Phytopharmacology 2012, 3(1) 145-149

Diuretic activity of *Ageratum conyzoides* extract in rats

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Received: 18 March 2012, Revised: 1st April 2012, Accepted: 1st April 2012

Abstract

The diuretic effect of the aqueous extract of the leaves of *Ageratum conyzoides* was evaluated in albino Wistar rats. Thirty rats in six groups were used for the study. Aqueous extract at three dose levels (200, 400 and 600mg/kg), Frusemide and Acetazolamide were used as standard drugs while distilled water was used for control. Parameters studied included total urine volume after 24 hours, pH and urine concentration of sodium, potassium and chloride ions. We found LD$_{50}$ to be greater than 5000mg/kg. The leaf extract exhibited significant ($P<0.01$) diuretic activity. At the end of 24 hours, 200, 400 and 600mg/kg of extract produced 7.60±0.68, 7.00±0.45 and 6.80±0.49ml of urine respectively. The pH of urine produced by the extract and standard drugs were all alkaline. At a dose of 600mg/kg body weight, there was significant ($P<0.01$) increase in concentrations of sodium, potassium and chloride ions. This could be important in the treatment of cardiovascular diseases such as hypertension. The results suggested that the leaf extract of *A. conyzoides* has diuretic properties similar to Acetazolamide.

Keywords: *Ageratum conyzoides*; diuretic; urine electrolytes

Introduction

*Ageratum conyzoides* is a tropical plant that is common in West Africa and some parts of Asia as well as Brazil (Shirwalkar *et al.*, 2003). It is an annual herb which grows erectly to approximately 1 meter in height and producing flowers of purple, pink to white, less than 6mm in diameter. About 30 to 50 flowers are arranged in close terminal inflorescences. The stems and leaves are curved and covered with fine white hair with the leaves being ovate and up to 7.5cm long. The plant has photoblastic easily dispersible seeds which are ovate, about 7.5cm long and lost in about 12 months. It grows abundantly in both Africa and Australian continents during wet season. The plant grows well in garden soils near to places of habitation as well as in waste places, abandoned farmlands or ruined sites. The plant easily adapts to different ecological conditions and has great morphological variation. It has a particular odour likened to an Australian male goat from where it got the name ‘goat weed’ or ‘billy goat weed’ (Okunade, 2002).
The plant has a long history of tradomedical uses in several countries particularly in many countries in Africa, Asia, and South America for the treatment of various diseases. Included in its folkloric use is its use as a purgative, febrifuge, for ophthalmia, colic, treatment of ulcers and wound dressing (Ming 1999, Okunade 2002, Kamboj and Saluja 2008). Similarly, the leaves are used in Nigeria for a number of ailments. For instance, in the eastern states of Nigeria, the pressed extract of the leaves is mainly employed to arrest bleeding from fresh cuts, and for wound dressing (Akah 1988). Amongst the Igede people of Benue State Nigeria, it is used for a variety of disease conditions such as skin disease, diarrhea, ear ache and infertility (Igoli et al 2005). There is the reported use in Brazilian folklore for the treatment of various ailments such as fevers, dermatitis, inflammation, rheumatism, and diarrhea and as a diuretic (Moura et al 2005). In this study the effect on urine volume, pH and urine electrolytes were used to evaluate diuretic activity of the leaves of Ageratum conyzoides.

Materials and Methods

Plant material

The leaves of Ageratum conyzoides were collected at Onyagede village of Ohimini Local Government Area of Benue State, Nigeria (August, 2011) and was identified by Mr. Joseph Jeffrey Azila of the Federal College of Forestry, Jos, Nigeria.

Preparation of extract

The leaves were left to air dry at room temperature and then grounded into a dried coarse powder using wooden mortar and pestle. The grounded powder was weighed and extracted in water using soxhlet apparatus giving a yield of 9.75% of dark brown residue which was stored in a screw-capped container away from moisture and kept in the refrigerator to be used when required.

