Original Article

Evaluation of the Diuretic Effect of the Chloroform Extract of the Benincasa Hispida Rind (Pericarp) Extract in Guinea-pigs

Pharmacology Section

T. JAYASREE, K. KIRAN KISHORE, M.VINAY, P. VASAVI, N. CHANDRASEKHAR, V.S. MANOHAR, ROHIT DIXIT

ABSTRACT

Background: Pharmacological research on the medicinal properties of phytochemicals has become mandatory to establish the claimed medicinal properties of herbs.

Aims: To evaluate the diuretic activity of the Benincasa hispida fruit rind extract (outer thick pericarp) and to compare its activity withthat of the control (normal saline) and the standard diuretic,hydrochlorothiazide in guinea-pigs.

Methods and Material: A total of 54 adult, male guinea-pigs were taken, whose weights ranged from 400-450gm. The guinea-pigs were divided into three groups of 18 each (control, standard and test). The control group received 0.9% normal saline/25 ml/kg orally. The standard group received hydrochlorothiazide –2.5 mg/kg body weight orally, along with normal saline, while keeping the volume of the fluid which was administered constant. The test group received the aqueous extract of the rind of Benincasa hispida at the dose of 90 mg/kg orally, along with normal saline-25 ml/kg. Urine was collected for a period of 5 hours by placing the animals in metabolism cages. The urinary volume, pH of the urine and the urinary excretion of sodium, potassium and chloride were measured and compared.

Results: The extract produced a significant increase ($p<0.001$) in the urinary volume. There was a significant increase in the sodium and chloride excretion and a decrease ($p<0.001$) in the potassium excretion.

Conclusion: The Benincasahispida rind (pericarp) extract possesses a significant diuretic activity with a potassium sparing effect.

Key Words: Benincasa Hispida, Diuretic activity, Urine volume

INTRODUCTION

Pharmacological research on the medicinal poperties of phytochemicals has become mandatory, to establish the claimed medicinal properties of herbs. The fruits of this plant are traditionally used as a laxative, diuretic, tonic, aphrodisiac andcardiotonic for urinary calculi, blood diseases, insanity, epilepsy, and also in cases of jaundice, dyspepsia, fever, and menstrual disorders [1]. Various in vitro as well as in vivo studies have shown that the Benincasacerifera extract has an antioxidant activity on tissues like the liver and the brain, but not a single study was performed on the kidney tissue [2]. In the present study, the aqueous extract of the Benincasahispida rind (pericarp) was evaluated for its diuretic property, which has not been evaluated so far.

The Plant

The fruit of Benincasa hispida (Thunb) Cogn, which is commonly called as ash guard, amember of the Cucurbitacae family, is employed as a main ingredient in kusmandalehyam, in the Ayurvedic system of medicine. It is a herbaceous, climbing plant, which needs artificial support. It is extensively cultivated in India. In the traditional system of medicine, all parts of benincasacerefera are used medicinally. Some scientific studies have been carried out, which have revealed its anti-inflammatory activity [3], diuretic activity [4] and its hypoglycaemic [5], anti alzheimer's [6], antidiarrheal [7], antioxidant [8], antiulcer [8-9], anti-obesity [10], antihistaminic [11] and anti cancer [12] activities and it is used in urinary disease. The major constituents of this fruit are triterpenoids, flavanoids, glycosides, saccharides, carotenes, vitamins, β sitosterin and uronic acid [13-15]. The fruit of Benincasa, as a whole, exerts a diuretic activity, which has been studied extensively, but that of the rind (pericarp) was not confirmed experimentally. The main aim of the present study was to evaluate the diuretic activity of its rind (pericarp) in guinea pigs.

Collection of the Plant

The fruit of Benincasa hispida was obtained from a local vegetable market in Khammam. The identification and the authentification of this plant were done in the Department of Botany, Government Degree College, Khammam.

Extraction Procedure

The preparation of the extract of the rind of Benincasa hispida was done in the Department of Pharmacology, Mamata Medical College, Khammam. The freshly peeled rind (pericarp) was cut into small pieces and driedin the shade. The dried rind was then finely powdered. The powdered rind was extracted by using chloroform water by the process of simple maceration [16].

