Evaluation of Diuretic Activity of Ethanolic Extracts of Cucurbita Maxima seeds in Rats

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Abstract

Background: Kidneys play an important role in water and electrolyte conservation and it forms an integral part of body homeostatic mechanism. Diuretics are drugs used to treat several conditions including cardiac failure, Nephrotic syndrome, cirrhosis of Liver and treatment of hypertension. In the present study we tried to evaluate the antidiuretic activity of extracts of Cucurbita maxima Duchesne (commonly known as pumpkin) in Rats.

Methods: Adult albinorats of either sex weighing in between 150-200gms were used. The diuretic activity of Cucurbita Maxima at the dosage of 150mg/kg and 300mg/Kg was compared with Standard drug Furosemide at the dosage of 20mg/Kg. Diuretic activity was measured by collecting total excreted urine (0-5hrs) the rats being kept in metabolic cage.

Results: The urinary volume during the period of the 5hr collection in Group I [Control] was 1.96±0.12 ml/kg. In the Group II which was treated with 20mg/kg of furosemide, there was a significant increase in the urinary volume i.e. 4.71±0.83 ml/Kg. In the test Group III treated with Cucurbita maxima at dosage of 150mg/Kg, the urinary volume was significantly greater than control. The urinary volume for the Test-1 group was found as 3.33±0.34 ml/Kg and Group IV treated with Cucurbita maxima at dosage of 300mg/Kg was found to be 3.55±0.51ml/kg.

Conclusions: Cucurbita maxima produced a significant increase in urinary volume, urinary and Serum electrolytes excretion when compared to control. The diuretic activity was more significant at 300 mg/kg body when compared to control. From this study, it may be concluded that the ethanolic extract of Cucurbita maxima produces significant diuresis however its effect is not as strong as standard drug Furosemide.

Key words: Cucurbita maxima, Furosemide, Diuretic

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Received on : 04/03/2016 Revised : 15/03/2016 Accepted : 20/03/2016

Introduction

Diuretic 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. Diuresis means increase in urine volume with loss of solute and water. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na reabsorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of ADH.

The main uses of diuretics are in the edema due to cardiac failure, renal disease (nephrotic syndrome) and cirrhosis of liver and in the treatment of hypertension. The plant Cucurbita Maxima Duchesne cucurbit or gourd family (commonly known as pumpkin) belongs to the family of Cucurbitaceae is widely cultivated throughout the world for use as vegetable as well as medicine. Both fruits and the aerial parts are commonly consumed as vegetable. It is a large climbing herb, annual or perennial. Its aerial part consists of flexible succulent stem with trifoliate leaves. Traditionally it is used in
most countries as anti-diabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents. Pumpkin seeds have been used as safe deworming and diuretic agents, and the seed oil as a nerverine tonic. Pumpkin seed oil has a strong antioxidant property, and has been recognized for several health benefits such as prevention of the growth and reduction of the size of prostate, reduction of bladder and urethral pressure and improving bladder compliance, alleviation of diabetes by promoting hypoglycemic activity, and lowering level of gastric, breast, lung, and colorectal cancer. There are only sparse data which indicates that Cucurbita Maxima possess diuretic activity. With this background we tried to evaluate the diuretic property of Cucurbita Maxima in Albino rats.

**Materials and Methods**

The study was carried out in Post Graduate research laboratory of the department of pharmacology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, after obtaining the permission from the Institutional Animal Ethics Committee (IAEC). Laboratory bred Albino rats of either sex were obtained from M/S Sainath Agencies, Hyderabad and placed in individual cages in central animal house of the Institute. Dried Pumpkin seeds (Cucurbita maxima) were purchased from super market (Hyderabad, Andhra Pradesh, India) and identified by the Department of Botany, Govt. S R R College of science, Karimnagar.

The seeds were ground to coarse particles and extraction was carried out with Soxhlet apparatus using 50% ethanol. Laboratory bred Albino rats of either sex weighing between 150 to 200gms were used for the study. The animals were housed under standard laboratory conditions and were acclimatized to the laboratory before carrying out the experimental work.

They were fed with Standard diet and water ad libitum. Cucurbita Maxima extract at a dose of 150mg/kg and 300mg/Kg body weight in 5mL of NaCl solution dose orally. Furosemide Lasix (Aventis Pharma, India) was used as a standard diuretic it was powdered and dissolved in 5ml Normal Saline to induce diuretic activity in rats the dose of 20 mg/kg body weight was given orally.

The rats were divided into 4 groups.

