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DIURETIC EFFECT OF CHLOROFORM EXTRACT OF BENINCASA HISPIDA RIND (Pericarp) IN SPRAGUE-DAWLEY RATS

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ABSTRACT : The diuretic activity of *Benincasa hispida* fruit rind extract (outer thick pericarp) was investigated and its activity was compared to control (normal saline) and standard diuretic hydrochlorothiazide in albino rats. Total of 54 adult male Sprague-Dawley rats were taken whose weights ranged from 175-225 gm. The rats were divided into three groups of 18 rats each (control, standard and test). Control group received 0.9% normal saline 25ml/kg orally. Standard group received hydrochlorothiazide 2.5mg/kg body weight orally along with normal saline keeping the volume of the fluid administered constant. Test group received aqueous extract of rind of *Benincasa hispida* at the dose of 100mg/kg orally along with normal saline 25ml/kg. Urine was collected for a period of 5 hours by placing the animals in metabolic cages. The urinary volume, pH, and urinary excretion of sodium, potassium and chloride were measured and compared. The extract produced significant increase ($p<0.001$) in urine volume, sodium and chloride levels, and significant decrease ($p<0.001$) in potassium excretion. *Benincasa hispida* rind extract possesses significant diuretic activity.

Key words: Benincasa hispida, diuretic, urinary volume.

INTRODUCTION

Diuretics are the first line drugs used for the treatment of hypertension. The fruit of *Benincasa hispida* (Thunb) Cogn, commonly called as white gourd or ash gourd belongs to family Cucurbitaceae. It is employed as a main ingredient in kusmanda lehyam in Ayurvedic system of medicine. The leyham (electuary) is used as rejuvenating agent and also in numerous nervous disorders. For centuries it has been used for various empirical applications in ailments such as dyspepsia, burning sensation, vermifuge, heart disease, diabetes and urinary disease [Asolkar LV et al, 2000, Anil Kumar D et al, 2002]. However, some scientific studies carried out reveal its anti-inflammatory [Gover JK et al, 1994], diuretic [Dong MY et al, 1995], hypoglycemic [G. R. Battu et al, 2007], anti-Alzheimer's [Chandan Roy et al, 2008], antidiarrheal [Vrushabendra Swamy Bhyrapur Mathad et al, 2005], antioxidant [Beena V et al, 2008], antiulcer [Beena V et al, 2008, Manish A et al, 2008], anti-obesity [A. Kumar et al, 2004], antihistaminic [D. Anil Kumar et al, 2002] and anticancer [Kumar A et al, 2002] activities. It is also used in disorders related to urinary tract**.** The major constituents of this fruits are triterpenoids, flavanoids, glycosides, saccharides, carotenes, vitamins, β sitosterin and uronic acid [Nadkarni AK, Wollen weber E et al, 1991, Yashizumi S et al, 1998]. It is documented that *Benincasa hispida* fruit juice was used in traditional medicine to decrease hypertension due to its diuretic effect. This study was done to confirm its diuretic effect.

MATERIALS AND METHODS

Collection of the plant

The study was done during April 2010 to August 2010. The fruit of *Benincasa hispida* was obtained from a local vegetable market in Khammam. The identification and authentication of the plant was done at the department of Botany, Government degree college, Khammam.

Extraction procedure

The preparation of extract from the rind of *Benincasa hispida* was done in the department of Pharmacology, Mamata Medical College, Khammam. Freshly peeled rind (pericarp) was cut into small pieces and shade dried. The dried rind was then finely powdered. The powdered rind was extracted with chloroform water by process of simple maceration [R. M. Mehta, 2002]

Animals

Adult male Sprague-Dawley rats, weighing between 175-225gm were used in the study. The animals were given free access to food and water. The experiment complied with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethics Committee (IAEC).

Drugs used

Tab. hydrochlorothiazide 2.5mg manufactured by Sun pharmaceuticals was used in the study.

Measurement of urinary volume and electrolytes

Collection of urine was done by placing the animals in metabolic cages. The collected urine was estimated for volume. The estimation of urinary electrolytes was done with digital spectrophotometer (mfd. by Electronics India, model 301) using electrolyte kit manufactured by M/S Excel Diagnostics Pvt. Ltd., Hyderabad.

