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Short Communication

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Anti-convulsant activity of *Benincasa hispida* fruit, methanol extract

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Abstract

Objective: To study the effect of methanol extract of *Benincasa hispida* fruit in various convulsive models in mice. Methods: Methanol extract of *B. hispida* was evaluated using chemo-convulsive agents such as pentylenetetrazole, strychnine and picrotoxin, and maximal electro seizures (MES) model in mice at dose levels ranging from 0.2 – 1g/kg, i.p. Results: The extract at 0.2-0.6 g/kg significantly ($p<0.001$) inhibited the hind limb extension induced by MES and at 0.4 and 0.6 g/kg, the extract significantly ($p<0.01$) increased the latency of convulsion and death induced by pentylenetetrazole and strychnine. However even at 1 g/kg, the extract failed to protect the convulsion induced by picrotoxin. Conclusion: On the basis of the present finding, we can conclude that the fruit *B. hispida* possess potential anticonvulsant activity.

Key words: *Benincasa hispida*, pentylenetetrazole, picrotoxin, strychnine, MES, anticonvulsant activity.

1. Introduction

Benincasa hispida (Thunb.) Cogn. is a fruit belonging to Cucurbitacecae family is widely used as a vegetable in India and other tropical countries. It is commonly called as ash gourd or ash-pumpkin. It is a hairy annual climber, which produces rounded fruits (gourds), which is large succulent, densely white hairy when young and has a thick white waxy deposition when mature. The fruit *B. hispida* is an important ingredient for Ayurvedic medicine "Kusmanda lehyam" which is widely used in epilepsy and other nervous disorders [1,2]. Isolated constituents of *B. hispida* reported were triterpenes, sterols and, glycoside [3], protease [4], esterase, peroxidase and, amylase [5] and volatile oils [6]. Very few studies such as the sedative nature [7] and suppression of the morphine withdrawal symptoms in mice [8] have been carried out to reveal the CNS activity of *B. hispida*. Preliminary studies in our lab on

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methanol extract of *B. hispida* revealed nootropic [9] and antidepressant activity [10] and loss of muscle tone or motor incoordination were not observed by traction and rota rod test [11]. We have undertaken in the present study on anticonvulsant property of methanol extract of *B. hispida* in various animal models.

2. Materials and methods

2.1 Plant material and extract preparation

Benincasa hispida fruit was obtained from the local market in months of August / September 2003 and its identify was further confirmed at Captain Srinvasmoorthy Drug Research Institute, Chennai. After peeling of the cuticle, the pulp freed of seeds was mashed with electric juicer and the product was macerated with methanol for seven days.

On eighth day, after filtration, the filtrate was evaporated under reduced pressure to afford a thick, brownish semi solid mass (yield 6.2 % w/w). The methanol extract of *Benincasa hispida* (MEBH) thus obtained was dissolved each time in distilled water for pharmacological testing.

2.2 Animals

Male Swiss albino mice (18-22 g) used in the study were bred and housed under standardized experimental conditions in the animal house of C. L. Baid Metha College of Pharmacy, Chennai, India. The animals were fed with standard diet (Hindustan levers, India) and water *ad libitum.* The experiments were performed after obtaining the approval of institutional animal ethics committee.

2.3 Drugs

Pentylenetetrazole, (PTZ; Sigma, Poole UK), picrotoxin (Kouch-Light lab UK) and strychnine (NP Chem, India) were used after diluting with distilled water.

2.4 Anticonvulsant activity

Different doses of MEBH were given ip 30 min before the administration of pentylenetetrazole (0.1 g/kg) [12], strychnine (0.003 g/kg) [13] and picrotoxin (0.003 g/kg) [14]. Latency of onset of convulsion and mortality time was recorded. For PTZ induced seizures both onset of tonic and clonic convulsion was noted. Cut off time of 30 min was assigned for the assessment of seizure activity.

The other anticonvulsant activity was examined by exposing the mice to maximal electro convulsive shock (MES; 50 mA for 0.2 sec) *via* small alligator clips attached to each pinna

Table 1.
Effect of methanol extract of *Benincasa hispida* on pentylenetetrazole (0.1 g/kg; ip) induced convulsions.

