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ACUTE DIURETIC ACTIVITY OF ALCOHOLIC EXTRACTS OF HYGROPHILA AURICULATA SEEDS IN NORMAL WISTAR ALBINO RATS

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ABSTRACT

Objectives: The present study was undertaken to investigate the diuretic properties of the seeds of Hygrophila auriculata (Schum) (Acanthaceae) in normal Wistar Albino rats. Methods: Alcohol extracts of seeds of H. auriculata (300mg/kg and 500mg/kg p.o.) were investigated for diuretic activity by measuring the total urine output over 24hrs and electrolytes (Sodium, Potassium and Chloride) estimation in Wistar rats (n=6). Frusemide (20 mg/kg, p.o), a high ceiling diuretic served as positive control and normal saline (25 ml/kg, p.o) as placebo control. Results: The urine volume was 3.33 ±0.13ml, 9.12 ± 0.25 ml, 3.18 ±33 ± 0.15ml and 5.52 ±0.18 ml for control, frusemide, extract at 300mg/kg and 500 mg/kg dose respectively. There was significant increase in sodium (198.33 ± 2.99 m.mol/L), potassium (97.67 ± 2.33 m.mol/L) and chloride ion excretion (132.67 ± 2.65 m.mol/L) in the 500mg/kg alcohol extract as compared to control group (107 ± 2.11 m.mol/L, 55 ± 4.09 m.mol/L & 87.33 ± 2.33 m.mol/L respectively) (P < 0.001). Conclusion: The alcoholic extract of H.auriculata showed significant diuretic properties. At both the doses employed there was a significant increase in electrolyte excretions, however increase in urine volume was significant only at 500mg/kg. Further studies elucidating mechanism of action and employing lower doses are warranted.
KEY WORDS

diuretic index, Lipschitz method, saluretic index

INTRODUCTION

Man has been using herbs and plant products for its medicinal use since times immemorial. The indigenous systems of medicines, developed in India for centuries, make use of many medicinal herbs. However, it is imperative that the traditional systems should be scientifically supported for their efficacy and safety.

*Hygrophila auriculata* (Schum) (syn) *Hygrophila spinosa* T.And. *Asteracantha longifolia* Nees. (syn) Acanthaceae is described in ayurvedic literature as Ikshura, Ikshugandha and Kokilaksha “having eyes like the Kokila or Indian cuckoo”. This annual marshy herb occurs commonly in moist places throughout India from the Himalayas to Ceylon. The flowers bright blue and the roots creamy yellow in colour, possess a peculiar slimy odour and have limy cooling taste. It has always occupied a prominent place in Hindu Materia Medica. The roots are considered as cooling, diuretic, stimulating and especially efficacious in dropsical conditions and in cases of stone or gravel in kidney. The whole plant, roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain, urinary infections, oedema and gout. It is classified in ayurvedic system as seethaveeryam, mathuravipaka and used for the treatment of premeham (Diabetes), athisaram (Dysentry) etc.¹,²

Earlier studies have shown that the plant possesses antitumor ³,⁴ anti-nociceptive ⁵ antibacterial⁶,⁷ antioxidant⁸, hepatoprorective⁹-¹¹, hypoglycemic ¹² and haematinic ¹³ effects. However, literature survey revealed that no scientific studies were conducted investigating the diuretic activity on the seeds of *Hygrophila auriculata* to substantiate their therapeutic claim. Hence in the present study the alcoholic extract of the seeds of this plant were examined for its diuretic activity.

MATERIALS AND METHODS

Animals:

Adult male Wistar albino rats (150-200 g) from our breeding stock were used for the study. Rodents were housed in clean polypropylene cages, with dust free rice husk as a bedding material; three rats per cage; under controlled laboratory conditions. (Temperature: 25° ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle as per CPCSEA guidelines) for a period of 7 days prior to the study. They were fed standard rat chow and water *ad libitum*. The experimental procedures described were approved by the Institutional Animal Ethics Committee.

