POVONIA ODARATA (LINN) CONFERS DIURETIC EFFECTS ON ALBINO RATS.

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Abstract:
Diuretics induce negative fluid balance and are useful in the treatment of diseases like edema and hypertension. In the present study ethanolic and aqueous extracts of Pavonia odorata was evaluated for diuretic activity of in male albino rats. Preliminary phytochemical studies carried out indicated the presence of flavonoids, saponins, glycosides, tannins, phenols and carbohydrates in the extracts of Pavonia odorata. Acute toxicity studies of the ethanolic and aqueous extract of the Pavonia odorata did not exhibit any signs of toxicity up to 2 g/kg body weight. Since there was no mortality observed at a higher dose, 100 and 200 mg/kg doses were selected for evaluation of diuretic activity. The diuretic activity of the extract was screened by quantification of urine volume and electrolyte concentration. Different doses of Pavonia odorata (100 mg/kg and 200 mg/kg) were administered orally to hydrated rats and the urine output was measured every hour, up to 3 hours. Frusemide (20 mg/kg) was used as standard drug, while normal saline (10ml/kg) was used as control. The treatment of Pavonia odorata at varying doses (100 mg/kg and 200 mg/kg) aqueous and alcoholic extract significantly (P>0.001) increases the urine output (68%, 81%) and (73%, 88%) the excretion of Na+ (90%, 93%) and (92%, 95%). Furthermore, a potassium-sparing effect at (36%,39%) and (40%,43%) was observed. Based on the observations it can be concluded that Pavonia odorata extracts exhibits diuretic property in a dose-dependent manner.

Key Words: Diuretic, Pavonia odorata, Frusemide.

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INTRODUCTION:
Diuretic agents are most widely prescribed categories of drugs in the world [1]. But synthetic diuretics have serious side effects such as diabetogenic effect, electrolyte imbalance, impotence and hyperuricemia [2,3]. Hence the searches for herbal drugs to act as diuretics are inevitable and can alter therapeutic efficacy without any adverse effects [4-6]. Diuretic plants induce the obstructive increase of the blood pressure thanks to the alkaloids [7,8]. An increase of blood pressure is a beneficial factor for the sportsman because a big number of molecules of oxygen and nutriments will be transported quickly toward the organs and the muscles requested by the movements [9]. Diuretics play an important role in the management of oedema and hypertension. This function is mainly an increase in net negative siddha are predominantly based on the use of plant materials[10]. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness [11]. Huge number of medicinal plants mentioned in ayurvedic system of medicine are known to possess diuretic properties such as Achyranthus aspera, Boerhavia diffusa, Anisochilus carnosus, Bixa orellana, Costus speciosus, Xanthium strumarium, Kigella pinnata, Bacopa monnieri, Barbara vulgaris, Abelmoschus esculentus, Steganotaemia araliacea, Benincasa hispida, Morena citrifolia[12,13,14]. Phytochemical screening of this plant revealed the presence of flavonoids, saponins, carbohydrates, tannins, and triterpenes [15]. The present work aims at measuring the diuretic extract of Povonia odarata in acute treatment.

DRUGS AND CHEMICALS:
Furosemide (Laxis), ethanol were used in this study. All substances were prepared immediately before use and the reagents were used as analytical grade.

Plant materials
Povonia odarata is mainly used to folk medicine for various treatments like Anti-Inflammatory Infectiveness diseases, and Skin disorders. Diuretics are commonly used for management of hypertension and electrolytic balance. The present study was to investigate the preliminary phytochemical screening of the various extracts of Povonia odarata aerial part were studied for diuretic activity. Plant material and extraction Dried aerial parts were collected in the month of May (2017) from cultivated areas of district virdhungar, Tamil nadu. Taxonomic distinguishing proof was produced using BSMPUS, Government of India, Tirunelveli District, Tamil Nadu. After removing the extraneous material, the aerial parts were crushed into a coarse powder with an electric grinder.

Extract preparation
Povonia odarata leaves powdered materials were extracted with aqueous and ethanol. In each experiment, the extract was diluted with water to desired concentration. Approximately, 500 g of the crushed material was soaked in one liter of hot water at room temperature (23–25 °C) for 3 days\ with occasional shaking (aqueous extract) and Approximately, 500 g of the crushed material was soaked in one liter of alcohol at room temperature (23–25 °C) for 3 days\ with occasional shaking (alcohol extract). The material was then filtered and the residue was again soaked in hot water alcohol for 3 days and this procedure was repeated thrice (total 9 days) and finally, the filtrate was evaporated in a rotary evaporator (under reduced pressure (−760 mmHg) to a thick, semi-solid pasty mass of dark brown color. Crude extract of povonia odarata (PO) was dissolved in distilled water and normal saline for use in in-vitro and in-vivo experimentation, respectively.