Toxicity evaluation in rats

The aqueous extract was tested for its acute toxicity in rats. To determine the acute toxicity, a single oral administration of 0.25, 0.5, 0.75, 1g/kg were administered to different groups of rats (2 rats were used in each group; control rats received normal saline). The animals were observed periodically for 48 hours. The parameters observed were hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions. No toxic effects and mortality was noted.

Optimization of effective doses:

Optimization of effective dose was carried out using increasing doses (25mg/kg, 50mg/kg, 100mg/kg and 200mg/kg) of the extract and compared to the control group which received normal saline (25ml/kg body weight), and standard group which received hydrochlorothiazide (2.5mg/kg body weight). Test drug caused variable diuresis in the dose range of 25 to 200 mg/kg body weight. When compared to control there was increase in urinary volume to 7.133 ± 0.73 ml/kg and 7.63 ± 0.77 ml/kg with 25 mg/kg and 50mg/kg respectively. There was increase in urinary volume to 10.93 ± 1.40 ml/kg with 100mg/kg and 10.97 ± 2.73 ml/kg with 200mg/kg. The urinary volume of the control group was 6.23 ± 0.56, whereas with the standard group the urinary volume was 13.37 ±0.95 which is higher to control and different doses of text drug. The sodium excretion with 25mg/kg, 50mg/kg and 200mg/kg was 137.45 ±6.77, 138.33 ±65 and 40.97 ±2.73. The chloride excretion was 132.5 ±3.905, 135.71 ±2.94 and 135.25 ±5.41 near to values of control group. The sodium and chloride excretion with 100mg/kg were 146 ±7.33 and 148.65 ±5.48 respectively. There was uniform decrease in potassium excretion with 25mg/kg, 50mg/kg, 100mg/kg and 200mg/kg which were 0.01 ±0.15, 2.16 ±0.47, 2.64 ±0.47, 2.44 ±0.09. With the standard group the sodium, potassium and chloride excretion were 168.4±3.39, 16±0.62 and 147.46±5.79 as shown in table-I. Further studies were conducted using the optimal dose of the test drug as 100mg/kg.

Table I: Comparison of the dose dependent effect of test drug on 5 hr excretion of urinary pH, urinary volume, Na+, K+ and Cl- excretion in Sprague-dawely Rats with control and standard groups represented as Mean ± SD

Treatment	Urinary pH	Urinary volume ml/kg	Urinary sodium excretion meq/kg	Urinary potassium excretion meq/kg	Urinary chloride excretion meq/kg
Control group NS (25ml/kg, n=9)	7.4±0.12	6.23 ± 0.56	141.63 ± 2.52	14.38 ± 0.48	135.39 ± 1.75
Hydrochlorothiazide (2.5mg/kg, n=9)	7.12±0.12	13.37±0.95	168.4±3.39	16±0.62	147.46±5.79
Benincasa hispida (50 mg/kg, n=9)	7.20±0.20	7.63 ± 0.77	138.33 ± 6.5	2.16 ± 0.47	135.71 ± 2.94
Benincasa hispida (100 mg/kg, n=9)	7.21±0.21	10.93 ± 1.40	146.61 ± 7.73	2.64 ± 0.47	148.65 ± 5.48
Benincasa hispida (200 mg/kg, n=9	7.23±0.23	10.97 ± 2.73	139.21 ± 2.53	2.44 ± 0.09	135.25 ± 5.41

All the values are represented as Mean ± SD. For all observations n = 9.

Experimental design:

The diuretic activity in rats was studied by modified Lipschitz test [H. Gerhard Vogel]. Adult male Sprague-Dawley rats weighing between 175-225gm were used. The room temperature was maintained between 27-29°C. Food was restricted 18 hours prior to the experiment with free access to water. All the animals were hydrated with 25ml/kg of 0.9% normal saline orally. The animals were divided into 3 groups with 18 rats in each group. In all the animals urinary bladder was emptied before administration of drug. First group of 18 rats were kept as control, which were given only 0.9% normal saline 25ml/kg body weight orally. The animals were then transferred to the metabolic cages; three animals per cage and time noted.

Second group of 18 rats were fed with normal saline 25ml/kg along with standard hydrochlorothiazide 2.5mg/kg orally and then transferred to the metabolic cages housing three animals per cage and time noted.

