DIURETIC AND CENTRAL NERVOUS SYSTEM DEPRESSANT EFFECTS OF THE ETHANOLIC EXTRACT OF *BENINCASA HISPIDA* SEEDS

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Summary

The seeds of *Benincasa hispida* (Thunb) COGN. (Family: Cucurbitaceae) were extracted with ethanol and was used to study acute toxicity, diuretic and CNS depressant effects. The extract was non lethal to the mice up to the dose of 5000 mg/kg *b.w.* At doses of 250 and 500 mg/kg *b.w*, the extract significantly ($P < 0.001$) increased the diuretic effect in a dose dependent manner in rats. Similarly, at doses of 250 and 500 mg/kg *b.w* the extract significantly ($P < 0.05$) produce CNS depressant effect in rats. These results indicate that ethanolic extract of *Benincasa hispida* possesses potent diuretic and CNS depressant effects and thus pharmacologically justifying its folkloric use in the management of diuresis and CNS depressant conditions.

Key words: *Benincasa hispida*, diazepam, furosemide, diuretic, depressant effects, acute toxicity.

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Introduction

Herbal medicines are most widely used in the traditional medicinal practice worldwide [1]. According to the WHO, more than 80% of the world's population-primarily those of developing countries relies on plants and plant-derived medicines for their healthcare [2]. Medicinal plants have also been used in the development of new drugs and continue to play an invaluable role in the drug discovery process [3, 4]. Diuretics play an important role in situations of fluid overload, like acute and chronic renal failure, hypercalciuria and cirrhosis of liver and also as an antihypertensive agent. A number of diuretics like mannitol, thiazides, frusemide, and ethacrinic acid are used in practice. Still there is a need for more effective and less toxic diuretic. Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system of medicine but they were not properly investigated [5, 6].

A large number of compounds, drugs are available which depress the central nervous system (CNS) and hypotonic effects [7, 8]. The general action of the hypnotics and sedatives is that of the depression of the CNS, which begins with the cortex and descends with increasing dosages to medullary centers. Certain compounds act at different points in the cortex and give the best therapeutic effect. The hypnotics and sedatives are usually classified into two categories: the barbiturates and non-barbiturates. Barbiturates reduce cerebral activity, which again reduces the cerebral metabolic rate probably by activating chloride channels and potentiating GABA's effects on these channels. Protection of the brain against hypoxia might theoretically occur by this mechanism, by vasoconstriction or by inhibiting calcium or glutamate [9].

Literature survey revealed that the plant extract has yet not been screened for its traditional diuretic and CNS depressant activity in experimental animals. Therefore the present study was carried out to provide pharmacological evidence for the folklore medicinal consideration of seeds of *Benincasa hispida* as diuretic and CNS depressant activity.

Benincasa hispida (Thunb) COGN. is employed traditionally to treat disorders such as dry-cough, fever, urethral discharges, biliousness, thirst. It acts as brain tonic and also possesses anti-helminthic property. In China, it is used in the treatment of appendicitis. Oil from seeds is soporific, good for the brain and liver and effective in the treatment of syphilis. Seed ash is a prized remedy for gonorrhea; ash is applied to painful wounds and swellings [10-12]. The present study was carried out to scientifically prove some of the folkloric use of this plant in conditions of diuresis and CNS depressant by exploring diuretic and CNS depressants effects.

Material and Methods

Collection and authentication of plant material

The seeds of *Benincasa hispida* were collected in the month of December 2006 and the seeds were authenticated by Dr. Marimuthu, Professor, Dept. of Botany, Govt. Arts and Science College, Salem and the specimen of *Benincasa hispida* bearing reference number 106/COL./219 was kept in the museum of Vinayaka Mission's College of Pharmacy, Salem for future reference [13].

Preparation of extract

The seeds of *Benincasa hispida* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No 40 and stored in an airtight container for further use. The dried powdered seed of *Benincasa hispida* was defatted with petroleum ether (60-80 ^{o}C) in a Soxhlet apparatus. The defatted powder material thus obtained was further extracted with chloroform, acetone, ethanol and water. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator. The extractive values are represented in Table [13].

Experimental Animals

Studies were carried out using male *Wistar* Albino rats (150-200 g). They were procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd., Bangalore, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee [13].

Preliminary Phytochemical Screening

The ethanolic extract of *Benincasa hispida* were tested for the presence of carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, gums and mucilages, saponins, proteins and free amino acids, phenolic compounds, tannins and flavonoids [13].