METHODS

Animals

Adult male guinea pigs who were aged 3-4 months and weighing between 800-1000 gms, were used in the study and they were obtained from the Central Animal House, Mamata Medical College, Khammam. The protocol was approved by the IAEC, MMC, Khammam. These animals were used for the study of the diuretic activity of the chloroform extract of the rind of Benincasa hispida. The animals were stabilized for 1 week; they were maintained understandard conditions at room temperature, 60 ± 5% relative humidity and 12 hr light and dark cycles. They were given a standard pellet diet and water ad-libitum. The animals were given free access to food and water.

Ethics

The experiment complied with the guidelines for the animal experimentation of our laboratory and was approved by the Institutional Animal Ethics Committee (IAEC).

Drugs Used

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

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 digital pH meter was calibrated by using standard buffer solutions at pH = 4 and 7. The electrodes were washed with a jet of distilled water and wiped with soft tissue paper. Then, the electrodes were dipped in the beaker which contained the urine sample. The pH reading was noted for the control, standard (hydrochlorthiazide) and different doses of test animals. The estimation of the urinary electrolytes was done by usinga digital spectrophotometer (Mfd by Electronics India, Model 301) by using an electrolyte kit which was manufactured by M/S Excel Diagnostics, Pvt.Ltd, Hyderabad.

Toxicity Evaluation in Albino Rats

The chloroform extract was tested for its acute toxicity in albino rats. To determine the acute toxicity, the extract was administered orally in an ascending order and in widely spaced doses i.e. 0.25 g/kg, 0.5 g/kg, 0.75 g/kg and 1 g/kg to different groups of albino rats (2 albino rats were used in each group; the control albino rats received normal saline). The animals were observed periodically for 48 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 1/10th of the maximum dose, i.e 100 mg/kg and the other 2 doses which were taken were half and double of the 1/10th dose, i.e 50 mg/kg and 200 mg/kg respectively. These doses were then compared with the control group which received normal saline –25 ml/kg body weight and with the standard group which received hydrochlorothiazide –2.5 mg/kg body weight for the evaluation of the diuretic activity.

The test drug caused variable diuresis in the dose range of 25 to 200 mg/kg body weight. When it was compared to the control, an increase in the urinary volume to 7.133 ± 0.73 ml/kg and 7.63 ± 0.77 ml/kg with 25 mg/kg and 50 mg/kg respectively was found. There was an increase in the urinary volume to 10.93 ± 1.40 ml/kg with 100 mg/kg and 10.97 ± 2.73 ml/kg with 200 mg/kg. The urinary volume of the control group was 6.23 ± 0.56, whereas that of the standard group was 13.37 ± 0.95, which was higher as compared to the control and to different doses of the text drug. The sodium excretion values with 25 mg/kg, 50 mg/kg and 200 mg/kg were 137.45 ± 6.77, 138.33 ± 65 and 40.97 ± 2.73. The chloride excretion values were 132.5 ± 3.905, 135.71 ± 2.94 and 135.25 ± 5.41, which was closer to the values of the control group.

The sodium and chloride excretion with 100 mg/kg were 146 ± 7.33 and 148.65 ± 5.48. There was a uniform decrease in the potassium excretion with 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg, whose values 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 values were 168.4 ± 3.39, 16 ± 0.62 and 147.46 ± 5.79, as shown in [Table/Fig-1]. Further experiments were conducted by using the optimal dose of the drug-90 mg/kg, as extrapolated according to the body surface area in guinea pigs.

Experimental Design

The diuretic activity in guinea pigs was studied by the modified Lipschitz test [17]. Adult, male guinea pigs who weighed between 400-450 gms were used. The room temperature was maintained between 27-29°C. Food was restricted 18hours prior to the experiment, but free access to water was allowed. All the animals were hydrated with 25 ml/kg of 0.9% normal saline orally. The animals were divided into 3 groups with 18 guinea pigs in each group. In all the animals, the urinary bladder was emptied before the administration of the drug. The first group of guinea pigs was kept as the control group, which was given only 0.9% normal saline/ 25 ml/kg of body weight orally. The animals were then transferred to the metabolism cages which housed 3 animals per cage and the time was noted.

The second group of 18 guinea pigs was fed with normal saline-25 ml/kg along with standard hydrochlorothiazide –2.5 mg/kg orally and they were then transferred to the metabolism cages which housed 3 animals per cage and the time was noted.