Animals

Rats (Wistar albino strain) weighing between 140 and 200g of either sex were used in the study. The rats were obtained from the animal house unit of the University of Jos, Nigeria. Animals were maintained under standard environmental conditions of temperature and humidity and allowed access to food and water ad libitum. Experiments on the animals were performed strictly in accordance with standard guidelines and The Institutional Animal Ethics Committee approved all procedures.

Acute toxicity

Acute toxicity was ascertained according to the method of Lorke (1983). The animals were observed for 24 hours for effect of toxicity and the number dead in each group within the period was noted.

Diuretic activity

Lipschitz et al. (1943) method was employed for the evaluation of diuretic activity. Test rats were randomly divided in 6 groups (Five in each). Frusemide and Acetazolamide
and aqueous extract of *A. conyzoides* at 3 dose levels (200mg/kg, 400mg/kg, and 600mg/kg) were used. The rats were allowed to acclimatize to the experimental conditions and fasted for 18 hours prior to the day of the experiment. On the day of experiment, animals were divided in five groups, which are, Group I: distilled water as a test control at 2ml. Group II: Frusemide at a dose of 5 mg/kg body weight. Group III: Acetazolamide at a dose of 5 mg/kg body weight. Group IV: *A. conyzoides* extract (200mg/kg body weight). Group V: *A. conyzoides* extract (400mg/kg body weight). Group VI: *A. conyzoides* extract (600mg/kg body weight).

All drugs and extract were administrated orally. Immediately after administration, the rats were kept in different metabolic cages. These are cages that are able to collect the urine in a graduated cylinder without faecal contamination. After twenty four hours, the volume of urine in each group was noted, pooled and analyzed for sodium, potassium and chloride ions.

**Statistical analysis**

Experimental data were expressed as Mean ± Standard Error of Mean. Statistical difference were analyzed using student’s *t*-test where *P*<0.01 was considered significant. SPSS version 15 software was used.

**Results**

Acute Toxicity results showed that the LD₅₀ was greater than 5000mg/kg. The urine volume, colour, pH and concentration of electrolytes are presented in Table 1 and table 2.

### Table 1. Effect of aqueous leaf extract of *Ageratum conyzoides* on Colour, Total Urine Volume and pH.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Colour</th>
<th>Total Urine Volume ml/24hrs</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Amber &amp; Cloudy</td>
<td>11.80±0.37</td>
<td>9.53</td>
</tr>
<tr>
<td>Frusemide (5mg/kg)</td>
<td>Pale Amber &amp; Turbid</td>
<td>39.80±0.58**</td>
<td>8.74</td>
</tr>
<tr>
<td>Acetazolamide (5mg/kg)</td>
<td>Amber &amp; Cloudy</td>
<td>4.20±0.66**</td>
<td>9.63</td>
</tr>
<tr>
<td>Extract (200mg/kg)</td>
<td>Amber &amp; Cloudy</td>
<td>7.60±0.68**</td>
<td>9.46</td>
</tr>
<tr>
<td>Extract (400mg/kg)</td>
<td>Amber &amp; Cloudy</td>
<td>7.00±0.45**</td>
<td>9.45</td>
</tr>
<tr>
<td>Extract (600mg/kg)</td>
<td>Deep Amber &amp; Turbid</td>
<td>6.80±0.49**</td>
<td>7.15</td>
</tr>
</tbody>
</table>

Values are represented as Mean± Standard Error of Mean

n = Number of Animals = 5

** P<0.01 Significant difference between standard drugs, leave extracts and control groups

### Table 2. Effect of aqueous leaf extract of *Ageratum conyzoides* on urine electrolytes.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Na⁺ mmol/L</th>
<th>K⁺ mmol/L</th>
<th>CL⁻ mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.4±0.36</td>
<td>97.64±0.26</td>
<td>86.0±0.45</td>
</tr>
<tr>
<td>Frusemide (5mg/kg)</td>
<td>61.6±0.75</td>
<td>106.00±1.14</td>
<td>193.6±1.08*</td>
</tr>
<tr>
<td>Acetazolamide (5mg/kg)</td>
<td>149.2±1.02**</td>
<td>215.00±1.00**</td>
<td>250.20±0.86**</td>
</tr>
<tr>
<td>Extract (200mg/kg)</td>
<td>90.8±0.11*</td>
<td>275.20±1.28**</td>
<td>214.00±1.64**</td>
</tr>
<tr>
<td>Extract (400mg/kg)</td>
<td>221.2±0.8**</td>
<td>298.60±1.03**</td>
<td>272.20±1.02**</td>
</tr>
<tr>
<td>Extract (600mg/kg)</td>
<td>261.8±0.97**</td>
<td>298.40±1.17**</td>
<td>270.20±0.86**</td>
</tr>
</tbody>
</table>