Diuretic Activity

Male rats (wister albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. The method of Lipschitz *et al* [14, 15] was employed for the assessment of diuretic activity. The experimental protocols have been approved by the Institutional Animal Ethical Committee. Four groups of six rats in each and were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control, received normal

saline (25ml/Kg, *p.o.*); the second group received furosemide (100mg/Kg, i.p.) in saline; the third, fourth groups received the Alcohol and Aqueous extract at the doses of 100 mg/Kg, respectively, in normal saline. Immediately after administration the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and feaces , kept at room temperature of 25± 0.5ºC throughout the experiment. The urine was collected in measuring cylinders up to 3 hrs after dosing. During this period, no food or water was made available to animals. The parameters taken for individual rat were body weight before and after test period, total concentration of Na^+, K^+, and Cl^- in the urine. Na^+, K^+ concentrations were measured by Flame photometry [16] and Cl^- concentration was estimated by titration [17] with silver nitrate solution (N/50) using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20 mg/Kg of furosemide per kg body weight in series of supportive experiments. Results are reported as mean ± SD, the test of significance ($p<0.01$ and $p<0.05$) was stastically.

Cocaine-induced hyperactivity experiments in rats

Male wistar albino rats were divided in to four groups of eight in each used. The animals were removed from the holding room and randomly assigned to treatment groups. Animals received either the vehicle or S. emarginatus (50, 100 & 200 mg/kg) for fifteen days and were placed in the activity cages. Following the 30 min of habituation period on the test day, the animals received cocaine (40 mg/kg i.p.) and were returned to the activity cages for a further 90 min. Activity was measured as light beam interruptions per 10 min period [18].

Effect of phenobarbitone sodium–induced sleeping time

Swiss albino mice were divided into four groups of eight in each. On the test day animals received 40 mg/kg (i.p.) phenobarbitone sodium 30 min after the administration of methanol extract of S. emarginatus at the dose of 50, 100 and 200 mg/kg and vehicle control 1 ml of 5% CMC. The sleeping time was recorded, and measured as the time interval between the loss and regaining of the righting reflex [19].

Statistical analysis

All the results are expressed as mean ± standard error. The data was analyzed statistically using ANOVA [20] at a probability level of $P < 0.001$.

Results

Present study shows that the alcoholic extracts of *Benincasa hispida seeds* possess good diuretic activity. Urine volume, cation and anion excretion were increased, Na^+/K^+ ratio of 2.04 and 2.18 were obtained for aqueous and alcoholic extract respectively. The normal value for Na^+/K^+ ratio is reported to be 2.05 – 2.83. The concentration of aldosterone is found to be dependent on Na^+/K^+ ratio.

If the Na^+/K^+ ratio falls below the normal in plasma the aldosterone secretion will be decreased and if the ratio rises above the normal value the aldosterone secretion will be increased. Significant increase in Na^+, K^+ and Cl^- ion excretion was observed in aqueous and alcoholic extract treated animals but it was less than the furosemide control. Further studies are required to assess the medicinal value of leaves of *Benincasa hispida as* a potential diuretic agent (Table 1).

Diuretics relive pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure [21]. Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that ethanol, aqueous and chloroform extract may produce diuretic effect by increasing the excretion of Sodium, Potassium and Chloride. The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to

maintain proper function of cardiac and skeletal muscles [22]. The regulation of Sodium, Potassium balance is also intimately related to renal control of acid-base balance. The Potassium loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended [23]. In present study ethanolic extracts showed elevated levels of Potassium in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. Active principles such as flavanoids, saponins and terpenoids are known to be responsible for diuretic activity [24-26]. Results of present investigation showed that ethanol is most effective in increasing urinary electrolyte concentration of all the ions i.e Sodium, Potassium and Chloride followed by chloroform and aqueous extracts while other extracts did not show significant increase in urinary electrolyte concentration. A complex set of interrelationships exists among the cardiovascular system, the kidneys, the central nervous system (Na +, appetite, thirst regulation) and the tissue capillary beds (distribution of extracellular fluid volume), so that perturbation at one of these sites can affect all the remaining sites. A primary law of the kidneys is that Na^+ excretion is a steep function of mean arterial blood pressure (MABP) such that small increase in MABP cause marked increase in Na^+ excretion [27]. One of the earliest strategies for the management of hypertension was to alter Na^+ balance by restriction of salt in the diet. Diuretic agents having antihypertensive effects were used alone and had greater efficacy than all other antihypertensive drugs.

