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Evaluation of diuretic activity of ethanol extract of *Benincasa hispida* stem in swiss albino rats

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ABSTRACT

All parts of the plant Benincasa hispida is traditionly used in different diseases because of their medicinal properties. The endocarp and rind of the fruit is diuretic. The present study was carried out to evaluate the diuretic effect of ethanol extract of Benincasa stem (EEBCS) in rats in comparison with standard drug Hydrochlorothiazide. Forty five male Swiss albino rats aged ten to twelve weeks of male sex weighing about 175–200 g were taken, and after acute toxicity studies three different doses were selected. The animals were divided into five different groups. The first group was kept as the control (Normal Saline), second as the standard (Hydro chlorthiazide) and the remaining three groups as Test I, Test II, and Test III and given different doses of the EEBCS. Diuretic and Natriuretic activities were carried out by administration of normal saline along with the treatment modules. The volume of urine (in ml) and the Na^+, K^+ and Cl^- content in the urine were measured. The results were expressed as mean ± SD. Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using primer of biostatistics McGraw–Hill software version 5.0.0.0 (2011). P-values < 0.05 were considered significant. The extract at 100 and 200 mg / kg, produced significant diuresis and increased sodium elimination but not potassium. Thus the study elucidates that aqueous extract of Benincasa stem possess significant diuretic, Saluretic, Natriuretic and carbonicanhydrase activity.

Keywords: *Benincasa stem*, Diuresis, Metabolic cages, Natriuretic, Potassium, Sodium.

INTRODUCTION

Herbal medicine is the oldest form of health care known to mankind and use of herbal drug for human health care is probably as ancient as mankind. Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs[1].The study of plant species with diuretic effects is still a fruitful research in search of new diuretics. The rind of the fruit is diuretic[2].According to previous ethno pharmacological survey carried out *Benincasa cerifera* exerts Reno protective activity probably by the radical scavenging activity[3].Diuretics are the drugs that increase the rate of urine flow; clinically useful diuretics also increase the rate of excretion of Na^+ (natriuresis) and an accompanying anion, usually Cl^-. Most clinical applications of diuretics aim to reduce extracellular fluid volume (edema) by decreasing total body NaCl content. Although continued administration of diuretic causes a sustained net deficit in total Na^+, the time course of natriuresis is finite because renal compensatory mechanisms brings Na^+ excretion in line with the Na^+ intake, a phenomenon known as diuretic braking. Diuretics alter the excretion of other cations (e.g. K^+, H^+, Ca^{2+}, and Mg^{2+}), anions (e.g. Cl^- HCo_3^- and H_2Po_4) and uric acid. In addition diuretics may alter renal hemodynamics indirectly mediated by local prostaglandins synthesis [4]. 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. It is documented that all parts of *Benincasa Hispida* i.e. fruit, pericarp and seeds except stem has diuretic effect. This study is done to elucidate diuretic effect of *stem extract of Benincasa Hispida.*

EXPERIMENTAL SECTION

Collection of the plant: The study was done during June 2012 to November 2012. The *stem of Benincasa hispida* was obtained from a vegetable garden near 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 *stem of Benincasa hispida* was done in the department of Pharmacology, Mamata Medical College at Khammam. Shade dried *stem of Benincasa hispida* without leaves was cut into small pieces and was then finely powdered. The powdered stem was extracted with ethanol by process of simple maceration [5].

Animals

Adult male Swiss albino 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) Registration number 285/CPCSEA.The guidelines for the investigation of experiments in conscious animals were followed in all tests.

Drugs

Tab. Hydrochlorothiazide 25mg, manufactured by Sun Pharmaceuticals was used in the study.

Toxicity Evaluation in Albino Rats

The ethanol extract was tested for its acute toxicity in albino rats. Acute oral toxicity was performed as per OECD-423 guide lines [6]. To determine the acute toxicity, the extract was administered orally in an ascending order and in widely spaced doses that is 0.25g/ kg, 0.5g/kg, 0.75g/kg and 1g/kg to different groups of albino rats. Two albino rats were used in each group; the control albino rats received normal saline). The animals were observed periodically for forty eight hours. The parameters which were observed were hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions. There were no toxic effects and mortality. The optimization of the effective dose was calculated by taking one tenth of the maximum dose, that is 100mg/kg and the other two doses which were taken were half and double of the one tenth dose, which is 50mg/kg and 200mg/kg respectively. These doses were then compared with the control group which received normal saline 25ml/kg body weight and with the standard group which received hydrochlorothiazide 2.5mg/kg body weight for the evaluation of the diuretic activity.

