DIURETIC EFFECT OF THE ETHANOLIC AND AQUEOUS EXTRACT OF SEEDLING OF BORASSUS FLABELLIFER

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Key words: Borassus flabellifer, Diuresis, Diuretics, Seedling.

Abstract - The aim of the study was to evaluate the diuretic activity of the ethanolic and aqueous extract of the seedling of Borassus flabellifer. Crude ethanolic and aqueous extract of seedling of Borassus flabellifer (family: Palmae) was prepared and investigated for diuretic activity in albino rats and that was compared with the standard drug frusemide (100mg/kg, per oral [p.o]). The assessment of diuretic activity of Borassus flabellifer seedling extracts in albino rats showed that the urinary levels of Na+, K+ and Cl- were significantly increased by both the ethanolic and aqueous seedling extract. However lower dose has significantly elevated the Na+ and K+ levels but not the Cl- levels.

INTRODUCTION

Diuresis in medicine is the increase in the production of urine. It may be due to increased fluid intake, decreased levels of antidiuretic hormone (vasopressin), renal disease, or the use of diuretics (Dirks and Sutton 1986).

Palms are believed to be among the oldest flowering plants in the world (Readhead, 1989) and Borassus flabellifer belong to the family Palmae. All parts of the toddy palm are used. In India it is called the tree with 800 uses (Davis and Johnson, 1987). The main product is the sap obtained from tapping the inflorescences which may be drunk immediately or be processed into sugar or be allowed to ferment for a few hours to become toddy. This mild palm wine with 5-6% alcohol content may later be converted into distilled ethanol (arrack) or vinegar (Fong, 1989). The soft upper 10 m of the trunk contains some starch, which may be harvested in times of food scarcity. The seedlings are sometimes grown for use as a starchy vegetable, and eaten boiled or raw. The growing point of the palm is also edible. The tender mesocarp of young fruits is cooked in curry. The ripe fruit has a yellow edible pulp with a distinctive aroma. The young solid or gelatinous endosperm of the seeds is also eaten fresh or in syrup. Palmyra palm jaggery (gur) is much more nutritious than crude cane sugar, containing 1.04% protein, 0.19% fat, 76.86% sucrose, 1.66% glucose, 3.15% total minerals, 0.861% calcium, 0.052% phosphorus; also 11.01 mg iron per 100 g and 0.767 mg of copper per 100 g. The fresh sap is reportedly a good source of vitamin B complex. Borassus flabellifer is used in folk medicines. Briefly, the young plant is said to relieve biliousness, dysentery, and gonorrhea. Young roots are diuretic and antihelmintic and a decoction is given in certain respiratory diseases. The ash of the spadix is taken to relieve heartburn and enlarged spleen and liver. The bark decoction, with salt, is used as a mouth wash, and charcoal made of the bark serves as a dentifrice. Sap from the flower stalk is prized as a tonic, diuretic, stimulant, laxative and anti phlegmatic and amebicide. Sugar made from this sap is said to counteract poisoning and it is prescribed in liver disorders. Candied, it is a remedy for coughs and various pulmonary complaints. Fresh toddy, heated to promote fermentation, is bandaged onto all kinds of ulcers. The cabbage, leaf petioles, and dried male flower spikes all have diuretic activity (Atchley, 1984). The pulp of the mature fruit relieves dermatitis. Male inflorescence used as contraceptive: Ash (after burning of male inflorescence) with powder of black peppers (Piper longum) & cow milk in the ratio of 2:1:1 is prescribed to women as contraceptive.

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In the present study we investigated the diuretic activity of ethanolic and aqueous extract of *Borassus flabellifer* seedling. Phytochemical analysis of *Borassus flabellifer* extracts were also carried out. The present study was undertaken during the period of November 2007 to March 2008 in the post graduate and research department of Biochemistry, Dr. N.G.P Arts and Science College, with collaboration of Kovai Medical Center and Hospital (KMCH), a multispeciality hospital.