The third group of 18 rats was taken as test group and the rind extract of *Benincasa hispida* which was obtained in liquid form was given orally along with normal saline at the dose of 100mg/kg, keeping the volume administered constant. Animals were subsequently transferred to metabolic cages housing, three animals per cage. The urine was collected in beakers for a period of 5 hours in all three groups. The rats were not given food or water during the experiment. At the end of 5 hours, the bladder of each rat was emptied by pulling the tail at the base to collect the residual urine. Urinary volume and urinary pH was noted and samples were taken for estimation of urinary electrolytes for sodium, potassium and chloride using spectrophotometer.

Statistical analysis:

All the values were expressed as Mean±SD. The differences were compared using one way analysis of variance (ANOVA) followed by Dunnet's t test. The p values <0.05 were considered significant.

Table –II. Effect of Benincasa Hispida extracts on urine volume, pH, sodium, potassium and chloride excretion

Treatment N=8	pH of urine	Urine volume ml/5hr	Na+ meq/kg	K+meq/kg	Cl- meq/kg
Normal saline (25ml/kg)	7.4±0.12	6.23±0.56	141.63±2.52	14.38±0.48	135.39±1.7
Hydrochlorthiazide (2.5mg/kg)	7.12±0.12	13.37±0.95	168.4±3.39	16±0.62	147.46±5.79
Aqueous extract of Benincasa.Hispida (100mg/kg)	7.21±0.21	10.93±1.40	146.61±7.73	2.64±0.47	148.65±5.4
P-value		<0.001*	<0.001* <0.05**	<0.001* <0.05**	<0.001* <0.05**

Mean ± SD, n=18, when compared with standard by using one way ANOVA* followed by Dunnette's multiple comparison test**.

Urinary volume:

Urinary volume (UV) during the period of 5 hour collection in 18 control animals was 6.22 ± 0.78 ml/kg. In standard group which were treated with 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in UV i.e. 13.37 ± 0.95 ($P < 0.001$). In test group, though the UV was significantly greater than the control group, it was lesser than the standard group. The UV for the test group was found to be 10.85 ± 1.57 ml/kg ($P < 0.001$).

Urinary pH:

Urinary pH with control was 7.2 ±0.12, with hydrochlorothiazide 7.12 ±0.12 whereas with 100mg/kg was 7.21 ±0.21. The changes in the pH were not significant when compared with control and standard.

Urinary sodium:
Urinary sodium (Na^+) during the period of 5 hour collection in control animals was 145.9±8.18 meq/kg. In standard group which were treated with 2.5 mg/kg of hydrochlorothiazide there was a significant increase in Na^+ excretion i.e. 168.4 ± 3.39 meq/kg (P<0.001) and in test group the Na^+ excretion was also significantly greater than the control group but lesser than the standard group i.e.148.5 ± 8.17 meq/kg (P<0.001).

Urinary potassium:
Urinary potassium excretion (K^+) during the period of 5 hour collection in control animals was found to be 14.59±1.44 meq/kg. In standard group, which received 2.5 mg/kg of hydrochlorothiazide, there was significant increase in K^+ excretion i.e. 16 ± 0.62 meq/kg (P<0.001) but there was a significant decrease in K^+ excretion in test group i.e. 2.71 ± 0.38 meq/kg (P<0.001).

Urinary chloride:
Urinary chloride (Cl^-) during the period of 5 hour collection in control animals was 134.8±4.11meq/kg. In standard group which were treated with 2.5 mg/kg of hydrochlorthiazide there was a significant increase in Cl^- excretion i.e. 147.46 ± 5.79 meq/kg (P<0.001) and in test group the Cl^- excretion was also significantly greater than the control group but lesser than the standard group i.e.137.41 ± 3.51 meq/kg (P<0.001).

DISCUSSION:

There is increasing interest in the health and wellness benefits of herbs and botanicals. This is with good reason as they might offer a natural safeguard against diseases and be a substantial treatment for certain diseases. Diuretics are one of the groups of drugs used for the treatment of hypertension. A number of herbs cause diuresis but most promising at the present time are *Foeniculum vulgare, Fraximus excelsior, Hibiscus sabdariffa, Spergularia purpurea* [CI Wright et al, 2007] Diuretics relieve pulmonary congestion and peripheral edema. They decrease plasma volume and subsequently venous return to heart. This decreases cardiac work load, oxygen demand and plasma volume, thus lowering blood pressure. They are the first line of drugs in the treatment of mild to moderate hypertension along with sodium restriction in the diet.