Experimental design:

The diuretic activity in rats was studied by modified Lipchitz test [7].Adult male Swiss albino rats weighing between 175-225gm were used. The room temperature was maintained between 27-29oC. 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 five groups with nine rats in each group. In all the animals urinary bladder was emptied before administration of drug. First group of nine 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 nine 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 nine rats was taken as test group and the ethanol extract of stem of *Benincasa hispida* which was obtained in liquid form was given orally along with normal saline at the dose of 50mg/kg, keeping the volume administered constant. Animals were subsequently transferred to metabolic cages housing, three animals per cage. The fourth group of nine rats was given 100mg/kg of test dose orally along with normal saline, keeping the volume administered constant. The fifth group of nine rats was given 200mg/kg of test dose orally along with normal saline, keeping the volume administered constant. The urine was collected in beakers for a period of five hours in all three groups. The rats were not given food or water during the experiment. At the end of five 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. The rats were kept back again in metabolic cages and urine was

collected after twenty four hours and subjected for urinary analysis including Natriuretic activity and carbonic anhydrase inhibition activity.

Measurement of Urinary Volume and Electrolytes

The collection of urine was done by placing the animals in metabolism cages. The collected urine was estimated for volume. The pH was measured by using a digital pH meter. The pH reading was noted for the control, standard (hydrochlorothiazide) and different doses of test animals. The estimation of the urinary electrolytes was done by using a digital spectrophotometer (Mfd by Electronics India, Model 301) by using an electrolyte kit which was manufactured by M/S Excel Diagnostics, Pvt.Ltd, and Hyderabad.

Saluretic, Natriuretic and Carbonic Anhydrase Inhibition

The sum of Na^+ and Cl^- excretion was calculated as a parameter of Saluretic activity. The ratio Na^+/ K^+ was calculated for Natriuretic activity. The ratio $Cl^- /Na^+ + K^+$ (ion quotient) was calculated to estimate carbonic anhydrase inhibition [7].

Statistical analysis

The results are expressed as mean values ± S.D (standard Deviation) Statistical comparison was carried out by analysis of variance (ANOVA). The difference between the means of treated groups and the non-treated control group was evaluated by the Dunnette's Multiple Comparisons Test. The results were considered statistically significant when P was < 0.05.

RESULTS

All the data subjected to 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.

Table1A: Comparison of effect of different doses of test drug on 5hr excretion of urinary pH, urinary volume Na^+ K^+ Cl^- excretion with control and standard groups represented as Mean ±SE

Treatment groups n=9	Urinary pH	Urinary volume ml/kg	Urinary Na^+ excretion mg/kg	Urinary K^+ excretion mg/kg	Urinary Cl^- excretion mg/kg
Group I(control) (Normalsaline25ml/kg	8	7.14±0.049	78.97±0.028	19.9 ± 0.1	267.9 ± 0.21
Group-II(Standard) HT 2.5mg/kg	8	19 ± 1*	145 ± 5*	18.5 ± 05*	116.2±0.40**
Group-III(Test-I) EEBC 50mg/kg	9	9.5± 0.04*	54.5 ± 0**	19.5± 0.14*	158.3±0.29**
Group-IV(Test-II) EEBC -100mg/kg	8	7.75± 0.04*	205 ± 15*	26.25± .33*	201 ± 15**
Group-V(Test-III) EEBC -200mg/kg	7	11 ±1*	301 ±1**	25.84±0.34*	145 ± 0.13**

*EEBC-Ethanol extract of stem of Benincasa,*significant P<0.05, **Highly Significant P<0.000, HT-Hydrochlorothiazide*