Animals
Adult male albino rat weighing about 200-250g were used in this study. Assessment of diuretic activity Adult albino rats of either sex having weights in the range of 200-220 g were divided into six groups of six animals each. Animals were screened for any visible signs of disease and only the healthy animals were selected for the study. The whole experiment was carried out in same environmental conditions. Temperature of the room was also kept constant to 25±5 °C.

Group divided

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control (Normal saline 10ml/kg) for 7 days.</td>
</tr>
<tr>
<td>Group II</td>
<td>Furosemide (20 mg/kg, p.o.) for 7 days.</td>
</tr>
<tr>
<td>Group III</td>
<td>Received aqueous extract of Povonia odarata at the dose of 100mg/kg orally for 7 days.</td>
</tr>
<tr>
<td>Group IV</td>
<td>Received aqueous extract of Povonia odarata at the dose of 200mg/kg orally for 7 days.</td>
</tr>
<tr>
<td>Group V</td>
<td>Received alcoholic extract of Povonia odarata at the dose of 100mg/kg orally for 7 days.</td>
</tr>
<tr>
<td>Group VI</td>
<td>Received alcoholic extract of Povonia odarata at the dose of 200mg/kg orally for 7 days.</td>
</tr>
</tbody>
</table>
All the doses were made in same volume of normal saline in order to administer same volume in each group. Reference and control drugs Furosemide, a high-ceiling loop diuretic, were used as the reference drug (positive control). Normal saline was used as control drug. Animals were also given pelleted food and drinking water ad libitum. Group I (control group) was given normal saline 10 ml/kg. Group II (reference group) was given 20 mg/kg of furosemide and test groups (III and IV) were given 100, 200 mg/kg of <i>povonia odarata</i> alcohol extract and (V and VI) were given 100, 200 mg/kg of <i>povonia odarata</i> aqueous extract respectively.

**Diuretic activity**

Male albino rats weighing about 200-250gm were divided into four groups of six animals each. The dosages of drugs were administered to the different groups.

**Evaluation of diuretic activity**

Oral route was used for the administration of drugs because of its benefits over other routes i.e. ease of administration and freedom to administer large volume of fluids compared with other routes. Specially designed to separate urine and feces. The urine collected in graduated vials was measured at the end of 6 hr and expressed as ml/100g of body weight per 6 hr [16]. Determination of electrolyte levels of sodium and potassium in fresh urine samples were estimated using calibrated Flame Photometer Before estimating urinary sodium and potassium levels, samples were filtered to remove debris and shedding. Concentration of electrolytes was expressed in mEq/L [17,18,19]. Determination of urine pH of the fresh urine samples from all the six groups was measured with the help of a calibrated pH meter (Model: WTW-Series pH720) [20,21,22]. Assessment of acute toxicity Acute toxicity test of <i>TP.Cr.</i> was performed on albino mice of 200-250 g body weight. Animals were divided in different groups of five mice each. The control group of mice was given normal saline (10 ml/kg), while other groups received increasing doses of extracts up to 100 mg/kg and 200 mg/kg [23]. All the treatments were administered by oral gavage. Animals were observed closely for 2 hr, then at 30 minute intervals for 6 hr for any visible sign of toxicity (salivation, lacrimation, ptosis, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of hair), stress (erection of fur and exopthalmia), behavioural abnormalities (such as impairment of spontaneous movement, climbing, cleaning of face and ataxia, and other postural changes) and aggressive behaviour (biting and scratching behaviour, licking of tail, paw and penis, intense grooming behaviour and vocalization) and diarrhea and then mortality was noted at end of 24 hr [24]. According to this method, the animals were deprived of food and water for 18 hours prior to the experiment and each animal is placed in an individual metabolic cage 24h prior to commencement of the study for adaptation [25]. In this study animals were divided into four groups of five animals each. Group I animals were received normal saline (10 ml/kg, p.o.) for 7 days ,Group II animals were received the standard diuretic, Frusemide (20 mg/kg, p.o.) for 7 days and group 3&4 animals were received alcoholic extracts of <i>Povonia odarata</i>,100,200 mg/kg and group 4&5 animals were received aqueous extracts of <i>Povonia odarata</i>,100,200 mg/kg body weight for 7 days respectively. On seventh day, immediately after administration of the extracts, Frusemide the rats were paired and placed in metabolic cages. Urine was recollected in a graduated cylindrical tube and its volume was recorded at 1-h intervals for 3h. Finally the Electrolytes (Na+, K+) concentrations and pH were estimated from pooled urine sample of each pair of rat at the end of the experiment, 3h after administration[26,27]