The third group of 18 guinea pigs wastaken as the 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 90 mg/kg, by keeping the volume which was administered, constant. The animals were subsequently transferred to the metabolism cages which housed 3 animals per cage. The urine was collected in beakers for a period of 5 hours in all three groups. The guinea pigs were not given food or water during the experiment. At the end of the 5 hours, the bladder of each guinea pig was emptied by

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 (25 ml/kg)	7.4 ± 0.12	6.23 ± 0.56	141.63 ± 2.52	14.38 ± 0.48	135.39 ± 1.75
Hydrochlorothiazide (2.5 mg/kg)	7.12 ± 0.12	13.37 ± 0.95	168.4 ± 3.39	16±0.62	147.46 ± 5.79
Benincasahispida (50 mg/kg)	7.20 ± 0.20	7.63 ± 0.77	138.33 ± 6.5	2.16 ± 0.47	135.71 ± 2.94
Benincasahispida (100 mg/kg)	7.21 ± 0.21	10.93 ± 1.40	146.61 ± 7.73	2.64 ± 0.47	148.65 ± 5.48
Benincasahispida (200 mg/kg)	7.23 ± 0.23	10.97 ± 2.73	139.21 ± 2.53	2.44 ± 0.09	135.25 ± 5.41

[Table/Fig-1]: Comparison of the dose dependent effect of test drug on 5 hr excretion of urinary pH, urinary volume, Na+, K+ and Cl- excretion in guinea pigs with control and standard groups represented as Mean ± SD

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

pulling the tail at the base, to collect the residual urine. The urinary volume and the urinary pH were noted and samples were taken for the estimation of the urinary electrolytes viz sodium, potassium and chloride, by using a spectrophotometer.

STATISTICAL ANALYSIS

All the values were expressed as Mean ± SD. The differences were compared by using the One Way Analysis Of Variance (ANOVA), followed by Dunnet's t test. P values of <0.05 were considered to be significant.

Results

As shown in [Table/Fig-2] and [Table/Fig-3].

Urinary Volume

The urinary volume (UV) during the period of the 5hr collection in the 18 control animals was 13.03 ± 1.02 ml/kg. In the standard group which was treated with 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in the UV i.e. 28.47± 1.31($P < 0.001$). In the test group, though the UV was significantly greater than that in the control group, it was lesser than the UV in the standard group. The UV for the test group was found to be 24.83± 2.64 ml/kg ($P < 0.001$).