Values are represented as Mean± Standard Error of Mean

n = Number of Animals = 5

** P<0.01 - Significant difference between standard drugs, leave extracts and control groups.
Discussion

The passage of urine is a desirable physiological process that helps the removal of waste/undesirable product from the body. In certain disease conditions like Congestion Heart Failure, nephritic syndrome, cirrhosis, renal failure, hypertension and pregnancy toxaemia; there are needs to increase the volume of urine production and a diuretic agent is used to achieve this (Agunu et al., 2005). Some herbal remedies have been found useful from time immemorial for the management of such disease conditions.

The term diuresis has two separate connotations: a reference to the increase in urine volume over time and also loss of solutes (electrolytes) and water (Irwin, 1990). These are mechanisms that are involved in suppression of renal tubular reabsorption of electrolytes.

The results showed that leaves of *A. conyzoides* have diuretic activity as observed at the end of 24 hours. As for the cumulative urine output the loop diuretic agent (Frusemide) induce the highest volume of urine at 39.80±0.58ml than that of the extract which produced 7.60±0.68ml at a dose of 200mg/kg body weight, 7.00±0.45ml at a dose of 400mg/kg and 6.80±0.49ml at a dose of 600mg/kg body weight (Table 1). Acetazolamide (a carbonic acid inhibitor) had the lowest urine output of 4.20±0.66ml, but it was also observed that it had the highest pH of 9.63.

When water re-absorption is reduced, there is increase in urine volume and the pH becomes more alkaline. This is evident in all groups. As stated by Nosiri et al (2011), this is aid to be accompanied by increases urinary concentration of *HCO₃⁻*, *Na⁺* and *K⁺* ions. The increase in *Na⁺* and *K⁺* ions is particularly noted with the acetazolamide and various doses of the extract.

The control of plasma sodium is important in regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscle. The regulation of *Na⁺*, *K⁺*, *Cl⁻* is intimately related to renal control of acid-base balance as well as the regulation of blood volume and pressure (Sharma and Verma 2011). This is important in the treatment of hypertensive disorders and even as the diuretic effect which has been demonstrated is required, particular attention must be paid to these other issues. This is more so as the potassium loss and other life threatening electrolyte disorders and toxicities that occurs with many diuretics (Meera et al 2009) may lead to hypokalemia for which potassium-sparing diuretics are recommended.

Table 2 showed that the extract demonstrated a significant (*P* < 0.01) increase in levels of *Na⁺*, *K⁺* and *Cl⁻* than that of the standard drugs and control. It can also be said that the effect of administration is dose dependent; and *K⁺* excretion was above the normal range of 40 – 90 mmol/L. This can cause hypokalemia and associated with kidney failure.

The leave extract of *A. conyzoides* can be said to have similar mechanism of action as acetazolamide. However based on effects on urine pH and concentrations of the different ions in the urine, this mechanism of action may be different from that of Frusemide. The presence of alkaloids, saponins and flavonoids as reported in literature (Kamboj and Saluja 2008) may likely be contributory to the diuretic effect. We conclude that the aqueous leave extract of *A. conyzoides* increased urine volume slightly and acts similarly to that produced
by carbonic anhydrase inhibitor (Acetazolamide) which supports its ethnopharmacological use as a diuretic.

Acknowledgement

We appreciate the technical assistance of the staff of the Animal House Unit, University of Jos.

Conflict of interest

The authors declare no conflict of competing interest.

References