Table IB 5 hrs Saluretic, Natriuretic and Carbonic anhydrase inhibition

Treatment Group n=9	Saluretic activity	Natriuretic activity	Carbonic anhydrase inhibition
Group-I(Control) Normal saline 25ml/kg	346.87	3.96	2.7
Group-II(Standard) HT 2.5mg/kg	261.2	7.8	0.7
Group-III(Test-I) EEBC 50mg/kg	213.18	2.88*	2.14
Group-IV(Test-II) EEBC -100mg/kg	464	7.86*	0.86**
Group-V(Test-III) EEBC -200mg/kg	334.8	7.34*	0.67**

EEBC--Ethanol extract of stem of Benincasa, significant P<0.05, **Highly Significant P<0.000 HT-- Hydrochlorothiazide.*

EVALUATION

The sum of Na^+ and Cl^- excretion is calculated as Parameter for Saluretic activity. The ratio Na^+/K^+ is calculated for Natriuretic activity. Values greater than 2.0 indicate a favorable Natriuretic effect. Ratios greater than 10.0 indicate a potassium-sparing effect. The ratio $Cl^-/Na^+ + K^+$ (ion quotient) is calculated to estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed.

Table-IIA Comparison of effect of different doses of test drug on 24 hr excretion of urinary pH, urinary volume, Na+, K+ and Cl- excretion with control and standard groups represented as mean ±SEM

Treatment Group n=9	Urinary pH	Urinary volume ml/kg	Urinary Na^+ excretion meq/kg	Urinary K^+ excretion meq/kg	Urinary Cl^- excretion meq/kg
Group-I(Control) Normalsaline25ml/kg	8	14.25 ± 0.25	63.17 ± 0.017	21.65± 0.05	264.2± 0.28
Group-II(Standard) HT 2.5mg/kg	8	5 ± 1*	173.2± .44**	21 ± 1	158.2±0.25**
Group-III(Test-I) EEBC 50mg/kg	8	3.4± 0.4**	184.5±4.5 *	20.78 ± 0.18*	177.3±0.09**
Group-IV(Test-II) EEBC -100mg/kg	8	2.4 ± 0.2**	301 ± 1**	32.39 ±0.49*	264.5 ± 0*
Group-V(Test-III) EEBC -200mg/kg	10	5.6±0.3**	260.8±0.23**	31.92 ±002**	216 ± 0.11**

EEBC-Ethanol extract of stem of Benincasa, significant P<0.05, **Highly Significant P<0.000, HT- Hydrochlorothiazide*

Table—IIB 24 hrs Saluretic, Natriuretic and carbonic anhydrase inhibition

Treatment Groups n=9	Saluretic activity	Natriuretic activity	Carbonic anhydrase inhibition
Group-I(Control) Normal saline 25ml/kg	327.35	2.91	3.11
Group-II(Standard) HT 2.5mg/kg	331.4	8.24	0.81*
Group-III(Test-I) AEBC 50mg/kg	361.8	8.878*	0.86*
Group-IV(Test-II) AEBC -100mg/kg	294.6	0.9	4.2
Group-V(Test-III) AEBC -200mg/kg	476.8	8.17*	0.737**

*EEBC-Ethanol extract of stem of Benincasa,*significant P<0.05, **Highly Significant P<0.000 HT- Hydrochlorothiazide*

EVALUATION: The sum of Na^+ and Cl^- excretion is calculated as Parameter for Saluretic activity. The ratio Na^+/K^+ is calculated for Natriuretic activity. Values greater than 2.0 indicate a favorable Natriuretic effect. Ratios greater than 10.0 indicate a potassium-sparing effect. The ratio Cl^-, $Na^+ + K^+$ (ion quotient) is calculated to estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed.

Results: 5 hr Urine collection (Table IA)

Urine Volume

Table IA shows that the increase in urine volume following the administration of the extracts. Ethanol extract produced a significant increase in urine volume (UV) as compared with the control group. In test groups I, II and III though the UV was significantly greater than the control group, it was lesser than the standard group. The UV in the test group II is less than the other two groups.

Electrolyte Excretion (**Table 1A)**

Table 1A shows the urinary electrolyte content following the administration of the extracts. There is significant dose dependent increase in Na^+ and K^+excreation in three test groups. Cl^-excretion is highly significant in all Test groups compared with the control group. Changes in parameters like pH were not significant when compared to control group.