Urinary Ph

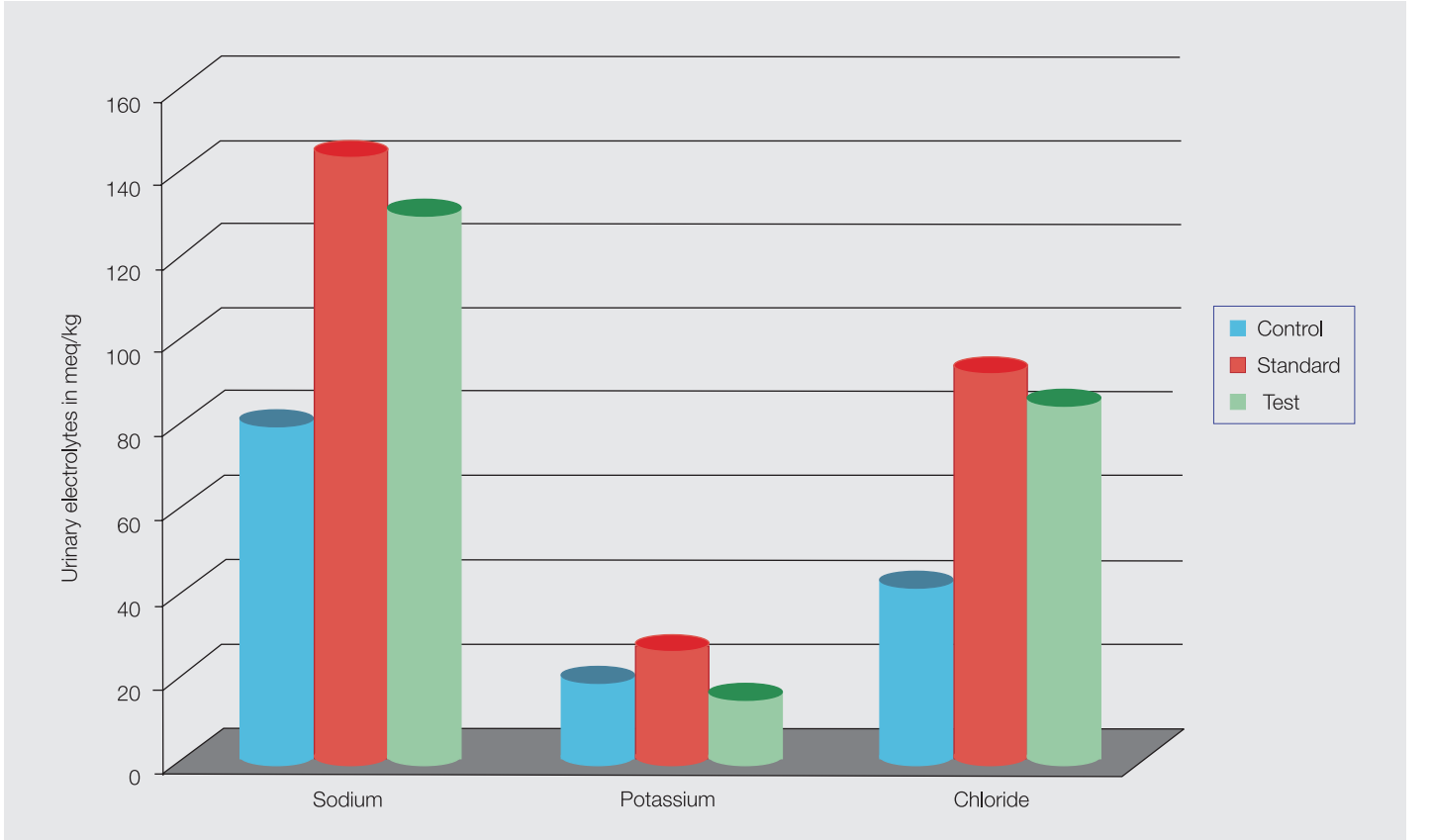
The urinary pH of thecontrol group was 7.2 ± 0.12 and that of the group which was hydrochlorothiazide, was 7.12 ± 0.12, whereas that of the group which was given the 90 mg/kg extract, was 7.21 ± 0.21. The changes in the pH were not significant when they were compared with that of the control and the standard.

Urinary Sodium

Urinary Sodium (Na^+) during the period of the 5hr collection in the control animals was 79.54 ± 4.77 meq/kg. In the standard group

Treatment n= 18	pH of urine	Urine volume ml/5hr	Na^+meq/kg	K^+meq/kg	Cl^-meq/kg
Normal saline (25 ml/kg)	7.2 ± 0.12	13.03 ± 1.02	79.54 ± 4.77	18.72 ± 1.66	41.31 ± 2.56
Hydrochlorthiazide (2.5 mg/kg)	7.12 ± 0.12	28.47 ± 1.31	144.51 ± 4.47	25.50 ± 2.85	92.47 ± 3.65
Aqueous extract of Benincasa Hispida (90 mg/kg)	7.21 ± 0.21	24.83 ± 2.64	129 ± 3.33	14.51 ± 0.94	84.05 ± 2.89
P-value		<0.001*	<0.001* <0.05**	<0.001* <0.05**	<0.001* <0.05**

[Table/Fig-2]: Effect of BenincasaHispida extracts on urine volume, pH, sodium, potassium and chloride excretion
All values are expressed as Mean ± SD, n=18
When compared with standard by using one way ANOVA* followed by Dunnette's multiple comparison test**.
Graph 1: Effect of BenincasaHispida extracts on sodium, potassium and chloride excretion



[Table/Fig-3]: Effect of Benincasa Hispida extracts on sodium, potassium and chloride excretion

which was treated with 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in the Na^+ excretion i.e. 1144.51 ± 4.47 meq/kg (P<0.001) and in the test group, the Na^+ excretion was significantly greater than that of the control group, but lesser than that of the standard group i.e. 129.99 ± 3'33 meq/kg (P<0.001).

Urinary Potassium

Urinary potassium excretion (K^+) during the period of the 5hr collection in the control animals was found to be 18.72 ±1.66 meq/kg. In the standard group which received 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in the K^+ excretion i.e. 25.50 ± 2.85 meq/kg (P<0.001), but there was a significant decrease in the K^+ excretion in the test group i.e. 14.51 ± 0.04meq/kg (P<0.001).

