DIURETIC ACTIVITY OF EXTRACT OF
SALVIA OFFICINALIS L.

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Abstract
Salvia officinalis L. is a perennial woody shrub; mainly dried leaves used as raw material in medicine, perfumery and food industry. The aim of this study was to investigate diuretic effects of leaf extract of Salvia officinalis L. in normal rats. Methanolic extract of Salvia officinalis L. leaves were administered to experimental rats orally at doses of 50 and 100 mg/kg p.o., Hydrochlorothiazide (10 mg/kg) was used as positive control in study. The diuretic effect of the extract was evaluated by measuring urine volume & excretion of sodium-potassium content. Urine volume was significantly increased by methanolic extract in comparison to control group. While the excretion of sodium was also increased by extract (100mg/kg). The methanolic extract had the additional advantage of a potassium-conserving effect. We can conclude that methanolic extract of Salvia officinalis L. produced notable diuretic effect which appeared to be comparable to that produced by the reference diuretic HCTZ (Hydrochlorothiazide). The present study provides a quantitative basis for explaining the folkloric use of Salvia officinalis L. as a diuretic agent.

Keywords: Diuretic activity, Salvia officinalis L., Hydrochlorothiazide, medicinal plants.

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Received: 05/02/2011   Accepted: 04/03/2011
Introduction
Salvia officinalis L. called sage is a popular plant belongs to the family of Labiatae. The plant has an industrial interest [1, 2] and especially the Dalmatian sage Salvia officinalis L. which is considered to possess the finest and the most characteristic aroma [3]. More recent studies on the biological activity of sage showed that the plant possesses some antimicrobial and antioxidant properties. It has tonic stimulant properties and it is also used in perfumery, in cosmetics [4, 5] and for liquors. The literature conclude that the top of the aerial part contribute mainly to the biological activities like antioxidant property [6-8].

There is no report on the diuretic studies of the extract of dried leaves of Salvia officinalis L. so far, though it is used in folk medicine. Thus it was considered worthwhile to take up such an investigation in detail. The present study was therefore aimed to explore diuretic effects of methanolic extract of the leaves of Salvia officinalis L.

Materials and methods
Collection of leaves of the Salvia officinalis L. was done in the month of March. Taxonomic identification of the plant was done by Department of Botany, University of Pune. Leaves of the Salvia officinalis L. were dried in shade for 10-12 days. After complete drying, leaves were pulverized to a coarse powder of 40 mesh size in a mechanical grinder.

Extraction procedure
The leaves were powdered and then defatted with petroleum ether at 60-70°C. The powdered material was then air-dried and subjected to soxhlet extraction for 18 h at 50-55°C to obtain alcoholic extract of leaves of Salvia officinalis L. The extract was thereafter concentrated under vacuum and then air-dried [9-11].

Animals
Adult male Wistar rats, each in the weight range of 180 – 200 g, used. The animals were randomly allocated to four treatment groups of 6 animals each and kept in cages and housed under standard conditions of temperature, humidity and dark light cycle (12h – 12h).

Experimental protocol
Diuretic activity was determined by the methods of Kau et al [12], with minor modifications. The rats were randomly divided into four groups of six animals each as follows:
(1) Control – given 5 ml/kg body weight of de-ionized water;
(2) Methanolic extract of leaves of Salvia officinalis L. – 50 mg/kg body weight;
(3) Methanolic extract of leaves of Salvia officinalis L. –100 mg/kg body weight; and
(4) Hydrochlorothiazide (Ref. std.) – 10 mg/kg body weight.

In all cases, the volume of the dose was administered 5 ml/kg body weight. The animals were fasted overnight (18 h) prior to the test but with free access to water only, then were given an oral loading of normal saline (0.9%) of 0.05 ml per g body weight. Immediately after administration, the rats were paired and placed in metabolism cages. Urine was collected in a graduated cylinder and its volume was recorded at 2 h intervals for 8 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 gm of body weight. Electrolyte (Na+ and K+) concentrations estimated (as described below) from the urine sample of each pair of rats at the end of the experimental period (8 h.) and expressed as mequiv./100 g body weight [13, 14].

Measurement of urine output and analysis of electrolytes
Na+ and K+ concentrations were measured using a Toshniwal group model TCM-35 flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na+ and K+.

Statistical analysis
The results were expressed as mean values ± S.E.M. (standard error of mean) for pairs of rats.
Statistical comparison was carried out by analysis of variance (ANOVA).

