DIURETIC EFFECTS OF ABUTILON INDICUM (LINN.) LEAVES IN RATS

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ABSTRACT
The present study was aimed to investigate the diuretic effects of aqueous and ethanol extracts of Abutilon indicum (Linn.) leaves (Family - Malvaceae) using an acute model in Wistar albino rats. A single individual dose of aqueous and ethanol extract of A. indicum leaves (200 mg/kg and 400 mg/kg, p.o., each) and furosemide (25 mg/kg, p.o.) as reference diuretic drug were administered orally to dehydrated rats. Control group rats were fed with normal saline (25 ml/kg, p.o.). The parameters measured for diuretic activity were total urine volume and urine concentration electrolytes such as sodium, potassium and chloride. The rats treated with aqueous and ethanol extracts of A. indicum leaves in a dose of 200 and 400 mg/kg showed higher urine output when compared to the control group. Aqueous extracts of A. indicum leaves showed a significant (P<0.001) increase in the excretion of electrolytes when compared to the control group. This study demonstrates diuretic action of extracts of A. indicum leaves and supports its folklore use in various urinary ailments.

INTRODUCTION
Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug induced diuresis is beneficial in many life threatening disease conditions such as, congestive heart failure, hypertension, nephritic syndrome, cirrhosis, and renal failure. Commonly used diuretics, like thiazides and furosemide proved to be very effective in promoting sodium excretion, but these have been associated with many adverse effects, like electrolytes imbalance, acid-base and water imbalance, hyperuricemia, carbohydrate, lipid metabolism and drug interactions. Therefore, there is a need to look for safer diuretics. So, herbal diuretics can be considered as better therapeutic option, because of their synergistic and/or side effects neutralizing potential as compared to diuretics used now-a-days which produce several adverse effects due to their strong saluretic effects.

Abutilon indicum (Linn.) Sweet (Family - Malvaceae), is commonly called as Country Mallow (English), Kanghi (Hindi) and Atibala (Sanskrit). It is a hairy herb or under-shrub 1.0 - 1.5 m high, annual or more often perennial with golden yellow flowers, flowering mostly throughout the year found abundantly throughout the hotter parts of India, as a common weed on road sides and other waste places in plains and hills, upto an elevation of 600 m. The plant is used as drug in Ayurvedic and Unani medicines as febrifuge, anthelmintic, demulcent, diuretic, anti-inflammatory (in urinary and uterine discharges, piles, lumbago). In addition to above traditional uses, various pharmacological studies have revealed that A. indicum has hepatoprotective, hypoglycemic, analgesic, anti-inflammatory, anticonvulsant, and anti-ulcer effects. The present study is an attempt to evaluate efficacy of the leaves of this indigenous herb in its different concentrations for diuretic activity.
MATERIAL AND METHODS

Chemicals and Drugs
All the chemicals and reagents were procured from S. D. Fine Chemicals, Mumbai, India. All the chemicals were of analytical grade. Reference diuretic drug furosemide (Lasix) was procured from the local market.

Collection of Plant Material
Leaves of *Abutilon indicum* (Linn.) were collected from the field area of Boranada, Jodhpur, Rajasthan. Taxonomical identification and authentication of the plant was done at Botanical Survey of India, Arid Zone Regional Center, Jodhpur, Rajasthan. A voucher specimen was deposited in the herbarium for future reference with No. LMC/PM/RCK-001.

Preparation of Extracts
After authentication, fresh leaves were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The powdered leaves (125 g) were extracted successively with 1 litre each of petroleum ether (40-60° C), ethanol and water for 72 hours in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them. On the basis of various phytochemical tests, aqueous (AIAE) and ethanolic (AIEE) extracts were used for the study of diuretic activities in rats. Both AIAE and AIEE were found to be non-toxic upto 4 g/kg and 3 g/kg body weight respectively, in studies performed for determining LD<sub>50</sub>.

Animals Used
Healthy adult male Albino rats of Wistar strain weighing 150 - 200 g were obtained from the Animal House of Lachoo Memorial College of Science & Technology, Pharmacy Wing, Jodhpur. The animal house was well ventilated and animals had 12 h day and night schedule with temperature between 27 ± 1° C. The animals were housed in standard polypropylene hygienic cages (three animals per cage). The animals were fed with standard rat pellets and water *ad libitum*. The current work was carried out after approval by Institutional Animal Ethical Committee (IAEC), Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur [Reg.No.541/02/C/CPCSEA].