Urinary Chloride

Urinary Chloride (Cl^-) during the period of the 5hr collection in the control animals was 41.31 ± 2.56 meq/kg. In the standard group which were treated with 2.5 mg/kg of hydrochlorothiazide, there was a significant increase in the Cl^- excretion i.e. 92.47± 3.65 meq/kg (P<0.001) and in the test group, the Cl^- excretion was significantly greater than that of the control group but lesser than that of the standard group i.e. 84.05 ± 2.89 meq/kg (P<0.001).

DISCUSSION

Herbs and botanicals offer a natural safeguard against diseases and are a substantial treatment for certain diseases.Diuretics have proved to be extremely valuable in the treatment of mild to moderate hypertension and also in enhancing the effect of other antihypertensive agents. Diuretics relieve pulmonary congestion and peripheral oedema. They are used to induce forced diuresis in cases of barbiturate poisoning and also in the prevention of recurrent renal calculi [18]. A number of herbs cause diuresis, but most promising ones at the present time are Foeniculumvulgare, Fraximus excelsior, Hibiscus sabdariffa and Spegulariapurpurea [19]. They decrease the plasma volume and subsequently the venous return to the heart. This decreases the cardiac work load, the oxygen demand and the plasma volume, thus lowering the blood pressure and they are the first line of drugs in the treatment of mild to moderate hypertension along with sodium restriction in the diet. It has beendocumented 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 ethnopharmacological 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 ischaemia/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 [20].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 [21]. Most of the experimental evaluations of diuretics were done on rats and dogs [22]. We used guinea pigs in our study, as there were only few studies on the evaluation of the diuretic action in vivo in guinea pigs.

Benincasa hispida belongs to the family, Cucurbitacae and it is also known as wax gourd, Chinese winter melon and fuzzy melon (English). Because of the diversity in the languages and the dialects, this plant has different vernacular names like Pethakaddu (Hindi), Boodidagummadikaya (Telugu), Boodagumbala (Kannada), Chalkumra (Bengali) and Kusmanda (Sanskrit). Benincasa hispida is found throughout Asia in the tropical regions and is used as both food and medicine. It is cultivated throughout the plains of India and on hills up to 4000ft high. Its seeds, fruits and fruit juice are used. The constituents of the fruit are moisture: 96%, protein: 0.4%, Fat: 0.1%, carbohydrates: 63.2%, minerals: 0.3% andvitamin B: 211U/100g.

Acute toxicity studies which have been conducted on albino rats did not show any change in the behavioural pattern and no mortality was observed at the given doses.

It was observed that in albino rats, the maximum diuretic response was obtained at 100 mg/kg (oral dose). An increase of dose to 200mg/kg did not produce any further diuretic effect, as shown in [Table/Fig 1]. Hence, the dose of 100 mg/kg has been considered as an effective dose and further experimentation was done by usingthe dose of 90 mg/kg, which was extrapolated according to the body surface area inguinea pigs. There was an increase of 118% (P<0.001) and 90% (P<0.001) in the urinary volume in the standard and the test groups respectively. The sodium excretion was increased by 81% (P<0.001) and 63% (P<0.001) in the standard and the test groups respectively. There was a percentage increase of 36% (P<0.001) and a decrease of 23% (P<0.001) in the urinary Potassium excretion in the standard and the test groups respectively. There was a percentage increase of 123% (P<0.001) and 103% (P<0.001) in the urinary chloride excretion in the standard and the test groups respectively. All the values which have been mentioned above are in comparison withthe controls.

Similar studies which were done with ethanol and an aqueous extract of Benincasa cerifera showed almost the same results [23], except that there was adecrease in potassium loss in our study. Those studies showed a significant increase in the Na+ and K+ excretion as compared to that in thecontrol group. So, in our study, 90 mg/kg of Benincasa hispida rind extract showed a significant loss of sodium and chloride and a significant decrease in potassium loss. This may be due to the use of a large number of animals i.e. 54 animals in three groups, i.e.18 in each group. The K^+ sparing effect of this extract may be due to other ingredients which are present only in the rind (pericarp) of the fruit. The mechanism of this effect has been assumed to be due to the aldosterone antagonist action, as well as the Na channel blockade in the collecting ducts, which has to be further elucidated.