**MATERIAL AND METHODS**

*Borassus flabellifer* seedling was collected from taroor, and pudussery village of Palakkad district, Kerala, India. It was then dried and powdered and extracted with 70% ethanol and water in soxhlet extractor and the extracts were evaporated to remove the solvents and were lyophilized for further drying. The extracts were then subjected to test for the identification of its active constituents (Iyengar, 1999).

**Evaluation of diuretic activity**

**Animals**: The albino rats and mice (for acute toxicity study) of either sex were obtained from Animal House, Kovai Medical Center Research and Educational Trust (KMCRET), Coimbatore. All the animals were stored in standard polypropylene cages and maintained at 27°C ± 2°C under 12 hrs dark/light cycle (Lipschitz et al. 1943). The animals were fed with standard rat feed (Gold Mohur Lipton India Ltd) and water was given ad libitum. Ethical clearance for handling of the animals and the procedures used in the study was obtained from the institutional animal’s ethical committee prior to the beginning of the study.

**Acute toxicity study**

The acute toxicity of extracts of *Borassus flabellifer* seedling was determined as per fixed dose method. It was observed that the test extract was not mortal even at 2000mg/kg dose hence; 1/10th (200mg/kg) of this dose was selected for further study.

**Diuretic activity**

Albino rats of either sex weighing 150 to 200 gm were divided in to four groups of six animals each. The animals were fasted for 24 hrs and water was given ad libitum during fasting. On the day of experiment the animals of group I was administered with saline (25mL/kg, p.o.) and this group served as control. Similarly the animals of group II, were administered with furosemide 100 mg/kg (standard), and animals of group III, IV, were administrated with test extracts (200mg/kg) orally respectively (dissolved in saline and suitably diluted so as to adjust the volume of administration to 25 mL/kg). Immediately after the respective treatments the animals were placed in metabolic cages (3 animals in one metabolic cage) and urine was collected in the measuring cylinder up to 5 hrs. The volume of urine was measured, Na+, K+ and Cl- were estimated in the urine for assessing diuretic activity by flame photometry (Lipschitz et al. 1943).

**Statistical Analysis**

All the results were statistically analysed using statistic software graph pad system by applying student’s t-test. The values given to software are obtained by applying the formula Mean ± Standard Deviation.

**Student’s t-test**

\[
t = \sqrt{\frac{n_1 n_2 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}{n_1 + n_2 - 2}}
\]

\[S_1, S_2 - \text{standard deviations of the two groups}\]

**Statistical significance**

If ‘t’ value is 1.960 and above but below 2.576, then \(p<0.05\)- significant at 5% level.
If ‘t’ value is 2.576 and above then \(p<0.01\)-significant at 1% level (Gupta, 2003).

**RESULTS AND DISCUSSION**

The *Borassus flabellifer* seedling extracts were subjected to identification of phyto constituents. Table 1 shows the presence of carbohydrates, proteins, aminoacids, flavanoids, phyloterosols, saponins and fixed oils in ethanolic extract of seedling, whereas the aqueous extract of seedling showed the presence of carbohydrates, proteins, aminoacids, flavanoids, phyloterosols and saponins.

**Determination of Diuretic activity of seedling extracts of Borassus flabellifer**

The method of Lipschitz et al.(1943) was employed for the assessment of diuretic activity of extracts of Borassus flabellifer seedling. The urinary levels of Na+, K+ and Cl- were significantly increased by both the ethanolic and aqueous seedling extract. However lower dose has significantly elevated the Na+ and K+ levels but not the Cl- levels. The diuretic activity demonstrated by the test extracts at 200 mg/kg was significantly lesser than the standard drug furosemide. Table 2 shows the Diuretic Effect of *Borassus flabellifer* seedling extracts in albino rats.
The results indicate that the urinary levels of Na+, K+ and Cl- were significantly increased by both the ethanolic and aqueous seedling extracts. However lower dose has significantly elevated the Na+ and K+ levels but not the Cl- levels. The changes in the urinary level are accompanied by the changes in the concentration of Na+, K+ and Cl-.