Effects on Natriuretic, Saluretic and Carbonic Anhydrase Inhibition (Table IB)
From the Table 1B, the ethanol extract of *Benincasa hispida* showed potent Saluretic activity with Test II and Test III groups comparable with control and standard and significant Natriuretic effect is seen with all test groups. Also there is highly significant increase in carbonic anhydrase inhibition with Test II and Test III groups.

24 hr urine collection (Table IIA)
Urinary volume: Urinary volume (UV) during the period of 24 hour collection was a significant in all test groups in test III The UV in test III was greater than the standard group.

Urinary sodium: Urinary Na^+ excretion in all test groups was significantly increased when compared with standard and control but it was highly significant in test group II and Test group III.

Urinary potassium: Urinary K^+ excretion increased significantly in Test II &III when compared with standard and control and highly significant in Test III.

Urinary chloride: Urinary Cl^- excretion was significant in all Test groups when compared with standard and control but highly significant in Test I and Test III groups.

24 hrs Saluretic, Natriuretic and Carbonic anhydrase inhibition (Table IIB)
Test groups I and III have high Saluretic activity when compared with control and standard and also has significant Natriuretic activity. Carbonic anhydrase inhibition action was significant in Test I group but highly significant with Test III group.

DISCUSSION

Drugs are produced synthetically by modern technology. Hybridization techniques are being used for synthesizing monoclonal antibodies for various diseases. Lately Gene therapy and Stem cell therapy had come in to practice to treat different diseases. Still plants are indispensable source of medicinal preparations even today. It is an era of phytomedicines which link traditional and modern medicines. Mankind has a long history in the use of herbal medicines. Rig-Veda and Ayurveda (4500-1600 BC) reveal that ancient Indians had a rich knowledge of the use of medicinal plants. India unquestionably occupies the top most position in the use of herbal drugs since ancient times utilizing nearly 600 plant species in different formulations. Great majorities of people in India have been depending on crude drugs for the treatment of various diseases as evidenced from well documented indigenous system of medicines, Ayurveda and Unani. The Materia Medica of these systems contains a rich heritage of indigenous herbal drugs [8]. Different parts of Benincasa hispida plant has been studied extensively for various disorders. The fruit of *Benincasa hispida* (Thumb) Cogan, commonly called as white gourd or ash gourd belongs to family Cucurbitaceous. It is employed as a main ingredient in kusmanda lehyam in Ayurvedic system of medicine. The lehyam (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 [9][10] however, some scientific studies carried out reveal its anti-inflammatory[11],diuretic[12] ,hypoglycemic[13],[anti-Alzheimer's[14], anti diarrheal[15],antioxidant[16], antiulcer [17], anti obesity[18],antihistaminic[19] and anticancer[20] activities. It is also used in disorders related to urinary tract**.** The major constituents of this fruits are triterpenoids, flavonoids, glycosides, saccharides, carotenes, vitamins, β sitosterin and uronic acid [8, 21, 22].The parts used are seeds, fruits and fruit juice. It is documented that *Benincasa hispida* fruit juice was used in traditional medicine to decrease hypertension due to its diuretic effect. It has been documented that the juice of Ash gourd i.e. *Benincasa hispida* was used in traditional medicine to decrease hypertension and for the prevention of recurrent renal calculi. According to a previous ethno pharmacological survey which was carried out on *Benincasa cerifera*, it was reported to exert a renoprotective activity, probably by its radical scavenging activity. The pretreatment with *Benincasa cerifera* prevented renal ischemia/reperfusion-induced lipid peroxidation and protected the kidneys from severe increase in the ROS products, the depletion of superoxide dismutase and reduced glutathione in rats which were exposed to the renal I/R [23].The effect of *Benincasa hispida* on the renal excretory function was studied in adult, male guinea pigs by the method which was described by Klatt et al[24]. The present study was done to confirm its diuretic and also for its Saluretic, Natriuretic and carbonic anhydrase inhibition activity of ethanol extract of stem of *Benincasa Hispida* which has not been evaluated so far. Diuretics are the first line drugs used for the treatment of hypertension.