- **Group I:** Consisted of 6 rats which served as normal control group and were given Normal saline orally. 5mL of NaCl solution per kg body weight.
- **Group II:** Consisted of 6 rats which served as standard group received Furosemide at a dose of 20 mg/kg body weight in 5mL of NaCl solution orally.
- **Group III:** Consisted of 6 rats which served as test group-1 and were given Cucurbita Maxima extract at a dose of 150mg/kg body weight in 5mL of NaCl solution dose orally.
- **Group IV:** Consisted of 6 rats which served as test group-2. Test group received Cucurbita maxima extract 300mg/kg body weight in 5mL of NaCl solution dose orally.
Furosemide was administered orally through gavage in rats for 6 days at a dose of 20mg/kg body weight in group II. The urine and serum electrolyte were monitored from 7 to 12 days for 0-5 hrs. Cucurbita maxima dried seeds was administered orally at a dose of 150 mg/kg and 300 mg/kg body weight to groups III, and IV respectively through oral feeding tube. The diuretic activity of urine electrolytes and serum electrolytes were monitored from 13 to 24 days for 0-5 hrs. Fifteen hours prior to the experiment food and water were withdrawn. Diuretic activity was measured by collecting total excreted urine (0-5 hrs) of rat kept in metabolic cage.

The cages together with the funnel and measuring cylinder used in the studies were coated with liquid paraffin before each experiment to facilitate the collection of urine with a minimum loss. For estimation of serum electrolytes blood was withdrawn from tail vein of the rat through a sterile syringe. Very gentle aspiration was done, in order to avoid vein collapse. Urine and blood electrolytes are estimated through flame photometer.

**Results**

The urinary volume during the period of the 5hr collection in the control animals was 110.50 ± 13.2. In the standard group which was treated with Furosemide was 146.67± 13.61mg/kg, there was a significant increase in the Na⁺ excretion when compared with control. In the Test Group III, the Na⁺ excretion was 258.87 ± 6.74 and in the Test group IV the Na⁺ excretion was significantly greater than that of the Group I the calculated p values were found to be significant Table 2. Similarly there was significant increase in serum potassium excretion in Group IV as compared to control group I and standard group II indicating that at 300mg/kg dose Cucurbita maxima causes loss of potassium the calculated p values were highly significant. On serum chloride levels in urine in Group IV were higher when compared to control and standard drug Furosemide which indicate that Cucurbita maxima at dose of 300mg/Kg caused more chloride loss. The calculated p values are given in the table 2.

Table 3 shows the effect of various groups on the serum electrolyte levels. The Group II Furosemide group shows significant decrease in the serum Na⁺ levels as compared to control Group I 139.50±4.84. Group III and Group IV also decreased serum Na⁺ levels 123.50±4.09 and 109.67±12.21 respectively and the calculated p values were found to be significant. Similarly serum potassium levels decreased significantly in the group II group III and group IV table 3. Serum chloride levels decreased markedly in Group II and slightly lesser decrease in levels of Group III and Group IV. The calculated p values were significant when compared to control.
Table 1: Showing comparison of mean values of all the parameters in all four groups

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Body weight</th>
<th>Total urinary volume mL/5hr</th>
<th>Urine Electrolytes (mEq/L)</th>
<th>Serum Electrolytes (mEq/L)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Na+</td>
<td>K+</td>
<td>Cl-</td>
</tr>
<tr>
<td>Group I</td>
<td>133.67 ± 7.23</td>
<td>1.96 ± 0.12</td>
<td>110.5 ± 45.88</td>
<td>183.83 ± 139.5</td>
<td>9.48 ± 0.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>±13.2 ± 9.7</td>
<td>±5.38 ± 4.85</td>
<td>±4.85</td>
</tr>
<tr>
<td>Group II</td>
<td>133.7 ± 8.5</td>
<td>4.71 ± 0.83</td>
<td>146.7 ± 126.9</td>
<td>180.50 ± 41.0</td>
<td>2.45</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>±8.5 ± 6.5</td>
<td>±5.8 ± 5.76</td>
<td>±0.2</td>
</tr>
<tr>
<td>Group III</td>
<td>139.67 ± 5.67</td>
<td>3.35 ± 0.34</td>
<td>258.87 ± 115.6</td>
<td>435.3 ± 123.5</td>
<td>3.87 ± 0.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>±6.7 ± 7.5</td>
<td>±4.50 ± 5.0</td>
<td>±0.4</td>
</tr>
<tr>
<td>Group IV</td>
<td>134.3 ± 5.55</td>
<td>3.55 ± 0.51</td>
<td>286.67 ± 276.6</td>
<td>362.3 ± 109.6</td>
<td>3.30 ± 0.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.65</td>
<td></td>
<td>±5.01 ± 7.56</td>
<td>±7.00 ± 12.2</td>
<td>±2.6</td>
</tr>
</tbody>
</table>

Table 2: Effect on urinary electrolytes excretion

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dose</th>
<th>Na+ (mEq/L)</th>
<th>K+ (mEq/L)</th>
<th>Cl- (mEq/L)</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>5mL of NaCl</td>
<td>110.5±13.2</td>
<td>4.59±0.33</td>
<td>143.83±5.38</td>
<td>7.25±0.104</td>
</tr>
<tr>
<td>Group II</td>
<td>20mg/kg body weight in 5ml</td>
<td>146.67±13.61**</td>
<td>6.98±0.45**</td>
<td>160.50±4.63**</td>
<td>6.27±0.12**</td>
</tr>
<tr>
<td>Furosemide</td>
<td>150 mg/kg body weight in 5ml of NaCl</td>
<td>258.87±6.74**</td>
<td>5.67±0.27*</td>
<td>150.33±4.50*</td>
<td>6.31±0.01**</td>
</tr>
<tr>
<td>Group III</td>
<td>Cucurbita maxima</td>
<td>286.67±5.00***</td>
<td>6.67±0.60**</td>
<td>162.3±7.00***</td>
<td>6.3±0.09**</td>
</tr>
<tr>
<td>Group IV</td>
<td>Cucurbita maxima</td>
<td>109.67±12.21**</td>
<td>3.30±0.84**</td>
<td>87.33±7.26*</td>
<td>7.4±0.100</td>
</tr>
</tbody>
</table>