Volume of urine

The volume of urine in the control (2.69 ± 0.02 mL), in standard drug Furosemide (10.33 ± 0.2 mL), in seedling aqueous (5.90 ± 0.13) and in seedling ethanolic extract (5.76 ± 0.13 mL) are presented in the Table 2. The volume of urine in standard, and the seedling ethanol and aqueous extract are increased which is satisfactorily significant at 1% level when compared to the control. Ethanol and aqueous extract of seedling significantly increased the urinary out put (Lipschitz et al. 1943).

Sodium

The concentrations of sodium in the control (60.25 ± 3.65 meq/L), in standard drug Furosemide (156.50 ± 3.40 meq/L), in seedling aqueous (123.62 ± 2.51) and in seedling ethanolic extract (129.49 ± 2.76 meq/L) are presented in the Table 2. The concentration of sodium in standard, in seedling ethanol and aqueous extract is increased which is statistically significant at 1% level when compared to the control. Ethanol and aqueous extract of seedling increased the sodium concentration (Lipschitz et al. 1943).

Potassium

The concentrations of potassium in the control (1.13 ± 0.12 meq/L), in standard drug Furosemide (5.67 ± 0.29 meq/L), in seedling aqueous (4.40 ± 0.19) and in seedling ethanolic extract (3.96 ± 0.29 meq/L) are presented in the Table 2. The concentration of potassium in standard, in seedling ethanol and aqueous extract are increased, which is statistically significant at 1% level when compared to the control. Ethanol and aqueous extract of seedling increased the potassium concentration (Lipschitz et al. 1943).

Chloride

The concentrations of chloride in the control (0.19 ± 0.03 meq/L), in standard drug Furosemide (0.51 ± 0.02 meq/L), in seedling aqueous (0.43 ± 0.02) and in seedling ethanolic extract (0.38 ± 0.03 mL meq/L) are presented in the Table 2. The concentration of chloride in standard, in seedling ethanol and aqueous extract are increased, which is statistically significant at 1% level when compared to the control. Ethanol and aqueous extract of seedling has increased the chloride concentration in urine (Lipschitz et al. 1943). But in lower concentrations the concentration of chloride is not significantly increased.

CONCLUSION

Urinary levels of Na+, K+ and Cl- were significantly increased by both the ethanolic and aqueous

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Table 1. Phytochemical of *Borassus flabellifer* seedling extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Carbohydrates</th>
<th>Glycosides</th>
<th>Proteins &amp; Amino acids</th>
<th>Fixed oils &amp; fats</th>
<th>Sterol &amp; triterpenoids</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
<th>Tannins</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Seedling</td>
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<tr>
<td>Aqueous</td>
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<tr>
<td>Seedlings</td>
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</tbody>
</table>

+: Present; -: Absent

Table 2. Diuretic effect of *Borassus flabellifer* seedling extracts in Albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Volume of Urine in (mL)</th>
<th>Concentration of elements (meq/L)</th>
<th>Na+</th>
<th>K+</th>
<th>Cl-</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2.69 ± 0.02</td>
<td>60.25 ± 3.65</td>
<td>1.13</td>
<td>0.12</td>
<td>0.19</td>
</tr>
<tr>
<td>II</td>
<td>Furosemide</td>
<td>10.33 ± 0.2***</td>
<td>156.50 ± 3.40***</td>
<td>5.67</td>
<td>0.29***</td>
<td>0.51</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous extract</td>
<td>5.90 ± 0.13</td>
<td>123.62 ± 2.51</td>
<td>4.40</td>
<td>0.19</td>
<td>0.43</td>
</tr>
<tr>
<td>VI</td>
<td>Ethanol extract</td>
<td>5.76 ± 0.13***</td>
<td>129.49 ± 2.76***</td>
<td>3.96</td>
<td>0.29***</td>
<td>0.38</td>
</tr>
</tbody>
</table>

*** Represents P value is less than 0.001; All values are mean ± SD (n = 6 number of mice)
seedling extract. However lower dose has significantly elevated the Na+ and K+ levels but not the Cl- levels. The diuretic activity demonstrated by the test extracts at 200 mg/kg was significantly lesser than the standard drug furosemide.

REFERENCES


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