**Results & discussion**

The results of the evaluations carried out on the extracts are listed in Tables 1 and 2. Table 1 shows the urinary volume (ml/100g/8h), while Table 2 shows the excretion of electrolytes (Na+ and K+ content, mequiv./100g/8 h.) in urine obtained from the rates of different treated groups.

**Urine volume**

Table 1 shows that the reference diuretic, HCTZ, increased urine volume by 54%. The extract also caused an increase in urine volume, for the methanolic extract, the increase in urine volume at doses level of 50 mg/kg body weight and 100 mg/kg body weight were 22 % (P < 0.01) and 44 % (P < 0.001) respectively [where, $F = 5.564732$] compared to the control group (Fig. 1).

**Electrolyte excretion**

Methanolic extract 100 mg/kg produced significant increase in Na+ excretion (P < 0.001) when compared to control group (Fig. 2). Only HCTZ (Ref. std.) produced significant increases in potassium excretion.

According to previous survey carried out the leaves of *Salvia officinalis* L. largely used for the treatment of hypertension and renal disease, but to the best of our knowledge, no previous pharmacological or clinical study has been carried out to test the diuretic activity of this plant. Methanolic extract of *Salvia officinalis* L. showed a dose-dependent increase in urine excretion, (100mg/kg) showed an increase of 44 % grouping urine volume. Thus the diuretic effect of extract indicated by increase in both water excretion and excretion of sodium ion. Methanolic extract (100mg/kg) showed significant result in excretion of water & sodium ion, which proved it strong diuretic agent, but active constitute responsible for the diuretic effect can not be concluded on the basis of this study. The preliminary phytochemical investigation revealed the presence of phytosterol, alkaloids in methanolic extract which can be responsible for diuretic activity but need to confirm by further study.

**Conclusion**

Methanolic extract of *Salvia officinalis* L. (100mg/kg) increased urine volume significantly and also potentiate excretion of Na+ in urine output, thus the results obtained in this study provides a quantiative basis to explain the traditional folkloric use of *Salvia officinalis* L. as a diuretic agent.

**Acknowledgement**

The authors are thankful to Dr. Raj Singh, Sr. Scientist, CAZRI, Jodhpur for providing plant material.

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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg b.w.)</th>
<th>Urine volume ± S.E.M. (ml/100gm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 (ml)</td>
<td>4.55 ± 0.13</td>
</tr>
<tr>
<td>HCTZ a</td>
<td>10</td>
<td>7.48 ± 0.18***</td>
</tr>
<tr>
<td><em>S. offic.</em> L. (Me OH) b</td>
<td>50</td>
<td>5.55 ± 0.13**</td>
</tr>
<tr>
<td><em>S. offic.</em> L. (Me OH) b</td>
<td>100</td>
<td>6.79 ± 0.14***</td>
</tr>
</tbody>
</table>

**p < 0.01 and ***p < 0.001 compared with the control group (ANOVA).**

a Hydrochlorothiazide

b Methanolic extract of *Salvia officinalis* L.

$F = 5.564732$
Table 2- Effect of methanolic extract of *Salvia officinalis* L. and HCTZ on sodium and potassium excretion in urine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg b.w.)</th>
<th>Sodium (meq./100g/8 hr) x10^-2</th>
<th>Potassium (meq./100g/8 hr) x10^-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 (ml)</td>
<td>44.16 ± 1.62</td>
<td>16.00 ± 1.37</td>
</tr>
<tr>
<td>HCTZ^a^</td>
<td>10</td>
<td>87.50 ± 1.22***</td>
<td>28.76 ± 1.65***</td>
</tr>
<tr>
<td><em>S. offic.</em> L. (Me OH)^b^</td>
<td>50</td>
<td>61.12 ± 1.27</td>
<td>16.83 ± 1.60</td>
</tr>
<tr>
<td><em>S. offic.</em> L. (Me OH)^b^</td>
<td>100</td>
<td>77.66 ± 1.76***</td>
<td>17.83 ± 1.07</td>
</tr>
</tbody>
</table>

**p < 0.01 and ***p < 0.001 compared with the control group. (ANOVA).**

^a^ Hydrochlorothiazide

^b^ Methanolic extract of *Salvia officinalis* L.

Fig. 1. Effect of methanolic extract of *Salvia officinalis* L. and HCTZ on urine volume.
Fig. 2. Effect of methanolic extract of *Salvia officinalis* L. and HCTZ on excretion of sodium ion in urine.

References: