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Preliminary pharmacological screening of *Benincasa hispida* Cogn.

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Abstract

Objective :To carry out the preliminary pharmacological screening of methanolic extract of fruit of *Benincasa hispida* Cogn. Materials and methods: The extract was tested for effect on general pharmacological effects like spontaneous motor activity, muscle relaxant activity, antihistaminic effect and effect on barbiturate induced hypnosis. Results: The extract caused reduction in spontaneous motor activity with no muscle relaxant activity. The extract significantly potentiated the barbiturate induced hypnosis, and showed significant antihistaminic activity. Conclusion: The methanolic extract of fruit of *Benincasa hispida* has antihistaminic activity and could be further explored.

Keywords: *Benincasa hispida,* Cucurbitaceae, histamine.

1. Introduction

Benincasa hispida cogn. (Cucurbitaceae) is a tendril climber which is cultivated throughout India in plains and hills. [1] Fruits of *Benincasa hispida* are traditionally used as nutritive, in insanity, epilepsy and other nervous diseases [2]. Two 'triterpenes' namely 'alonusenol' and 'multiflorenol' from methanol extract of *B. hispida* fruit was found to have potential inhibitory effect on the histamine release from the rat exudates cells induced by antigen antibody reaction [3].

This study was designed to evaluate the potential CNS and antihistaminic effect of *B.hispida* in experimental animals.

2. Materials and methods

2. 1 Collection and identification of B. hispida fruit

Well mature fruit of *B.hispida* was purchased from the local market in the month of September and its authenticity was confirmed by comparing with standard herbarium specimen [4].

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2.2 Preparation of methanolic extract of B. hispida fruit

The pulp of the fruit was mashed to a fine slurry in a grinder, and extracted with methanol by maceration. A semisolid mass was obtained (yield 2.95% w/w) after complete elimination of solvent under reduced pressure below 60° C. This extract was stored in a refrigerator and protected from direct sunlight.

2.3 Experimental Animals

Adult albino mice (20 - 30 g), albino rats (130 - 150 g) and Guinea pigs (200 - 300 g) obtained from Kings Institute, Guindy, Chennai were used throughout the study. The animals were maintained on a 12 h light / dark cycle and allowed free access to food and water.

2.4 Toxicity study

An acute toxicity study relating to the determination of LD_{50} was performed with different doses of the extract according to the method described by Karber. [5]

2.5 General behaviour profile

General behavioural profile was evaluated by the method of Dixit and Varma (1976) [6] and Mukherjee et al (1996) [7]. Adult albino male mice were divided into five groups (n=10). The methanolic extract (200, 400 and 600 mg/kg, i.p.) was administered to groups 1, 2 and 3, respectively. Group 4 and 5 were treated with Chlorpromazine (5 mg/kg) and normal saline (10 ml/kg), respectively. Activity was observed at 30 min intervals for 1 h and at 1 h intervals for 4 h.

Awareness, alertness and spontaneous activity - These responses were tested by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behaviour.

Touch response - This was noted when the mice were touched with a pencil or forceps (i.e. on the side of the neck, abdomen and groin).

Sound response - Albino mice normally utter no sound; vocalization may indicate a noxious stimulus.

Pain response - This was graded when a small artery clamp was attached to the base of the tail.

2.6 Muscle relaxant activity

Muscle relaxant activity was determined using the traction test and the rotarod test.

Traction test - This test was conducted in groups of ten animals, 30 min. after injection

Table 1
Effect of *B. hispida* extract and Chlorpromazine on general behavioural profile.

Behaviour type	**Methanolic Extract (mg/kg)**			**Chlorpromazine (5 mg/kg)**	**Saline (10 ml/kg)**
	200	**400**	**600**		
Spontaneous activity	+	+++	++++	++++	-
Alertness	+	++	++	+++	-
Awareness	+	++	++	++	-
Sound response	++	+++	+++	++++	-
Touch response	++	+++	++++	++++	-
Pain response	++	+++	++++	++++	-

- No effect ; + slight depression ; ++ moderate depression ; +++ strong depression ; ++++ very strong depression.

Table 2
Percentage effect of *B. hispida* extract and Diazepam on muscle relaxant activity.

Treatment n = 10	Dose mg/kg	Traction Test	Rotarod Test
S	-	0	0
Di	4	100	100
Be	400	0	0
Be	800	0	0
Be	1500	0	0
Be	3000	0	0

S - Saline ; Di - Diazepam ; Be - *B.hispida* extract.

of either normal saline (10 ml/kg), diazepam (4 mg/kg) or methanolic extract (400, 800, 1500 and 3000 mg/kg). The forepaws were placed on a small twisted wire rigidly supported above with a bench top. Untreated mice grasped the wire with forepaws and placed at least one hind foot on the wire within 5 sec. Inability to put up at least one hind foot constituted failure in the traction test [8].

