Anthelmintic and Anticonvulsant studies of ethanolic extract of *Benincasa hispida* seeds.

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Summary

The ethanolic extract of *Benincasa hispida* seeds was studied for its anthelmintic activity using earthworms (*Pheretima posthuma*) and anticonvulsant activity in *Swiss* albino mice. Maximal electroshock (MES) seizures were induced by exposing mice to 150 mA current for 0.2 seconds. The ethanol extract of *Benincasa hispida* were prepared in a soxhlet apparatus. Ethanol extract took less time to produce paralysis and death of earthworms and the activity increased with increasing concentrations. *Benincasa hispida* ethanol extract at the dose levels of 250 and 500 mg/kg p.o. produced significant ($P<0.01$) anticonvulsant effect. Phenytoin sodium (25 mg/kg, *i.p*) was used as positive control. However, 500 mg/kg dose offered greater anticonvulsant activity but the activity was less than the positive control.

Keywords: Maximal electroshock, mebendazole, epilepsy, anticonvulsant activity, phenytoin.

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Introduction

The uses of plant and animal parts for medicines have long been in existence and are widely documented in ancient India, China and Egypt. These ancient indigenous practices were discovered by a series of "trial and error" which then could not be substantiated by proven scientific theories. However, these practices have produced results of proven efficacies compared to conventional modern medicine [1]. In recent times, herbal medicines have become indispensable and are forming an integral part of the primary health care system of many nations. A survey (1977) in USA indicates an expected 20% annual growth in herbal medicine in the next 5 years with an estimated 80% of the world population living in the developing countries would rely on plants for health [2]. In view of this large dependence on traditional health practices, the World Health Organization (WHO) in 1978 recognized the implicit role of herbal medicine in the treatment of various disorders. Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterized by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, asynchronous and firing of nerve impulse by the neurons in the brain [3]. Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per 100,000 [4]. It has been observed that the currently available antiepileptic drugs were unable to control seizures effectively in as much as 25% of the patients [5]. The conventional antiepileptic agents like phenytoin, carbamazepine and sodium valproate carry with them several serious side effects notably neurotoxicity [6]. As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. Newer antiepileptics like gabapentin, vigabatrin, lamotrigine, etc are used supplemental to the conventional agents [3]. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in terms of drug related toxicity. The aim of treating epilepsy is not only to abolish the occurrence of seizures but also to lead a self sustained life. The aim of our study is to explore the anthelmintic and anticonvulsant activity of the seeds of *Benincasa hispida* using earth worms and albino mice.

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 [7].

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 °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 [7].

Experimental animals
Male *Swiss* albino mice (18-23 g) were procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were grouped and housed in polypropylene 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 a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Preliminary phytochemical screening
The ethanolic extract of *Benincasa hispida* was 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 [7].

Anthelmintic activity
Earthworms (*Pheretima posthuma*) collected from moist soil were washed with normal saline to remove sand and fecal matter adhering and were then used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [8]. Mebendazole was diluted with normal saline to obtain 20 mg/ml was poured into petridish and served as standard. The ethanol extract of *Benincasa hispida at* concentrations of 5, 10 and 20 mg/ ml were prepared with normal saline. Control group was tested only with normal saline. Six Earth worms of size about 3-5 cm were placed in each petridish at room temperature. The time taken to complete paralysis and death were recorded [9]. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors.

Anticonvulsant tests
Twenty four adult Swiss albino mice were divided into four groups of six animals in each. Group I mice received distilled water and served as a negative control, group II received 25 mg/kg *i.p.* phenytoin sodium serving as positive control, mice of group III and IV received 250 and 500 mg/kg of *Benincasa hispida* ethanol extract respectively. Maximal electroshock (MES) were induced by passing 50 mA, alternating current for 0.2 s through electrodes on mice eye sockets [10, 11]. The number of animals protected from tonic hind limb extension was determined in each dose group.

Results and Discussion

The dried seeds of *Benincasa hispida* (Thunb.) COGN. were extracted with solvents of increasing polarity by Soxhlet apparatus. The percentage yield of the seeds of *Benincasa hispida* (Thunb.) COGN. were found to be 3.2% , 1.3% , 1.1% , 7.2% and 4.1% with petroleum ether, acetone, chloroform, alcohol and water, respectively. The percentage yield of the ethanolic extract of *Benincasa hispida* (Thunb.) COGN. were found to be greater (7.2%) than extracts with other solvents [7].