The role of Benincasa hispida as a diuretic has been conformed in our study. The active principles which are responsible for the diuretic effectof the extracts of this plant have not yet been elucidated, but a preliminary phytochemical 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. 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 [24]. The overall mechanism seems to be the inhibition of the tubular reabsorption of water and anions [25] 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 a antihypertensive.

CONCLUSION

The test drug produced a significant increase in the excretion of the 3 parameters (urinary volume, urinary Na^+ and urinary Cl^-) and a significant decrease in urinary K^+ excretion. The diuretic effect which was produced by the test drug was less ascompared to that

which was produced by hydrochlorothiazide. From this study, it may be concluded that the aqueous extract of Benincasa hispida produces mild diuresis and that it has a K^+ sparing action, which supports the traditional use of the Benincasa fruit extract in the treatment of different oedemas and also as a antihypertensive which produces diuresis.

However, further studies are needed to elucidateits exact mechanism of action.

REFERENCES

[1] Asolkar LV, Kakker KK, Chahre OJ. Glossary of Indian medicinal plants, National Institute of Science and Communication, New Delhi, 2000:119

[2] Anil Kumar D, Ramu P. Effect of the methanolic extract of Benincasa hispida against histamine and acetylcholine induced bronchospasm in guniea pigs. *Indian J Pharmacol* 2002; 34:365-6.

[3] Gover JK, Rathiss. Anti-inflammatory activity of the fresh juice of Benincasahispida. *Indian J Pharmacol* 1994;26:66.

[4] Dong MY, Lumz, Yin QH, Feng WM, XuJX,Xu WM. Study of Benincasa hispida contents effective for protection of kidney. *Jiangsu J Agricultural Sciences* 1995;1(3):46-55.

[5] G.R. Battu, S.N. Mamidipalli, R. Parimi, R.K.Viriyala, R.P.Patchula, L.R. Mood. Hypoglycemic and Anti-hyperglycemic Effect of the Alcoholic Extract of Benincasa hispida in Normal and in Alloxan Induced Diabetic Rats. *Pharmacognosy Magazine*, 2007;3(10): 101-105.

[6] Chandan Roy, T.K. Ghosh, Debjani Guha. Dose Dependent activity of Benincasahispida on Colchicine Induced Experimental Rat Model of Alzheimer's Disease, *International Journal of Pharmacology*, 2008; 4(4):237-244.

[7] Vrushabendra Swamy Bhyrapur Mathad, Sridhar Chandanam, Sreenivasa Rao Thirumala Setty, Dhanapal Ramaiyan, Balamuralidhar Veeranna, Ashoka Babu Vechham Lakshminarayana Settry. Antidiarrheal Evaluation of Benincasahispida (Thunb) Cogn Fruit Extracts, *Iranian Journal of Pharmacology and Therapeutics*, 2005; 4(1):24-27, 2005

[8] Beena V. Shetty, AlbinaArjuman, AparnaJorapur, RajashreeSamanth, Sudhir Kumar Yadav, Valliammai N, Anna DeepthyTharian, Sudha K, Gayathri M. Rao. Effect of the extract of Benincasa hispida on oxidative stress in rats with indomethacin induced gastric ulcers. *Indian Journal of Physiology & Pharmacology*, 2008;52 (2):178–182.

[9] Manish A. Rachchh, Sunita M. Jain. Gastroprotective effect of the Benincasa hispida fruit extract. *Indian Journal of Pharmacology*, 2008; 40(6):271-275.

[10] A. Kumar, R. Vimalavathini. Possible anorectic effect of the methanol extract of Benincasa hispida (Thunb). Cogn, fruit. *Indian Journal of Pharmacology*, 2004; 36 (6):348-350.

[11] D. Anil Kumar, P. Ramu. Effect of the methanolic extract of Benincasahispida against histamine and acetylcholine induced bronchospasm in guinea Pigs. *Indian Journal of Pharmacology* 2002; 34:365-366

[12] Kumar A, Rama II. Antiulcer properties of the methanolic extract of Benincasa hispida (Thunb.) Cogn. *Indian Drugs* 2002; 39:9-13.

[13] Nadkarni AK In: Indian Materia Medica. PapularPrakashan. Bombay, India 1976, p. 185-6.

[14] Wollen weber E, Faure R, Gaydou EM. A rare triterpene as a major constituent of the "wax" on the fruits of Benincasa hispida. *Indian drugs* 1991; 28(10):458-460.