Plant extract:

Seeds of the plant were obtained from the local areas in and around Mangalore. The seeds were identified and authenticated as seeds of *H. auriculata* L. in the Dept. of Botany, St. Aloysius College, Mangalore and a specimen of the seeds was deposited in the Herbarium museum of college. The seeds were shade dried and ground to a fine powder in an electric grinder to give a coarse powder. Plant extract was then obtained using soxhlet’s apparatus using ethanol as the solvent. The obtained solvent extract was concentrated using rotary vacuum evaporator and dried in desiccators.
Drugs: Frusemide (Sanofi Aventis Co.) was used as a reference diuretic drug.

Acute toxicity study:
The test was carried out as suggested by Ganapaty et al. The Wistar albino rats, of either sex, weighing 100-200 g were divided into different groups comprising six animals each. The control group received normal saline (2 ml/kg, p.o.). The other groups received 100, 200, 300, 600, 800, 1000, 2000, 3000 and 4000 mg/kg of the test extract respectively. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. Thereafter, they were kept under observation up to 14 days after drug administration to find out mortality, if any.

Evaluation of Diuretic activity:
Each animal was placed in an individual metabolic cage 24h prior to commencement of the animal for adaptation. The method of Lipschitz et al, 1943 was employed for the assessment of diuretic activity. According to this method, male albino rats weighing between 120-150 g, deprived of food and water for 18 hours prior to the experiment, were divided in eight groups of six rats in each. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 5ml/100g body weight to impose a uniform water and salt load. The first group of animals, serving as control received normal saline (25 ml/kg, p.o.); the second group received frusemide (20 mg/kg, p.o.) in saline. (Frusemide, a high ceiling diuretic was taken as the positive control). The remaining groups received the test extract at doses, 300 mg/kg and 500mg/kg respectively. Doses of extract were based on acute toxicity studies.

Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faeces, kept at 20°C±0.5°C. At the end of 5 h the volume of urine collected was measured. During this period, no food and water was made available to the animals. The parameters noted include body weight before and after test period, total urine volume, and concentration of Na⁺, K⁺ and Cl⁻ in the urine. Na⁺ and K⁺ concentrations were determined by flame photometer and Cl⁻ concentrations were estimated by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator.

Statistical Analysis:
The results were expressed as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. A value of P < 0.05 was considered as statistically significant.

RESULTS

Acute toxicity tests:
The test extract showed no signs of acute toxicity as evidenced by the absence of mortality or visible adverse effects in the animals during the study period. No macroscopic alterations were noted in the viscera of the treated rats.

Effect on urine volume:
There was no evidence of dehydration and the animals were found normal at the observed 5hr and 24hr intervals. The reference diuretic frusemide, significantly increased the urine output when compared to control (P < 0.01), the diuretic index being 2.74. The test drug at 300mg/kg showed no increase in the urine volume when compared to the control. As the dose was increased to 500mg/kg, the extract however, showed significant diuresis as showed in table 1 (P < 0.01). The diuretic index of the test drug at 500mg/kg (1.66) was less than that of frusemide (2.74).
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Urine volume (ml/100g/24 hrs)</th>
<th>Diuretic index (24 hr interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.33 ±0.13</td>
<td>-</td>
</tr>
<tr>
<td>Frusemide</td>
<td>9.12 ± 0.25*</td>
<td>2.74</td>
</tr>
<tr>
<td>H.auriculata extract (300mg/kg)</td>
<td>3.1833 ± 0.15</td>
<td>0.95</td>
</tr>
<tr>
<td>H.auriculata extract (500mg/kg)</td>
<td>5.52 ±0.18*</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Values are expressed in mean±SEM; *P <0.01 compared with control group (ANOVA followed by Dunnett’s test) †Diuretic index = volume of test group/volume of control group

Effect on urinary electrolyte excretion:

As indicated in table 2, the test drug, when compared to the control group, showed a significant increase in the excretion of sodium, potassium and chloride excretion in dose dependent manner. These changes were also reflected in a dose dependent increase in the Na/K ratio. The increase in the electrolyte excretions was more than that of the standard drug, frusemide at 500mg/kg dose of the extract indicating a stronger saluretic effect than frusemide.

Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na⁺ m.mol/L</th>
<th>K⁺ m.mol/L</th>
<th>Cl⁻ m.mol/L</th>
<th>Na/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>107 ± 2.11</td>
<td>55 ± 4.09</td>
<td>87.33 ± 2.33</td>
<td></td>
</tr>
<tr>
<td>Frusemide</td>
<td>167.83 ±7.00**</td>
<td>92.5 ±2.06**</td>
<td>132.5 ±1.8**</td>
<td>1.57</td>
</tr>
<tr>
<td>H.auriculata extract (300mg/kg)</td>
<td>143.33 ±10.26*</td>
<td>95 ±1.75**</td>
<td>104.67 ±3.66*</td>
<td>1.34</td>
</tr>
<tr>
<td>H.auriculata extract (500mg/kg)</td>
<td>198.33 ± 2.99**</td>
<td>97.67 ± 2.33**</td>
<td>132.67± .65**</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Values are expressed in mean±SEM; **P <0.01 compared with control group (ANOVA followed by Dunnett’s test) ‡P < 0.001 compared to the control group
‡Saluretic index = Concentration of test group/concentration of control group

DISCUSSION

The present study revealed that ethanolic extract of H.auriculata seeds significantly increased the urinary output as well as urinary electrolyte concentration at 500mg/kg; however, there was no significant increase in urinary output at 300mg/kg of the extract. The increase in urine volume was moreover less compared to the frusemide treated rats. However, at
500mg/kg dose, the *H. auriculata* seed extract treated group showed an increase in Na\(^+\) & K\(^+\) concentrations which was more than that of frusemide treated group indicating that it has a better saluretic activity than frusemide. The absence of acute toxicity confirmed the safe nature of the ingestion of the seeds of this plant.

Diuresis has two components: increase in urine (water secretion) and a net loss of solutes (i.e. electrolytes) in the urine\(^{20}\). These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. The reference drug frusemide, increases urine output and urinary excretion of sodium by inhibiting Na\(^+\) K\(^+\)2Cl\(^-\) symporter (co-transport system) in the thick ascending loop of Henle\(^{20}\). The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles\(^{21}\). The regulation of Na\(^+\)/K\(^+\) balance is also intimately related to renal control of acid-base balance. The K\(^+\) loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended\(^{22}\).

In the present study, frusemide showed strong diuresis accompanied with high natriuresis, chloruresis, and kaliuresis (\(p < 0.01\)). Further there was low Na\(^+\)/K\(^+\) ratio, as it inhibits Na\(^+\) K\(^+\) and Cl\(^-\) co-transport at the thick ascending loop of Henle. K\(^+\) excretion was increased perhaps due to high Na\(^+\) load reaching the distal tube. However, *H. auriculata* seed extract (500mg/kg) induced both marked natriuresis and kaliuresis (\(p < 0.01\)), but the Na\(^+\)/K\(^+\) ratio was more than that of frusemide, indicating the weak kaliuresis or K\(^+\) saving property of the extract\(^{23}\). The above results raise the possibility of existence of diuretic activity by inhibiting tubular reabsorption of water and sodium ion. It is a good indicator for the efficacy of *H. auriculata* seed extracts as diuretics. These results are concordant with a previous study conducted on the whole plant extract\(^{24}\).

Chopra & co-workers investigated *H. auriculata* plant and isolated a basic amorphous residue which gave alkaloidal tests. Besides this they also isolated potassium salts and sugars. The diuretic and soothing properties of the plant are probably due to the potassium salts and large extent of mucilage present in the plant\(^2\). Studies revealing preliminary phytochemical screening of both aqueous and alcoholic extract and different fractions of alcoholic extract of the whole plant have revealed the presence of flavonoids, terpenoids and tannins as major phytoconstituents present in alcoholic extracts/fractions of the whole plant\(^{24}\). *H. auriculata* also contains various groups of phytoconstituents viz. phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, vitamins, glycosides etc\(^{25}\). Active phytprinciples such as flavonoids, saponins and terpinoids are known to be responsible for diuretic activity\(^{26-28}\). These active principles might also contribute to the diuretic activity of *H. auriculata* seed extract. Isolation of these active principles and study of their exact mechanism of action needs to be investigated.

**CONCLUSION**

The alcoholic extract of *H. auriculata* seeds showed significant diuretic properties. At both the doses employed there was a significant increase in electrolyte excretions, however increase in urine volume was significant only at 500mg/kg. Further studies elucidating mechanism of action and chronic dose studies are warranted.
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