The preliminary phytochemical screening of the dried seed extract showed the presence of carbohydrates, phenolic compounds, amino acids and proteins, flavonoids and sterols. In acute toxicity study, ethanolic extract of *Benincasa hispida* (Thunb.) COGN. showed to be safe and no mortality was observed at a dose as high as 5 g/kg.
The data revealed that ethanolic extract of *Benincasa hispida* showed significant anthelmintic activity at 20mg/ml concentration. Results are comparable with standard drug Mebendazole. Table No. 1 reveals that ethanolic extract of *Benincasa hispida* showed best anthelmintic activity than compared with other two concentration of the same extract.

In the maximum electroshock induced seizure model, ethanolic extract of *Benincasa hispida* 250 and 500 mg/kg doses and diazepam showed significant ($p<0.01$) reduction in duration of convulsion, but extract of *Benincasa hispida* 500 mg/kg exhibit anticonvulsant effect more than that of 250 gm/kg body weight shown in Table No. 2. The most popular and widely used animal seizure models are the traditional maximum electroshock-induced seizure. The maximum electroshock-induced seizure test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures.
The maximal electroshock test is the most widely used animal model in antiepileptic drug discovery, because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high [12, 13]. The maximal electroshock test identifies agents with activity against generalized tonic clonic seizures using clinically established antiepileptic drugs [12]. The pharmacology of acute maximal electroshock dose not differs from the pharmacology of generalized tonic-clonic seizures in genetic models with chronic epilepsy, e.g., Audiogenic seizure susceptible mice and rats or epileptic gerbils [13]. In addition to identifying drug activity against generalized tonic-clonic seizures, it has often been proposed that the maximal electroshock test predicts anticonvulsant drug effects against partial seizures. The anticonvulsant activity of *Benincasa hispida* 250 and 500 mg/kg, in maximal electroshock model indicates that *Benincasa hispida* might be precipitate the tonic and clonic seizures in dose dependent manner.
From the above study it was concluded that, the *Benincasa hispida* exhibits anthelmintic activity in dose dependent manner by paralyzing the earth worms and anticonvulsant activity and the probable mode of action may be due to GABAaminergic mediation, glycine inhibitory mechanism and inhibit the electrical kindling effect.

Table No. 1 Anthelmintic activity of Ethanolic extracts of *Benincasa hispida* (THUNB.) COGN. using Indian adult earthworms (*Pheretima posthuma*).

Treatment	Time taken for Paralysis in min.	Time taken for death in min.
Mebendazole (STD)	3.8 ± 0.51	3.1 ± 0.4
EEBH 20mg	12.2 ± 2.3^{ns}	10.2 ± 1.2^{ns}
EEBH 40mg	5.2 ± 1.0*	3.1 ± 1.02*
EEBH 60mg	2.2 ± 0.5*	1.3 ± 0.43*

Results are expressed as mean ± SEM from six observations. Control worms were alive up to 24 hrs of observation. ^{ns} represents non significant; *represents significant
EEBH is ethanolic extract of *Benincasa hispida*.

Table No. 2 Anti-convulsant activity of extracts of seeds of *Benincasa hispida* (THUNB.) COGN. against maximal-electro shock (MES) induced convulsions.

Group	Treatment	Time in sec. in various stages of convulsion				Recovery/ Death	% Protection
		Flexion	Extensor	Clonus	Stupor		
I	Normal saline (10 ml/kg)	4 ± 0.71	9 ± 1.31	4 ± 1.05	102 ± 6.39	Recovery	-
II	Phenytoin sodium (25 mg/kg)	2 ± 0.78[c]	0	2 ± 0.63	5 ± 0.48[d]	Recovery	89.37
III	EEBH (250 mg/kg)	3 ± 0.71	3.02 ± 0.8[b]	3 ± 0.71	14.2 ± 3.64[d]	Recovery	63.78
IV	EEBH (500 mg/kg)	3 ± 0.81	1.1 ± 0.84[c]	3 ± 0.84	5.6 ± 1.54[d]	Recovery	77.78

Values are expressed by Mean ± SEM for 6 animals.
[a]P > 0.02 as compared to control.
[b]P < 0.02 as compared to control.
[c]P < 0.01 as compared to control
[d]P < 0.001 as compared to control
Data was analyzed by student's't' test.
EEBH is ethanolic extract of *Benincasa hispida*.

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