Rotarod test - Untreated mice were placed on a horizontal wooden rod (32 mm dia.) rotating at a speed of 5 rpm. Only mice remaining on the rod for 3 min or more, in 3 successive trials were selected for the study. The mice were divided into six groups (n=10). Groups 1, 2, 3 and 4 were injected intraperitonealy with the methanolic extract (400, 800, 1500 and 3000 mg/kg). Groups 5 and 6 were treated with normal saline (10 ml/kg) and diazepam (4 mg/kg), respectively. Each group was placed on the rod at intervals of 30, 60, 90, 120 and 150 min. Mice failing more than once to remain on the rotating rod for 3 min constituted a positive result. [9]

2.7 Antihistaminic activity

Antihistaminic activity of methanolic extract was evaluated in Guinea pigs as per the method suggested by Vanproosdij [10, 11]. A cut off period of 25 min was used following the administration of drug and the percentage protection offered is calculated using the relation $(1 - T_1/T_2)$ 100 where T_1 is the mean of the control pre-convulsion time before and two days after the adminstration of the drug and, T_2 is the pre-convulsion time determined with the administration of drug.

2.8 Potentation of barbiturate induced hypnosis

This experiment was performed in group of six rats. 30 min. after intraperitoneal injection of saline (10 ml/kg) or the methanolic extract (200, 400 and 600 mg/kg) or chlorpromazine (5 mg/kg), all animals were subjected to Thiopentone sleeping time test (35 mg/kg i.p.)

Table 3
Effect of *B. hispida* extract and Diphenhydramine on histamine induced pre-convulsion time.

Treatment n = 4	Dose mg/kg	Effect on Pre-convulsion time T_1	Effect on Pre-convulsion time T_2	% Protection
DP	4	7.0 ± 0.14	14.20 ± 0.09***	57.4
Be	50	7.0 ± 0.04	13.16 ± 0.08**	46.15
Be	100	7.0 ± 0.81	> 25***	> 72

DP - Diphenhydramine ; Be - *B.hispida* extract, values represent mean ± SEM.
** P < 0.01, *** P < 0.001 as compared to pre-convulsion time before drug treatment; Student's *t* - test.

Table 4
Effect of *B. hispida* extract and Phenobarbitone on Barbiturate induced hypnosis.

Treatment n = 6	Dose $mgkg^{-1}$	Sleep Time (min)
S	-	31.3 ± 3.18
Pe	50	86.4 ± 3.81 ***
Be	200	60.5 ± 3.72 ***
Be	400	106.5 ± 3.5 ***
Be	600	145.0 ± 5.0 ***

S - Saline ; Pe - Phenobarbitone ; Be - *B.hispida* extract, values represents mean ± SEM. *** P < 0.001 compared with saline treated group.

and sleep time (the time from the loss to the recovery of righting reflex) was measured to the nearest minute.

2.9 Statistical analysis

All the value are represented as mean ± SEM. Statistical analysis of data were performed using Student's *t* - test to study the differences among the means. When probability (P) was less than 0.05, the differences were considered as significant [12].

3. Results

3.1 Toxicity study

The plant extract was non-toxic and did not cause death in doses up to 3.0 g/kg.

3.2 Effect on general behavioural profile

The results obtained from the different experiments are shown in Table 1. The methanolic extract affected spontaneous activity, sound and touch responses at doses above 400 mg/kg, and produced moderate or slight depression relating to awareness and alertness. The methanolic extract caused a significant depression of these responses comparable to standard drug chlorpromazine.

3.3. Effect on histamine induced pre-convulsion

The methanolic extract delayed the histamine induced pre-convulsion time in a dose dependent manner($p< 0.001$) (Table 3).

3.4 Effect on barbiturate induced hypnosis

Methanolic extract significantly potantiated barbiturate induced hypnosis in a dose dependent manner (Table 4).

4. Discussion

The results indicate that the methanolic extract influences general behavioural profiles, as evidenced in the spontaneous activity, touch, sound and pain responses.

The methanolic extract significantly potentiated the barbiturate induced sleeping time, possibly through a CNS depressant action [13] or tranquilizing action. [7] The possible CNS activity of the methanolic extract was further tested against other common psychopharmacological tests. (i.e., rotarod and traction test). Methanol extract showed no muscle relaxant activity in doses up to 3.0 g/kg.

The *in vivo* antihistaminic effect of methanolic extract supports the *in vitro* studies.[3]

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