PRELIMINARY STUDIES ON DIURETIC EFFECT OF **SPILANTHES ACMELLA** LEAVES EXTRACTS IN RATS

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**ABSTRACT**

Petroleum ether, chloroform and alcohol extracts of leaves of *Spilanthes acmella* were evaluated for its diuretic activity using modified method of Rao. The leaves were grouped into different groups of six animals each. All the animals received priming dose of 0.9% sodium chloride solution (20 ml/kg body weight p.o.). The first group of animals, served as control, received normal saline (20 ml/kg body weight p.o.); the second group received the standard drug frusemide (10 mg/kg body weight p.o.) in 0.9% sodium chloride solution and The other three groups received petroleum ether, chloroform, and alcohol extracts of *Spilanthes acmella* leaves in a dose of 500 mg/kg body weight suspended in 0.9% sodium chloride solution (p.o.). The urine volume was recorded for all the groups for 5h. and electrolyte concentration (Na+, K+ and Cl–) were measured. The extracts showed increase in total urine volume and electrolyte excretion (sodium Na+, potassium K+ and chloride Cl–). So, Out of the different extracts, the alcohol extract (500 mg/kg) significantly and markedly increased the urine output (p < 0.01). The pattern of diuresis induced by the alcohol extract was almost similar to that produced by the frusemide. These findings suggest the possible traditional use of this plant in hypertension as diuretics are used in the management of hypertension.

**Keywords:** *Spilanthes acmella*, Frusemide, Diuretic activity, Electrolyte excretion.

**INTRODUCTION**

Diuretic agents have very wide application in the treatment of various chronically diseases associated with edema. They are generally prescribed for the treatment of hypertension, congestive heart failure, glaucoma, diabetes insipidus and liver ailments. The modern era of diuretic therapy began in 1949 when sulphamamide was discovered to possess diuretic and natriuretic properties. (*Spilanthes acmella* is commonly known as akarkara, is used medicinally in Indochina, Philippine islands, Launenon and Madagascar; *Spilanthes acmella* introduced from Brazil and often cultivated in gardens in many part of India. Leaves are opposite, broadly ovate-lanceolate, 2.5-5 by 1.3-3.8cm, sub obtuse, irregularly crenate-serrate or sometime entire, glabrous or nearly so, base usually acute petioles 0.6-1.6cm long, pubescent. Trichomes present on both the surfaces. Upper surface is darkening than the lower one. Midribs prominent on lower surface. Stems are glandular and hairy with pungent taste. The whole plant is acid in taste. The leaves are used as immunomodulatory, adaptogenic, diuretic, tooth paste, lithotriptic, antiscorobic, saliagnostic, antibacterial, tonic and digestive. The leaves contain alkaloids, carbohydrates, pungent amide, tannins, steroids, carotenoids, prostatin A, α-carotene and β-carotene, essential oils, sesquiterpenes, and amino acids etc. Preliminary studies have reported as diuretic, antiinflammatory and analgesic, vasorelaxant and antioxidant. However, no systematic pharmacological studies have been carried out in order to confirm its diuretic activity. Hence, in the present study diuretic activity of petroleum ether, chloroform and alcohol extract of leaves of *Spilanthes acmella* was investigated to justify the rationale behind using this plant as diuretic in hypertension. The present investigation was undertaken to confirm traditional medicinal use of the plant.

**MATERIALS AND METHODS**

**Plant Material**

Leaves of *Spilanthes acmella* (Family-Compositae) collected from local areas of Hubli, Karnataka (India) and authenticated by Dr. Ganesh Hegde, Professor and Head, Dept. of Botany, Karnatak University, Dharwad, Karnataka and voucher specimen has been deposited at the herbarium for further reference.

**Processing of plant material**

Dried coarse powder (40-mesh) leaves (500g) of *Spilanthes acmella* was subjected to successive extraction in a soxhlet apparatus using petroleum ether (60-80°C), chloroform and alcohol. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. Filter it rapidly through whatman No. 1 filter paper. The extract was concentrated to ¾ of its original volume by rotary evaporator. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste and dried over a desiccator. The yield was 11.40% w/w, 5.32% w/w and 17.52% w/w for petroleum ether, chloroform, and alcohol extract, respectively were subjected to preliminary phytochemical analysis.

**Phytochemical investigation**

Qualitative Phytochemical tests were done by Harbone method for different extracts of *Spilanthes acmella* leaves to identify the various phytoconstituents. The results of preliminary phytochemical investigation are shown in Table 1.

**Drugs and chemicals**

All the drugs, chemicals, and reagents were procured from S.D. Fine Chemicals, (Mumbai, India). All the chemicals were of analytical grade.

**Acute toxicity studies**

Healthy albino mice of either sex weighing 25-30g maintained under controlled conditions of temperature (20–25°C) and humidity (55%) were used for toxicity study as per Up & Down or Staircase method. The maximum no-lethal and the minimum lethal dose are thus determined using only about 10 mice, once the approximate LD50 or the range between the maximum non-lethal and minimum lethal dose is found, a final and more reliable LD50 assay is planed using at least 3 or 4 dose levels within this range with longer number of animals in each group. LD50 is expressed in term of mg/kg. The maximum no-lethal dose was found to be 5000mg/kg body weight; hence 1/10th of the dose was taken as effective dose (500mg/kg body weight) for the different extracts of *Spilanthes acmella* leaves for diuretic activity.

**Evaluation of diuretic activity**

**Treatment**

Albino Wistar male rats (200-250g) procured from CPCSEA approved breeder (Reg. no. 126/1999/CPCSEA dated 29.6.1999)
were used for diuretic studies. Animals were kept at room
temperature (26 ± 2°C) for one week to acclimatize to laboratory
conditions before starting the experiment; they were given free
access to water and standard rat feed but 18 h prior to the
experiment, the rats were deprived of food but water ad libitum.