Values are in Mean±SD *P<0.05 Vs control; **P<0.01 Vs control; ***P<0.001 Vs control

Table 3: Effect on Serum electrolytes excretion

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dose</th>
<th>Na+ (mEq/L)</th>
<th>K+ (mEq/L)</th>
<th>Cl- (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>5mL of NaCl</td>
<td>139.50±4.84</td>
<td>4.983±0.87</td>
<td>96.33±11.98</td>
</tr>
<tr>
<td>Group II</td>
<td>20mg/kg body weight in 5ml of NaCl</td>
<td>41±5.762***</td>
<td>2.45±0.26**</td>
<td>28.17±3.06***</td>
</tr>
<tr>
<td>Furosemide</td>
<td>150 mg/kg body weight in 5ml of NaCl</td>
<td>123.50±4.09**</td>
<td>3.87±0.60**</td>
<td>91.50±8.04*</td>
</tr>
<tr>
<td>Group III</td>
<td>Cucurbita maxima</td>
<td>109.67±12.21**</td>
<td>3.30±0.84**</td>
<td>87.33±7.26*</td>
</tr>
<tr>
<td>Group IV</td>
<td>Cucurbita maxima</td>
<td>300mg/kg body weight in 5mL of NaCl</td>
<td>109.67±12.21**</td>
<td>3.30±0.84**</td>
</tr>
</tbody>
</table>

Mean±SD *P<0.05 Vs control; **P<0.01 Vs control; ***P<0.001 Vs control

Chart 1: Percentage increase in urine Excretion in mL/5hr Group III and Group IV when compared to control
Discussion

India is a vast country with presence of various indigenous medicinal plants that are used extensively in traditional medical treatments however there is no standardization for herbal medicine until in 1991 WHO developed certain guidelines for assessment of herbal medicine with suggestions for standardizations. The recent growth of field of herbal medicine is extensive and it is getting popularized especially in developing countries due its natural origin, cheap costs and virtually negligible side effects. Cucurbita maxima plant (squash) belonging to the family Cucurbitaceae is very extensively found in India. It weighs about 2-3Kg has orange colour flesh and seeds. Cucurbita maxima seeds have many health benefits, as they are a good source of protein, zinc, and other vitamins and they are even said to lower cholesterol. The seeds used as anthelmintic, diuretic and nervous tonic and are useful in taeniasis, strangury and nervous debility. Seeds also used as abortifacient and insecticidal. Although only few studies have been conducted so far as diuretic activity of the Cucurbita maxima is concerned but in one study by Jose M A et al showed that cucurbita maxima aqueous extracts showed significant diuretic activity. However they showed that the concentration of Na+ and K+ in urine collected increased but it was not to a significant levels but in our study we found that both Na+ and K+ levels in both test groups of III and IV were increased significantly when compared to control. In a very similar study done by Saravanan V S et al who compared the diuretic activity of cucurbita maxima on rats comparing it with standard drug acetazolamide showed that cucurbita maxima possess significant diuretic activity at the dosage of 300mg/Kg when compared with control and its diuretic potential was comparable with acetazolamide. In the present study we compared the diuretic potential of Cucurbita maxima with standard drug Furosemide. We found the volume of urine increased. In Group I the control group the volume of urine collected was 1.96 ± 0.12 ml/5 hours in Group III and and Group IV it was about 3.35 ± 0.34 and 3.55 ± 0.51 respectively however it was only lesser as compared to standard drug furosemide which had volume of 4.71 ± 0.83 this shows that the Cucurbita maxima although has diuretic potential however its potential was not matched with high ceiling diuretic like furosemide. The probable underlying mechanism for Cucurbita maxima to act as diuretic could be due to suppression of renal tubular reabsorption of water, electrolytes and low molecular weight organic compounds into the blood stream. As a consequence promotes urine formation. The diuretic activity of Cucurbita maxima hydro-alcoholic extract may be due the presence of glycosides, flavonoids, polyphenols and alkaloids. In the present study the Group IV (300mg/Kg) showing more potent activity as compared to the group III (150mg/Kg) indicating The activity of cucurbita maxima also is dose dependent.

Conclusion

Cucurbita maxima produced a significant increase in urinary volume, urinary and Serum electrolytes excretion when compared to control. The diuretic activity was more significant at 300 mg/kg body when compared to control. From this study, it may be concluded that the ethanolic extract of Cucurbita maxima produces significant diuresis however its effect is not as strong as standard drug Furosemide.

Conflict of Interest: None declared
Source of Support: Nil
Ethical Permission: Obtained

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