[15] Yashizumi S, Murakam T, Kadoya M, Matsuda H, Yamahara J, Yoshikava M. Histamine release inhibitors from was guard the fruits of Benincasa hispida (thunb). *Yakugaku Zassi*. 1998;118(5):188-192.

[16] R. M. Mehta; Pharmaceutics I; Extraction Processes; Maceration; 2002; page 149.

[17] H. Gerhard Vogel. Diuretic and saluretic activity. Drug discovery and evaluation Pharmacological assay, 2nd edition. Germany, Springer-Verlag Berlin Heidelberg, 2002;323.

[18] R.S Satoskar, S.D Bhandarkar, NirmalaRege. Pharmacotherapy of hypertension. Pharmacology and pharmacotherapeutics, revised 21st edition. New Delhi, Popular Prakashan, 2009; 420.

[19] Cl Wright, L. Van buren et al. Herbal medicines as diuretics- A review of the scientific evidence, *Journal of Ethnopharmacology* 2007;114(1):1-31.

[20] Bhalodia Y, Kanzariya N, Patel R, Patel N, Vaghasiya J, Jivani N, Raval H. Renoprotective activity of Benincasa cerifera fruit extract on ischemia/reperfusion-induced renal damage in rat. *Iran J Kidney Dis*. 2009 Apr; 3(2);80-5.

[21] Klattp, Muschaweck, Method of collecting urine and comparative investigation of Quantities excreted by cats and dogs after furosemide. *Am J. Vet Res* 1997; 36:919-23

[22] Tanaka S, Kanda A, Ashida SI. Uricosuric and diuretic actitives of Dr-3438 in dogs and rabbits. *Japan J pharmacol* 1990; 54:307-14

[23] Ramadas. V. Pandhare, Effect of Aqueous and Ethanol Extracts of Benincasa cerifera on Diuresis and Urinary Electrolytes excretion in rats. *Drug Invention Today*, 2010;2(6):308-10.

[24] Maghrani M, Zeggwagh N, Haloui M, Eddouks M. Acute diuretic effect of aqueous extract of Retamaraetam in normal rats. *J. Ethnopharmacol*. 2005;99:31-35.

[25] Pantoja CV, chiang LCH, Norris BC, Concha JB. Diuretic, natriuretic and hypotensive effects produced by Allium sativum (garlic) in anaesthetized dogs. *J. Ethnopharmacol* 1993;31:325-331.

AUTHOR(S):

1. Dr T. Jayasree
2. Dr K Kiran Kishore
3. Dr. M.Vinay
4. Dr P Vasavi
5. Dr N Chandrasekhar
6. Dr. V S Manohar
7. Dr. Rohit Dixit

PARTICULARS OF CONTRIBUTORS:

1. Corresponding Author.
2. Dr K Kiran Kishore (MD- Asst Prof)
   Department of Pharmacology, Mamata Medical College, Khammam- 507002, Andhra Pradesh, India.
3. Dr. M.Vinay (MD- Asst Prof)
   Department of Pharmacology, Mamata Medical College, Khammam- 507002, Andhra Pradesh, India.
4. Dr P Vasavi (MD- Asst Prof)
   Department of Pharmacology, Mamata Medical College, Khammam- 507002, Andhra Pradesh, India.
5. Dr N Chandrasekhar (MBBS- Post graduate)
   Department of Pharmacology, Mamata Medical College, Khammam- 507002, Andhra Pradesh, India.
6. Dr. V S Manohar (MBBS- Post graduate)
   Department of Pharmacology, Mamata Medical College, Khammam- 507002, Andhra Pradesh, India.
7. Dr. Rohit Dixit (MBBS- Post graduate)
   Department of Pharmacology, Mamata Medical College, Khammam- 507002, Andhra Pradesh, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. T. Jayasree M.D, Professor & Head, Department of Pharmacology, Mamata Medical College,
Khammam- 507002, Andhra Pradesh, India.
Phone No: +919848127116; E-mail: drtjayasreemd@gmail.com

DECLARATION ON COMPETING INTERESTS:

No competing Interests.

Date of Submission: **Apr 1, 2011**
Date of per review: **May 4, 2011**
Date of acceptance: **May 9, 2011**
Online first: **May 21, 2011**
Date of Publishing: **Jun 13, 2011**