Diuretic activity

The modified method of Rao was employed for the assessment of
diuretic activity.19 Male healthy Wistar albino rats (200-250g) were
divided into different groups of six animals each. All the animals
received priming dose of 0.9% sodium chloride solution (20 ml/kg
body weight p.o.). The first group received vehicle saline (20 ml/kg
body weight p.o.), served as control; the second group received the
standard drug frusemide (10 mg/kg body weight p.o.), served as
standard. The other groups received doses of different extracts (500
mg/kg body weight p.o.), suspended in normal saline. After oral
administration, each animal was placed in an individual metabolic
cage specially designed to separate faeces and urine at room
temperature. The volume of urine collected was measured at the end
of 5 h and the total urine volume and concentrations of Na⁺, K⁺ and
Cl⁻ in the urine were determined. The concentration of the
electrolytes in urine were expressed in terms of mmol/L and the

Table 1: Preliminary phytochemical analysis of different extracts of Spilanthes acmella leaves

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Alcohol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* = Positive, ‐ = Negative

The results of different diuretic parameters are shown in Table 2.
Frusemide treated animals significantly (p < 0.01) increased the
urinary output (by 269%) and electrolyte excretion of Na⁺ (by
152%), K⁺ (by 185%) and Cl⁻ (by 136%) as compared to control.
Alcohol extract significantly (p < 0.01) increased the urinary output
(by 229%) and electrolytic excretion of Na⁺ (by 135%) and K⁺ (by
172%), without significant renal excretion of Cl⁻ as compared to
control. Chloroform extract also showed good diuretic action (p
<0.05). The diuretic action of petroleum ether extract was not
significant.

dThe observed Na⁺/K⁺ ratio for frusemide, chloroform extract and
alcohol extract were 1.41, 1.51 and 1.34 respectively, as compared to
1.70 for control. The present result shows significant diuretic
potency and their effect on electrolyte excretion of different extracts
of Spilanthes acmella comparable to the standard drug frusemide.

Table 2: Effect of oral administration of different extracts of Spilanthes acmella leaves on urinary volume and electrolyte excretion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Total Urine volume(ml)</th>
<th>Na⁺ (mmol/L)</th>
<th>K⁺ (mmol/L)</th>
<th>Cl⁻ (mmol/L)</th>
<th>Na⁺/K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20 ml/kg</td>
<td>5.2±0.48</td>
<td>80.32±3.71</td>
<td>47±2.92</td>
<td>107.78±6.62</td>
<td>1.70</td>
</tr>
<tr>
<td>Standard</td>
<td>10mg/kg</td>
<td>14.0±0.87**</td>
<td>122.83±4.63**</td>
<td>87±4.75**</td>
<td>147±6.13**</td>
<td>1.41</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>500mg/kg</td>
<td>4.9±0.61</td>
<td>72.10±5.21</td>
<td>52.89±3.74</td>
<td>109±6.81</td>
<td>1.36</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>500mg/kg</td>
<td>6.8±0.43 *</td>
<td>103.06±2.3 *</td>
<td>68.20±5.63 *</td>
<td>137±6.37 *</td>
<td>1.51</td>
</tr>
<tr>
<td>Alcohol extract</td>
<td>500mg/kg</td>
<td>11.9±0.71**</td>
<td>108.74±5.57**</td>
<td>81.0±5.46**</td>
<td>127.34±4.52**</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM [n = 6]; "p < 0.05 and **p<0.01 compared with control (ANOVA followed by Dunnett’s t-test).

DISCUSSION

The diuretic action of different extracts was evaluated using
frusemide which is a high-ceiling loop diuretic, under controlled
laboratory conditions. As diuretic therapy may lead to number of
life-threatening electrolytic disorders and toxicities, so safety profile
studies was carried out following a sub chronic administration of
extracts. Results showed that there was absence of mortality and
overt signs of toxicity.

This would amplify the heterogeneous array of diuretic curatives
available for safe and effective treatment of edema and cardiovascular
diseases.21 The results of the present study revealed

that alcohol extract induced diuresis was strong and accompanied
with high natriuresis, chloruresis, and kaliuresis (p < 0.01). Further
there was low Na⁺/K⁺ ratio, so the alcohol extract seem to be acting
like loop diuretics which inhibits Na⁺, K⁺ and Cl⁻ co-transport at thick
ascending loop of Henle. K⁺ excretion was increased perhaps due to
high Na⁺ load reaching the distal tube. However, chloroform extract
induced both marked natriuresis and kaliuresis (p < 0.05), but the
Na⁺/K⁺ ratio was more than that of frusemide, indicating the weak
kaliuresis or K⁺ saving property of chloroform extract.24 The
preliminary phytochemical analysis revealed that alkaloids,
carbohydrates, tannins, steroids, carotenoids, sesquiterpenes and
amino-acids are present in different extracts. These natural products
might be acting individually or synergistically to produce diuresis. It is also possible that the alcohol extract might manifest cumulative effect of several active principles in the extract. These findings suggest the possible traditional use of this plant in hypertension as diuretics are used in the management of hypertension.

CONCLUSION

In conclusion, different extracts of Spilanthes acmella have diuretic effect supporting the ethnopharmacological use as diuretics and our results have shown that the extracts administered at the dose of 500 mg/kg body weight (p.o.) have significant effects on urinary excretion of electrolytes and support the claims of diuretic efficacy of the title plant. The present study also provides basis for the traditional use of Spilanthes acmella in hypertension.

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