DIURETIC AND ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF
AERVA SANGUINOLENTA (L.) BLUME.

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ABSTRACT
The study was designed to evaluate the diuretic and anti-inflammatory potency of aqueous extract of whole plant of Aerva sanguinolenta in wistar albino rats. Different parameters viz. total urine volume, urine concentration of electrolytes such as sodium; potassium and chloride have been evaluated for assessment of diuretic activity. Anti-inflammatory was performed against carrageenan induced paw oedema method by using indomethacin as standard. The results revealed that the aqueous extract showed significant diuretic activity at a dose of 400 mg/kg body weigh by increasing the total volume of urine and concentration of sodium, potassium and chloride ions in urine and also extract showed significant anti-inflammatory activity.

KEYWORDS: Diuretic, Anti-inflammatory, Carrageenan induced, Aerva sanguinolenta

INTRODUCTION
Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics like opioids or non-narcotics like salicylates and corticosteroids like hydrocortisone. All of these drugs present have well known side and toxic effects1. Diuretics are the drugs that increase the rate of urine flow, sodium excretion and used to adjust the volume and composition of body fluids in a variety of clinical situations. Most of the diuretic drugs have the adverse effect on quality of life including impotence, fatigue, and weakness2. The severe side effects of these drugs evoked us to search for new anti-inflammatory and diuretic agents from natural botanical sources which have minimal drawbacks.

Aerva sanguinolenta(L.)Blume a member of Amaranthaceae Family, It is an erect or rambling and perennial under shrub or herb found throughout India, ascending up to 1800 m in Himalayas. Traditionally, the whole plant is used as a tonic, sedative, and dermatitis3. The decoction made of young branches of the plant used internally against haematuria and irregular or painful menstruation. The roots used for dysentery and paste of the roots applied externally for headache4. Bhoxas tribals of dehradun, tie a twig of the plant on the neck of sick cattle’s with the belief that it may get relief 5. Leaves are made in to paste and applied externally for the treatment of cuts and wounds6. The ethanol extract of the whole plant reported to possess neuroleptic activity7. As per literature survey there is no systematic study regarding diuretic and anti-inflammatory activity of A. sanguinolenta. Hence the present study was undertaken to investigate diuretic and anti-inflammatory activity of aqueous extract of whole plant of A. sanguinolenta.

MATERIAL AND METHODS

Plant collection and authentication
The entire plant of Aerva sanguinolenta (2 kg) was collected from Ciddipet, near Warangal and authenticated by Prof. V. S. Raju, Taxonomist, Kakatiya University, Warangal. A voucher specimen was deposited in the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam. The dried material was coarsely powdered.

Preparation of extract
The aqueous extract was obtained by decoction from 500 gm of coarse powder with 3 litre of distilled water followed by concentration under vacuum, which yielded a brown-sticky mass (yield: 14.264 % w/w).

Test animals for diuretic and anti-inflammatory activity
Adult wistar albino rats of either sex weighing between 185 to 225 g (for diuretic and anti-inflammatory study) and adult Swiss albino mice of either sex weighing 25 to 33 g (for the gross behavioral and toxicity studies) were
used for the study. The selected animals were maintained under standard diet and water under laboratory conditions (35°C ± 2°C).

**Gross behavioral and toxicity studies**
The study was carried out as per OECD guidelines. The selected mice were divided into eight groups of six animals each. The control group received 2 ml/kg distilled water orally. The other groups received the extract, at dose levels of 100, 200, 400, 800, 1000, 2000 and 3000 mg/kg in distilled water through oral route. After administration of the dose the animals were observed continuously for first four hours for behavioral changes and for mortality if any at the end of 72 h.

**ASSESSMENT OF DIURETIC ACTIVITY**
The diuretic activity of *A. sanguinolenta* was evaluated by the method suggested by Lipschitz et al. 8, 9. The animals were kept under fasting conditions and deprived of water for 18 h prior to the commencement of experiment. On the day of the experiment, all the animals were administered orally, normal saline at a dose of 25 ml/kg. Group I served as control, which received only normal saline (25 ml/kg). Group II received furosemide (100 mg/kg) and served as reference. Group III, IV and V received the aqueous extract at dose levels of 100, 200 and 400 mg/kg respectively. Immediately after dosing, the animals were placed in metabolic cages separately and kept at room temperature (33 ± 1°C). The urine samples were collected up to 6 h after dosing. During this period the animals were deprived of food and water. The different parameters taken for each individual rat were body weight, total urine volume, concentration Na⁺, K⁺, and Cl⁻ in urine. Na⁺ and K⁺ concentrations were measured by flame photometry10 and Cl⁻ concentration was estimated titrimetrically11. The results were shown in table 1.