Diuretic Activity
The method of Lipschitz was employed for the assessment of diuretic activity. In this method, male albino rats weighing between 150 - 200 g deprived of food and water for 15 h prior to the experiment, were divided into six groups of six rats in each. The Group - I of animals serving as control, was administered normal saline (25 ml/kg, p.o), the Group - II was administered furosemide (25 mg/kg, p.o.) in normal saline; Groups - III, IV, V and VI were administered different extracts separately at doses of 200 mg/kg and 400 mg/kg in a similar manner. Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faeces, kept at 27° ± 2° C. The volume of urine collected was measured at the end of 5 and 24 h; results are shown in Table 1. During this period, no food and water was made available to the animals. The parameters taken were the total urine volume and concentration of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> in the urine. Na<sup>+</sup> and K<sup>+</sup> concentrations were determined by flame photometer (Elico CL-361) and Cl<sup>-</sup> concentration was estimated by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator. The results are depicted in Table 2. The various indices studied for diuretic actions were as follows:

Diuretic index = Urine volume of test group / Urine volume of control group;
Saluretic index = Urinary excretion of electrolyte of test group / Urinary excretion of electrolyte of control group;
Natriuretic index = Urinary excretion of Na<sup>+</sup> / Urinary excretion of K<sup>+</sup>.

Statistical Analysis
Results are expressed as the mean ± SEM. Data obtained in the studies were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A value of *P*<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening
The results of the preliminary phytochemical screening of different extracts revealed the presence of alkaloids, steroids, terpenoids, flavonoids, glycosides, tannins, saponins and sugars in extracts of *A. indicum* leaves.

Diuretic Activity
Diuresis occurs mainly by two phenomena; net increase in urine volume (water excretion) and elevated excretion of electrolytes (solutes) in
the urine. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. The thiazide diuretics inhibit Na⁺/Cl⁻ symporter (co-transporter system) in the distal convoluted tubule by competing for the Cl⁻ binding site and thereby increasing the excretion of Na⁺ and Cl⁻, while the loop diuretic drug, furosemide, increases the urine output and urinary excretion of Na⁺ by inhibiting Na⁺/K⁺/Cl⁻ symporter in the thick ascending limb of Loop of Henle⁵. In the present study, 25 mg/kg dose of reference drug, furosemide, showed significant diuresis of more than 100%, when compared to the untreated control group over a period of 24 h in rats. In comparison, similar increase in urine volume excretion was found with the AIAE and AIEE in the dose (400 mg/kg), when administered orally in dehydrated rats (Table 1). A gradual onset of diuretic action was observed with AIAE (400 mg/kg) within first 5 h comparable to the reference diuretic drug. This effect lasted up to 24 h showing significant increase in urine excretion volume (Fig. 1). The intensity of diuresis induced by AIAE (400 mg/kg) in 24 h was almost similar to that of reference drug, furosemide and significant (P<0.001) in comparison to control group (Table 1).

An increase in the urinary excretion of electrolytes (Na⁺, K⁺ and Cl⁻) exhibited by the furosemide over 24 h period, was found significant (P<0.001) in comparison with the control group (Table 2). AIAE (400 mg/kg) also showed significant (P<0.001) increase in urinary excretion of Na⁺, K⁺ and Cl⁻ in comparison with the control group (Table 2). The results obtained on electrolytic excretion by both extracts of A. indicum suggest a difference in their diuretic actions (Table 2). The increase in urine volume along with marked increase in urinary Na⁺, K⁺ and Cl⁻ levels by the AIAE (400 mg/kg) were similar to that of furosemide. The diuretic actions of AIAE were similar to a loop diuretic, mainly due to its higher natriuretic, kaliuretic and saluretic actions⁵,²⁴-²⁵.

Loop diuretics are clinically used in patients with salt and water overload due to host of conditions. The observed mode of action of AIAE and AIEE in the present study, suggests that A. indicum leaves may be used as a non toxic natural therapeutic agent in the treatment of conditions such as pulmonary oedema, cardiac oedema and hypertension.