**Analytical method.**

Na+ and K+ concentrations were measured by flame photometer. The instrument was calibrated with standard solution containing different concentrations of Na+ and K+. pH was directly determined on fresh urine samples using a pH meter, urine volume measured with a micropipette.
Table 1: Effects of Oral administration of *povonia odarata* on urinary volume excretion.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Volume of urine (ml/3h) Mean ± SEM</th>
<th>Diuretic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>10ml/kg</td>
<td>01.53 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Frusemide</td>
<td>20 mg/kg</td>
<td>10.1 ± 0.53</td>
<td>8.121</td>
</tr>
<tr>
<td><em>Aqueous extract Povonia odarata</em></td>
<td>100 mg/kg</td>
<td>4.29±0.32</td>
<td>3.411</td>
</tr>
<tr>
<td><em>Aqueous extract Povonia odarata</em></td>
<td>200 mg/kg</td>
<td>6.66±0.38</td>
<td>4.352</td>
</tr>
<tr>
<td><em>Alcoholic extract Povonia odarata</em></td>
<td>100 mg/kg</td>
<td>6.98±0.72</td>
<td>4.562</td>
</tr>
<tr>
<td><em>Alcoholic extract Povonia odarata</em></td>
<td>200 mg/kg</td>
<td>9.31±0.11</td>
<td>6.084</td>
</tr>
</tbody>
</table>

Table 2: Effects of oral administration of *povonia odarata* on urinary electrolytic excretion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Electrolyte Concentration in PPM</th>
<th>Saluetic index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total sodium (mEq/l)</td>
<td>Na+</td>
</tr>
<tr>
<td>Normal saline</td>
<td>10ml/kg</td>
<td>15.71±0.53</td>
<td>-</td>
</tr>
<tr>
<td>Frusemide</td>
<td>20 mg/kg</td>
<td>47.41±0.74***</td>
<td>3.017</td>
</tr>
<tr>
<td><em>Aqueous extract Povonia odarata</em></td>
<td>100 mg/kg</td>
<td>19.47±0.47**</td>
<td>6.12</td>
</tr>
<tr>
<td><em>Aqueous extract Povonia odarata</em></td>
<td>200 mg/kg</td>
<td>34.85±0.94***</td>
<td>7.64</td>
</tr>
<tr>
<td><em>Alcoholic extract Povonia odarata</em></td>
<td>100mg/kg</td>
<td>19.47±0.47**</td>
<td>7.12</td>
</tr>
<tr>
<td><em>Alcoholic extract Povonia odarata</em></td>
<td>200mg/kg</td>
<td>34.85±0.94***</td>
<td>8.21</td>
</tr>
</tbody>
</table>

**RESULTS:**
The result of diuretic activity of the alcoholic extract of *Povonia odarata* at 100, 200 mg/kg showed that a dose dependent significantly increase of urinary water excretion and electrolytes concentration then compared with aqueous extract of *Povonia odarata* at 100, 200 mg/kg in normal rats. The results of 200mg/kg both extract treated group showed significant change in electrolytes concentration and urine volume (P ≤ 0.001) compared with control group. In the present study, alcoholic extract treated groups at different doses (100mg/kg and 200mg/kg) showed significant effect on urinary potassium and sodium ion concentration. On the above results, it can be concluded that the extract produces diuretic effect with increase in Electrolyte concentration in urine.

**CONCLUSION:**
Further studies are necessary to identify and isolate the active constituents responsible for the diuretic activity. These findings may provide a lead for further investigations of the overall pharmacological actions of *Povonia odarata* in more appropriate model.

**REFERENCES:**
1. Gurwitz JH, Field TS, Harrold LR, Rothschild J, Debellis K, Seger AC, Cadoret C, Fish LS, Garber L,