The effect on the CNS of the different dose of ethanolic extract of *Benincasa hispida* has produced a significant increase in the hypnotic effect induced by the phenobarbitone, in a dose dependent manner, thus suggesting a profile sedative activity. It is emphasized that the method employed for this assay is considered as a very sensitive way and denote agent with depressor activity on the CNS. The sedative effect recorded in this study may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts.

Administration of cocaine to rats, which releases both dopamine and noradrenalin, causes a cessation of normal 'ratty' behavior (exploration and grooming), and the appearance of repeated 'stereotyped' behavior (rearing, gnawing and so on) unrelated to external stimuli. These effects are prevented by dopamine antagonists and by destruction of dopamine-containing cell bodies in the midbrain, but not by drugs that inhibit the noradrenergic system. The cocaine-induced motor disturbances in rats probably reflect hyperactivity in the nigrostriatal dopaminergic system. Numerous studies have demonstrated that dopamine antagonists prevent the hyperactivity following cocaine administration in mice and rats. For example, haloperidol and clozapine in a dose-dependent manner attenuated the locomotor effects of cocaine [21]. SCH23390, the selective D_1 antagonist, also significantly suppressed the locomotor response produced by cocaine. These data suggest that both D_1 and D_2 receptors are involved in cocaine-induced hyperactivity [21-22].

In this study pharmacological evaluation of diuretic action of ethanolic extracts of *Benincasa hispida* was evaluated using furosemide under controlled laboratory condition. As diuretic therapy may lead to number of life threatening electrolytic disorder and toxicities, so safety profile studies are carried out following a sub chronic administration of extracts. This amplifies the heterogeneous array of diuretic curatives available for safe and effective treatment of edema and cardiovascular diseases [28]. Also, in the present study, ethanolic extracts of *Benincasa hispida* produced a partial reduction in the hyperactivity produced by cocaine.

Conclusion

The extract of Benincasa hispida seeds has diuretic and CNS depressant effect supporting the folkloric use in the management of diuresis and CNS depressant conditions. This effect may be explored in the use of the plant in the management of some cardiovascular diseases.

Table No. 1 Effect of ethanolic extract of *Benincasa hispida* seeds on excretory parameters.

Design	Dose	No. of Rats Used	Urine Volume (ml)	Electrolyte Excretion			Total Chloride µ Moles/kg
				Na^+ µ Moles/kg	K^+ µ Moles/kg	Na^+/K^+ ratio	
Normal Saline	25ml/kg	6	3.2±11	1987±22	906±21	2.230	531.44
EEBH	250mg/kg	6	5.1±1.2	3062±23*	1467±43*	2.520	2014±11
EEBH	500mg/kg	6	2.5±0.52	2080±32	1112±13	1.675	2110±36
Furosemide	100mg/kg	6	3.6±0.46	2899±05	1645±213	1.450	22.56±105

EEBH stands for ethanolic Extract of *Benincasa hispida* seeds
The values are expression of the mean standard error *P<0.001 vs Control

Table No. 2 Effect of Methanol extract of *Benincasa hispida* seeds on cocaine induced hyperactivity.

No. of light interruptions at an interval of 10 minutes	Experiment & Dose		
	CMC (5% ml)	Extract (250mg/kg)	Extract (500mg/kg)
10	215±2.13	180±0.85	165±0.74
20	170±1.63	155±0.52*	145±0.58*
30	130±1.28	100±0.43*	90±0.49*
40	175±1.68	100±0.27*	90±0.31*
50	185±1.75	110±0.99*	80±0.78*
60	160±1.56	100±0.96*	80±0.68*
70	140±1.37	95±0.88*	70±0.68*
80	130±1.28	80±0.78*	60±0.56*
90	100±0.98	70±0.63*	50±0.46*

Values are the number of entries in 3 min (mean + S.E.M., n = 8); *Significant difference between control group and treated group; P<0.05, ANOVA followed by Dunnett's Multiple comparison test.

Table No. 3 Effect of methanol extract of *Benincasa hispida* seeds on phenobarbitone sodium–induced sleeping time.

Experiment	Dose	Sleeping time (min)
CMC	5% 1ml	64±5.9
Extract plus Phenobarbitone sodium	250mg/kg	82±7.4*
	500mg/kg	112±7.3*

Values are expressed as mean + S.E.M., *n* = 8; * Significant difference between control group and treated

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