**CONCLUSION**

The findings from present study support the folklore use of A. indicum for its diuretic actions. The study demonstrated that the aqueous and ethanol extracts of A. indicum significantly increased the urinary output as well as urinary electrolyte concentrations (Na⁺, K⁺ and Cl⁻) at a dose of 200 and 400 mg/kg, p.o. in rats. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extracts increased sodium ion excretion to a greater extent than potassium, which is a very essential requirement of an ideal diuretic. Presence of phytoconstituents like flavonoids, terpenoids, saponins have been previously found to be responsible for diuretic activity in plants⁶-²⁷. The presence of the said constituents in different extracts of A. indicum may be responsible for the observed diuretic activity. Results obtained in the study suggest the use of A. indicum as an appealing alternative to presently available diuretic drugs. The exact mechanism exhibited by the extracts can only be established after further investigation.

**ACKNOWLEDGEMENT**

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**Table 1: Effect of Abutilon indicum leaves extracts on urine excretion volume**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg, p.o.)</th>
<th>5 h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine Excretion (ml/100 g)</td>
<td>Diuretic Index</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.29 ± 0.02</td>
<td>1.00</td>
</tr>
<tr>
<td>Furosemide</td>
<td>25</td>
<td>0.68 ± 0.04***</td>
<td>2.21</td>
</tr>
<tr>
<td>AIAE</td>
<td>200</td>
<td>0.55 ± 0.02*</td>
<td>1.70</td>
</tr>
<tr>
<td>AIEE</td>
<td>400</td>
<td>0.64 ± 0.06**</td>
<td>2.09</td>
</tr>
<tr>
<td>AIAE</td>
<td>200</td>
<td>0.45 ± 0.05</td>
<td>1.55</td>
</tr>
<tr>
<td>AIEE</td>
<td>400</td>
<td>0.56 ± 0.04*</td>
<td>1.77</td>
</tr>
</tbody>
</table>

n (number of pairs in each group) = 3; Values are mean±SEM; *P<0.05, **P<0.01, ***P<0.001, vs Control (Saline 25ml/kg, p.o.); one-way analysis of variance (ANOVA) followed by Dunnett’s t-test
Table 2: Effect of *Abutilon indicum* leaves extracts on urinary excretion of electrolytes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Concentration of ions (mEq/l/100 g)†</th>
<th>Saluretic index</th>
<th>Natriuretic index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na+</td>
<td>K+</td>
<td>Cl−</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>2.84 ± 0.08</td>
<td>1.07 ± 0.02</td>
<td>3.74 ± 0.04</td>
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<tr>
<td>Furosemide</td>
<td>25</td>
<td>5.56 ± 0.09***</td>
<td>1.45 ± 0.07***</td>
<td>5.62 ± 0.07***</td>
</tr>
<tr>
<td>AIAE</td>
<td>200</td>
<td>4.30 ± 0.10***</td>
<td>1.11 ± 0.03</td>
<td>4.10 ± 0.03**</td>
</tr>
<tr>
<td>AIAE</td>
<td>400</td>
<td>5.33 ± 0.14***</td>
<td>1.18 ± 0.05**</td>
<td>5.14 ± 0.05***</td>
</tr>
<tr>
<td>AIEE</td>
<td>200</td>
<td>3.41 ± 0.07*</td>
<td>1.11 ± 0.02</td>
<td>3.82 ± 0.03</td>
</tr>
<tr>
<td>AIEE</td>
<td>400</td>
<td>3.83 ± 0.05***</td>
<td>1.16 ± 0.03</td>
<td>3.88 ± 0.04</td>
</tr>
</tbody>
</table>

n (number of pairs in each group) = 3; Values are mean ± SEM; *P<0.05, **P<0.01, ***P<0.001, vs Control (Saline 25 ml/kg, p.o.); one-way analysis of variance (ANOVA) followed by Dunnett’s t-test; † Urine collected for 24 h after treatment

Figure 1: Cumulative urine output in control, reference and experimental groups. Histograms showing cumulative urine excretion (ml/100 g) in control, reference and experimental groups over 24 h. All values are expressed as mean ± SEM at 5 and 24 h intervals of urine collection; *P<0.05, **P<0.01, ***P<0.001, vs Control (Saline 25 ml/kg, p.o.); one-way analysis of variance (ANOVA) followed by Dunnett’s t-test

REFERENCES