Treatment (n=6)	Dose (g/kg; ip)	Onset of (sec) convulsion[a]		Death time [a] (sec)	Lethality [b] (%)
		clonic	tonic		
Control	-	55.2 ± 3.1	268.8 ± 32.5	302.7 ± 16.6	100
Diazepam	0.004	126.8 ± 12.0*	1086.0 ± 125.0*	1132.3 ± 105.0*	33
MEBH	0.2	58.5 ± 10.8	262.5 ± 35.8	315.3 ± 29.6	100
	0.4	94.7 ± 15.3*	905.7 ± 123.7*	999.0 ± 141.4*	100
	0.6	355.8 ± 35.1*	983.3 ± 126.8*	1200.0 ± 0.0*	0

[a] Values are mean ± SD; [b] % of death in 24 h; n=number of animals per group
*p<0.01 *vs.* control; ANOVA followed by Dunnett's multiple comparison test.

Table 2.
Effect of acute treatment of menthol extract of *Benincasa hispida* (MEBH) on mice against strychnine (0.003 g/kg, ip) and picrotoxin (0.003 g/kg, ip) and MES induced seizures.

Treatment	Dose (g/kg, ip)	Strychnine (n=6)		Picrotoxin (n=6)		Animals with incidence of hind limb extension induced by MES (n=10)
		Onset of convulsion (sec)	Death time (sec)	Onset of convulsion (sec)	Death time (sec)	
Control	-	160.8 ± 15.0	264 ± 39.8	206.7 ± 22.3	315.7 ± 49.3	10/10
Diazepam	0.006	278.8 ± 58.7*	880.0 ± 123.9*	345.0 ± 53.2*	713.5 ± 142.5*	-
Phenobarbitone	0.03	-	-	-	-	2/10#
MEBH	0.2	166.6 ± 26.6	235.0 ± 14.2	NT	NT	
	0.4	330.0 ± 62.9*	520.0 ± 72.7*	201.7 ± 21.4	319.2 ± 60.9	4/10#
	0.6	365.0 ± 85.7*	563.3 ± 219.7*	209.0 ± 18.3	343.2 ± 17.8	2/10#
	1.0	NT	NT	243.8 ± 26.7	418 ± 61.8*	1/10#

Values are mean ± SD; NT- Not Tested; n = number of animals per group
*p<0.01 *vs.* control; ANOVA followed by Dunnett's multiple comparison test. # p<0.001 *vs.* control; Fisher's exact test.

through an electroconvulsive meter (Inco, India) after 30 min administration of the extract. The presence or absence of hind limb extension was noted [15]. Control animals received ip, 10ml/kg of distilled water whereas MES group received phenobarbitone (0.03 g/kg), and diazepam (0.006 g/kg) tested against strychnine and picrotoxin groups and for PTZ group 0.004g/kg of diazepam. All reference drugs were administered ip, 30 min prior to the administration of convulsion stimuli.

2.5 Data analysis

Results were all expressed as mean ± SD. Statistical analysis were carried out using Fisher's Exact test and ANOVA followed by Dunnett's multiple comparison test wherever required.

3. Results

In PTZ induced convulsion MEBH (0.4 and 0.6 g/kg) increased significantly ($p<0.01$) the latency of onset of tonic and clonic convulsion in a dose dependant manner. MEBH at 0.6 g/kg provided 0 % lethality (Table 1). MEBH at doses of 0.4 and 0.6 g/kg significantly increased the latency of convulsion onset ($p<0.01$) and death onset respectively, induced by strychnine when compared to control (Table 2).

While even at 1g/kg, MEBH was not able to produce any significant increase in latency of convulsion or death induced by picrotoxin (Table 2). In MES induced seizures, MEBH at all dose levels (0.2-0.6g/kg) dose dependently was able to significantly ($p<0.001$) inhibit the hind limb extension (Table 2).

4. Discussion

The results reveal the protection against MES, PTZ and strychnine induced seizures by methanol extract of *Benincasa hispida* though not in picrotoxin induced convulsion. As picrotoxin is a GABA-A receptor blocker [14] and therefore from the present study and also taking into account form the previous study [11], wherein MEBH at various dose levels, produced no loss of muscle tone (traction test),

or loss of muscle co-ordination (rota-rod test), it can be suggested that MEBH has minor influence in the GABAergic system.

As these convulsive models involve various neurotransmitters with different mechanism, further pharmacological studies are required to reveal the mechanism through which the constituents of the extract act to bring about the anticonvulsant action. At present bioassay guided separation is attempted. Nevertheless the study validates the use of *B. hispida* in Indian traditional system of medicine for epilepsy.

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