) and furosemide (Sanofi India limited, Mumbai) etc.
**Plant material:** The fresh leaves of *Centella asiatica* belonging to the family Apiaceae, collected in month of January 2017 from the local areas of Azara, Guwahati, Assam, India. The plant was authenticated by Prof. P.P. Baruah, Department of Botany, Gauhati University. A voucher specimen (Acc-18232, Dated: 25/04/2017) was kept in Department of Botany, Gauhati University for future reference. The leaves were dried under shade for 3 weeks and the dried leaves were then ground into coarse powder using high capacity grinding machine and the grinded material was passed through sieve no. 40 and stored in air tight bottle, kept away from light, heat and moisture until use.\[9,10,11\]

The extraction process was carried out by maceration process. *Centella asiatica* leaves powder was soaked in the solvent for 48 hrs and filtered. The above filtrate collected and evaporated in a rotavapour at 40-50ºC under reduced pressure. A semisolid greenish material obtained was stored at 0-4ºC until used. The percentage yield was calculated and preliminary phytochemical tests were carried out for aqueous extracts of *Centella asiatica* as per the standard methods.\[11,12,13\]

**Animals:** Wistar albino rats weighing between 150-200gm each were used for this experiment. The animals were kept under standard conditions in the animal house. They were housed in polypropylene cages and maintained at 27±2ºC. The animals were given standard diet. The animal experiment was carried out in accordance with the guidelines of CPCSEA and was approved by the Institutional Animal Ethical Committee of Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowapara, Azara, Guwahati, Assam, India. [Approval No.: GIPS/IAEC/B.Ph/2017/17].

**Evaluation of diuretic activity:** The method of Lipschitz *et al.* was employed for the assessment of diuretic activity by using metabolic cage. According to this method, the animals should be deprived of food for 18 hours prior to the experiment. The animals were randomly divided into four groups of six animals each as follows:

- Group 1 (Normal) - given 5 ml/kg body weight of de-ionized water
- Group 2 (Standard) - received furosemide (10mg/kg, p.o.)
- Group 3 (Test) - received aqueous extract of *Centella asiatica* (250mg/kg, p.o.)
- Group 4 (Test) - received aqueous extract of *Centella asiatica* (500mg/kg, p.o.).\[14, 15, 16, 17\]

Before the oral administration of test drugs, the animals were dosed/hydrated with 25 ml/kg body weight of normal saline (0.9%). Immediately after test drug administration, animals were placed in metabolic cages specially designed to separate urine and faecal matter. During
the period of study no food, water was made available to the animals. The total volume of urine was collected and measured from control, standard and extract treated groups up to 18 hours of administration. The parameters monitored for individual group of rat were total urine volume and urine concentrations of Na⁺, K⁺ and Cl⁻. Concentration of Na⁺ and K⁺ were determined using flame photometer while Cl⁻ concentration was estimated titrimetrically using 0.02N AgNO₃ with 5% potassium chromate as an indicator, appearance of brick red precipitate was taken as the end point. The mean urine volumes were determined and diuretic potency was assessed by comparison of urine excretion due to extracts with respect to the standard drug furosemide.[14,15,16,17]

**Statistical analysis**

The data was expressed as mean ±SEM and statistically analysed using one way ANOVA followed by Dunnett’s test. The results were considered statistically significant when p< 0.05.

6. RESULT AND DISCUSSION

The % yield of the aqueous extract of *Centella asiatica* was found 10.71% and with greenish black appearance. The preliminary phytochemical screening revealed the presence of different phyto-constituents such as carbohydrates, proteins, tannins, glycosides, saponins and terpenoids etc.

The present study deals with pharmacological evaluation of aqueous extract of leaves of *Centella asiatica* for diuretic activity at doses of 250 mg/kg and 500 mg/kg body weight, per oral. The diuretic study was carried out as per the method of Lipsciz. et.al. The urine volume is expressed in ml and the electrolyte concentration was expressed in mEq/L. All the values are expressed as mean ± SEM.

The aqueous extract of leaves of *Centella asiatica* significantly (p<0.05) increased urine volume in rats at doses of 250mg/kg and 500 mg/kg body weight. The urinary excretion of sodium, potassium and chloride ions also increased when compared with normal group in a dose dependent manner.
TABLE-1: Diuretic activity of aqueous extract of leaves of *Centella asiatica*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Urine Volume (ml)</th>
<th>Electrolyte Concentration (mEq/L)</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25ml/kg</td>
<td>3.22 ± 0.221</td>
<td>45.62 ± 0.108</td>
<td>65.52 ± 0.152</td>
<td>55.52 ± 0.224</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>10mg/kg</td>
<td>11.32 ± 0.188**</td>
<td>68.50 ± 0.210**</td>
<td>94.81 ± 0.205**</td>
<td>80.21 ± 0.158**</td>
<td></td>
</tr>
<tr>
<td>Test – I</td>
<td>250mg/kg</td>
<td>5.61 ± 0.201*</td>
<td>55.62 ± 0.161*</td>
<td>75.52 ± 0.212*</td>
<td>65.63 ± 0.176*</td>
<td></td>
</tr>
<tr>
<td>Test – II</td>
<td>500mg/kg</td>
<td>9.44 ± 0.154**</td>
<td>60.57 ± 0.178**</td>
<td>80.67 ± 0.195**</td>
<td>78.59 ± 0.166**</td>
<td></td>
</tr>
</tbody>
</table>

* The data was expressed as mean ± SEM and statistically analysed using one way ANOVA followed by Dunnett’s test. The results were considered statistically significant when p < 0.05.

Figure-1: Effect of aqueous extract of leaves of *Centella asiatica* on urine volume.

Figure-2: Effect of aqueous extract of leaves of *Centella asiatica* on electrolyte concentration.
7. CONCLUSION
Present study shows that the aqueous extracts of leaves of Centella asiatica possess good diuretic activity. Hence from our overall investigation it can be concluded that aqueous extract of dried leaves of Centella asiatica has got remarkable potential to be used as a diuretic agent and it may be due to presence of terpenoids and saponins etc. but further studies are warranted to isolate the pure phyto-constituent responsible for diuretic activity.

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9. REFERENCES