Benincasa hispida (Thunb) Cogn., belongs to the family *Cucurbitaceae.* It is also known as wax gourd, Chinese winter melon and fuzzy melon (English). Because of diversity of languages and dialects, the plant has different vernacular names petha kaddu (Hindi), boodida gummadikaya (Telugu), boodagumbala (Kannada), Chalkumra (Bengali), kusmanda (Sanskrit). *Benincasa hispida* is found throughout Asia in tropical regions. It is used both as a food and as well as a medicinal product. It is cultivated throughout the plains of India and on hills up to 4000 feet. The parts used are seeds, fruits and fruit juice. The constituents of the fruit are moisture: 96%, protein: 0.4%, fat: 0.1%, carbohydrates: 63.2%, minerals: 0.3%, vitamin B: 211U/100g. The fruit is claimed to contain triterpenoids, flavanoids, glycosides, saccharides, vitamins, β sitosterin and uronic acid [Yazhizumi s et al, 1998, Chandan Roy et al, 2008, Grassman J et al, 2010]. It is documented that *Benincasa hispida* fruit juice was used in traditional medicine to decrease hypertension. In the present study aqueous extract of rind (pericarp) *Benincasa hispida* was evaluated for its diuretic property which has not been evaluated so far.

The effect of *Benincasa hispida* on renal excretory function was studied in adult male Sprague-Dawley rats. Acute toxicity studies conducted on rats did not show any change in the behavioral pattern. No mortality was observed at the given doses as well. It was observed in albino rats that maximum diuretic response was obtained at 100mg/kg (oral). An increase of dose to 200mg/kg did not produce further diuretic effect as shown in table 1. Hence, the dose of 100mg/kg has been considered as effective dose and further studies were done taking the same. There was an increase of 114% (P<0.001) and 74% (P<0.001) in urinary volume in standard and test groups respectively. The sodium excretion increased by 15% (P<0.001) and 2% (P<0.001) in standard and test groups respectively. There was a percentage increase of 9% (P<0.001) and decrease of 2 % (P<0.001) in urinary potassium excretion in both standard and test groups respectively. There was a percentage increase of 9% (P<0.001) and 2% (P<0.001) in urinary chloride excretion in standard and test groups respectively. All the values mentioned above are in comparison to control.

Similar studies done with ethanol and aqueous extract of *Benincasa cerifera* showed almost same results [Ramadas. V et al, 2010] except that there was decrease in potassium loss in our study. The studies done on *Benincasa cerifera* show significant increase in Na+ and K+ excretion compared with control group. In our study done with the extract with the dose of 100mg/kg there was a significant loss of sodium and chloride with significant decrease in potassium loss. The K^+ sparing effect of this extract may be due to the other ingredients present only in the rind (pericarp) of the fruit. The mechanism of this effect is assumed to be due to aldosterone antagonist action and Na+ channel blockade in the collecting ducts which has to be further elucidated. The difference may also be due to the variation of phytochemicals in both *Benicasa cerifera* and *Benincasa hispida*.

The claim of *Benincasa hispida* as diuretic is confirmed in our study. So, the phytochemicals such as flavanoids, saponins or organic acids [Maghrani M et al, 2005] could be responsible for this diuretic effect. The overall mechanism seems to be inhibition of tubular reabsorption of water and anions [Pantoja CV et al, 1993] and may be due to stimulation of regional blood flow in the kidney. The increased loss of Na^+ and water is the basis for its use as antihypertensive.

CONCLUSION:

The test drug has produced significant increase in excretion of three parameters (urinary volume, urinary Na^+, urinary Cl^-) and significant decrease in urinary K^+ excretion. The diuretic effect produced by the test drug was less in comparison with that of hydrochlorothiazide. From this study, it may be concluded that the aqueous extract of *Benincasa hispida* produces mild diuresis and has a K^+ sparing action, which supports the traditional use of Benincasa fruit extract in the treatment of different edemas and also as antihypertensive agent by producing diuresis.

Despite all the progress in synthetic chemistry and biotechnology, plants are still indispensable source of medicinal preparations, both preventive and curative. Tremendous progress has been made in research on herbal medicine viz. Pharmacognosy, Phytochemistry and Pharmacology of the plant products all over the world. Indeed "Phytomedicines" are beginning to link traditional and modern medicine. It is clear that modern science can no longer afford to ignore reports of any aboriginal uses of plants simply because they seem to fall beyond the limits of our credence. On the contrary these uses should stimulate examination under the impartial searchlight of modern scientific analysis. Hence for the overall development of the drug research, the Pharmacological research studies have a very important role to play.

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