The effect of ethanol extract of stem of *Benincasa hispida* on renal excretory function was studied in adult male Swiss albino 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. In 5 hrs urinary analysis it was observed that that maximum diuretic response was obtained at 200mg/kg (oral) and an increase in diuretic effect is more with 50mg/kg than of 100mg/kg dose as shown in table 1A. There was an increase of urinary volume in standard by 266.1 and 133.5, 108.5 and 154.06% in all test groups respectively. The sodium excretion increased by 183.6% ($P<0.05$), 69.0 % ($P<0.000$), 259.5% ($P<0.05$), and 381.1% ($P<0.000$) in standard and test groups respectively. The values in Test group I and Test group III are highly significant. There was a percentage increase of 92.9, 97.9, 131.9 and 129.8% ($P<0.05$) in K in standard and all test groups. The K+ excretion is more in Test II and III, whereas Cl -excretion was highly significant in standard as well as in all test groups (43.3, 59.08, 75.02 and 54.12%).It is evident that all the doses of EEBC has diuretic effect along with excretion of salts, but the action is high with 200mg/kg body weight. The K excretion is less with 50mg/kg body weight. The Natriuretic effect of EEBC is significant in all test groups but Saluretic effect is highest with 200mg/kg followed by 100mg/kg lastly with 50mg/kg body weight which is in correlation with similar study[25]. The carbonic anhydrase inhibition activity of EEBC is highly significant with 100mg/kg and 200mg/kg weight. All the values mentioned above are in comparison to control.

Similar studies which were done with ethanol and an aqueous extract of *Benincasa cerifera* fruit showed almost the same results [25]. Those studies showed a significant increase in the Na+ and K+ excretion along with urinary volume as compared to that in the control group. So, in our study, 50mg/kg and 200mg/kg of *Benincasa hispida* stem extract showed highly significant loss of sodium and chloride and a significant potassium loss. Whereas100mg/kg EEBC has only significant loss of electrolytes. This may be due to pharmacokinetic differences in the rate of absorption. We observed potent Saluretic action with all test doses and significant Natriuretic effect, highly significant carbonic anhydrase inhibition activity with 100 and 200mg/kg dosages as shown in Table IB

Analysis of 24 hr urinary excretion showed a significant increase in the Na+, K+ and Cl excretion in three test groups (50,100,200mg/kg) but highly significant increase with 200mg/kg dose.(Table II) and potent Saluretic activity with all three test doses, significant Natriuretic and carbonic anhydrase inhibition action with 50mg/kg dose. Significant Natriuretic and highly carbonic anhydrase inhibition action with 200mg/kg dose is observed. (Table II B).From 5hr and 24 hr urine analysis we conclude that 200mg/kg body weight is the most appropriate and effective dose as diuretic.

The role of *Benincasa hispida* stem as a diuretic has been conformed in our study. The active principles which are responsible for the diuretic effect of the extracts of this plant have not yet been elucidated, but a preliminary photochemical analysis of the extracts revealed the presence of polar compounds such as flavonoids and steroids. It may be suggested that these substances may be responsible, at least in part, for the observed diuretic activity and that they may act individually or synergistically. According to previous ethno pharmacological survey carried out *Benincasa cerifera* exerts renoprotective activity probably by the radical scavenging activity. [3] Previous studies have also demonstrated that there are several compounds which could be responsible for the diuretic effect of this plant, such as flavonoids, saponins or organic acids [27]. The overall mechanism seems to be the inhibition of the tubular reabsorption of water and anions [28] and this may be due to the stimulation of the regional blood flow in the kidney. The increased loss of Na+ and water is the basis for its use as an antihypertensive. Twenty four hour urinary electrolyte estimation also shows higher Saluretic, Natriuretic and carbonic anhydrase inhibition action which indicates long duration of action of ethanol extract of stem of *Benincasa hispida*.

CONCLUSION

Diuretic effect of different parts of *Benincasa hispida*, fruit, leaf, and pericarp are studied in recent years but not stem. The results obtained in this study confirmed that stem extract of Benincasa hispida has potent Saluretic, Natriuretic, and hyperkalaemic action along with carbonic anhydrase inhibition action. Traditional use of *Benincasa hispida* as diuretic is confirmed from this study. Further studies are to be conducted to elucidate above said actions.

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