**ASSESSMENT OF ANTI-INFLAMMATORY ACTIVITY**
*Carrageenan* induced rat paw oedema
The anti-inflammatory activity of the extract was assessed in selected healthy adult wistar albino rats by carrageenan induced hind paw oedema method12, 13 using indomethacin as reference standard. The selected animals were divided in to five groups each consisting of six animals. Group 1 served as control received 0.5%w/v sodium CMC (2 ml/kg), Group 11 received indomethacin (10 mg/kg) and Group 111 to V received aqueous extract of *Aerva sanguinolenta* (100, 200 and 400 mg/kg) respectively. After 1 h of the administration of the extracts and drug, 0.1 ml of 1%w/v solution of carrageenan was injected in the sub planter region of left hind paw to all the groups. The paw volume was measured at 1 h, 2 h and 3 h respectively by using plethysmograph as the volume of mercury displaced by the inflamed paw. The percent inhibition of edema as calculated for each group with respect to its vehicle-treated control group. The percentage inhibition was calculated by using the formula,

\[
\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

where Vc and Vt denote mean increase in paw volume of control and drug-treated animals respectively. The observations were presented in Table 2.

**Statistical analysis**
All values are expressed as mean ± S.E.M. Significance of difference between control and treated groups was determined by using one-way ANOVA followed by Student’s t-test.

**RESULTS AND DISCUSSION**
From the gross behavioral and toxicity studies, it was observed that the aqueous extract at tested dose levels produced increased urination and marked analgesia. No mortality was observed with the animals even after observation for a period of 72 h.

The studies on diuretic activity revealed that the extract of *A. sanguinolenta* has considerably increased the urine volume with significant increase in the cationic concentration at each increased dose. The Na⁺/K⁺ ratio indicates a dose dependent response with comparable results at the dose of 100, 200 and 400 mg/kg with that of the reference standard drug administered.

The aqueous extract of *A. sanguinolenta* showed significant anti-inflammatory activity at all tested dose levels. The percentage inhibition of paw oedema was found to be dose-dependent. However the percentage protection was found to be almost equal at a dose of 200 mg/kg and 400 mg/kg, which concludes that a dose of 200 mg/kg may be considered as optimum dose as comparable with reference drug indomethacin. Thus the present study justifies its use in the indigenous system of medicine and folklore remedies as anti-inflammatory and diuretic agent. Further study needed to isolate the active principles responsible for these activities and study of their exact mechanism of actions.

**ACKNOWLEDGEMENT**
The authors express their gratitude to the Director, principal and the management of Vaagdevi College of Pharmacy, Hanamkonda for the facilities and encouragement. The authors are thankful to Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal, for authentication of the plant.

**REFERENCES**
Table 1: Diuretic activity of aqueous extract of *A. sanguinolenta* whole plant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Urine volume (ml)</th>
<th>Na (mEq/lit)</th>
<th>K (mEq/lit)</th>
<th>Cl (mEq/lit)</th>
<th>Na/K ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>25ml/kg</td>
<td>1.43 ± 0.05</td>
<td>90.16 ± 1.35</td>
<td>62.18 ± 1.18</td>
<td>91.1 ± 1.52</td>
<td>1.45</td>
</tr>
<tr>
<td>II</td>
<td>Furosemide</td>
<td>100mg/kg</td>
<td>2.6 ± 0.06</td>
<td>122.77 ± 1.53*</td>
<td>90.41 ± 1.06*</td>
<td>97.15 ± 2.23</td>
<td>1.36</td>
</tr>
<tr>
<td>III</td>
<td><em>A. sanguinolenta</em> aquatic extract</td>
<td>100mg/kg</td>
<td>2.3 ± 0.04</td>
<td>93.58 ± 1.37</td>
<td>71.57 ± 1.21*</td>
<td>97.31 ± 1.96</td>
<td>1.28</td>
</tr>
<tr>
<td>IV</td>
<td><em>A. sanguinolenta</em> aquatic extract</td>
<td>200mg/kg</td>
<td>2.81 ± 0.05</td>
<td>127.89 ± 1.56*</td>
<td>75.78 ± 2.58*</td>
<td>90.2 ± 1.31</td>
<td>1.66</td>
</tr>
<tr>
<td>V</td>
<td><em>A. sanguinolenta</em> aquatic extract</td>
<td>400mg/kg</td>
<td>2.86 ± 0.03</td>
<td>177.38 ± 2.23*</td>
<td>76.46 ± 2.62*</td>
<td>91.6 ± 1.28</td>
<td>2.29</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM from six observations. *P < 0.001

Table 2: Effect of aqueous extract of *A. sanguinolenta* whole plant on carrageenan induced paw oedema in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Mean paw volume (ml ± SEM)</th>
<th>% inhibition at the end of 3 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Control</td>
<td>2 ml/ kg</td>
<td>1.08 ± 0.027</td>
<td>1.58 ± 0.046</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10 mg/kg</td>
<td>1.078 ± 0.024</td>
<td>1.026 ± 0.08</td>
</tr>
<tr>
<td><em>A. sanguinolenta</em> aq. extract</td>
<td>100 mg/kg</td>
<td>1.08 ± 0.03</td>
<td>1.16 ± 0.03</td>
</tr>
<tr>
<td><em>A. sanguinolenta</em> aq. extract</td>
<td>200 mg/kg</td>
<td>1.08 ± 0.012</td>
<td>1.04 ± 0.006</td>
</tr>
<tr>
<td><em>A. sanguinolenta</em> aq. extract</td>
<td>400 mg/kg</td>
<td>1.13 ± 0.04</td>
<td>1.068 ± 0.017</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM from six observations. *P < 0.001

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