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Original Article

ANXIOLYTIC EVALUATION OF *BENINCASA HISPIDA* (Thunb) Cogn. FRUIT EXTRACTS

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Abstract

Benincasa hispida (Cucurbitaceae), popularly known as 'ash gourd', is a large climbing or trailing herb, that has been widely used in India, China, Indochina and Malaya. According to the Sanskrit texts, it is useful in insanity, epilepsy, constipation, piles dyspepsia and other nervous diseases; fresh juice is given either with sugar or as an adjunct to other medicines for these diseases. In this work, the anxiolytic effects of alcoholic extract of *B. hispida* were evaluated in mice using elevated plus maze and light-dark transition test and spontaneous motor activity was measured in actophotometer. The extract administered orally was able to increase the percentage of time spent and the percentage of open arm entries in the elevated plus maze, as well as increase the time spent in the illuminated side of the light-dark test. The same extract was not able to modify the spontaneous motor activity measured in actophotometer. These results provide support for the potential anxiolytic activity of *B. hispida*.

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Key words: *Benincasa hispida*, anxiolytic, Elevated plus maze, light-dark test, actophotometer.

INTRODUCTION

According to World Health report (WHO 2001), approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020¹. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research worldwide has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models².

Anxiety is unpleasant feeling of apprehension or fearful concern. It can be a normal, reasonable and expected response to a stressful situation or perceived danger or it may be an excessive, irrational state that signifies a mental disorder³. The normal fear response to threatening stimuli comprises several components, including defensive behaviors, autonomic reflexes, arousal and alertness, corticosteroid secretion and negative emotions. In anxiety

states, these reactions occur in an anticipatory manner, independently of external event. The distinction between a pathological and a normal state of anxiety is not clear-cut but represents the point at which the symptoms interfere with normal productive activities⁴ and some degree of anxiety is a part of normal life. The disability and health costs caused by anxiety disorders are comparable to those of other common medical conditions such as, diabetes, arthritis or hypertension⁵.

B. hispida is medicinally used in India, China, Indochina and Malaya. It is probably a native of Japan and Java, cultivated more or less throughout India and in warm countries. The fruit of *B. hispida* (Thunb) Cogn. Commonly called as ash gourd, belonging to cucurbitaceous is employed as a main ingredient in kusmana lehyam, in Ayurvedic system of medicine. The lehyam is used as rejuvenate agent and also numerous nervous disorders. According to the Sanskrit texts, it is useful in insanity, epilepsy, constipation, piles dyspepsia and other nervous diseases^{3,4}. Though some scientific studies

have been carried out reveal its Anti-ulcer⁶ anti-diarrhoeal⁷ anti-angiogenic⁸ anti-inflammatory⁹ anticancer¹⁰ anti-asthmatic¹¹ antioxidant and angiotensin converting enzyme inhibitor¹² anorectic¹³, nootropics¹⁴ and hypoglycemia¹⁵ activities. The major constituents of this fruits are triterpenoids, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, β -sitosterin and uronic acid^{16,17}.

In the light of above information, the present investigation was undertaken to evaluate the anxiolytic potential of alcoholic extract of *B. hispida* fruit.

METHODS AND MATERIALS

Plant Material

The fresh fruits were collected during October-November from the local market of Raichur and were identified by botanist of our college.

Extract Preparation^{6,7}

After removing the outer skin and the seeds, the fruit of *B. hispida* was mashed using an electric juicer to afford a soft mass. For the preparation of an alcoholic extract, 100ml of fresh juice was mixed with 500ml of ethanol and kept covered for seven days at room temperature with daily occasional stirring. The mixture was then filtered and the filtrate was heated (below 55°C) and evaporated under reduced pressure. Later the extract was dried completely using a lyophilizer (Lyotap, Germany), brownish sticky mass was obtained, which was protected from direct sunlight. The yield of the extract was 0.733gm/100ml of the fresh juice.

Animals Used

Swiss albino mice of either sex weighing between 20-30g were procured from Shri Venkateswara Enterprises, Bangalore and were acclimatized for seven days under standard husbandry conditions. The animals were fed with commercial diet (Amrut laboratories, Pranava Agro Industries Ltd., Sangli, India) and water *ad libitum*.

The approval of the Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy Raichur, Karnataka (Registration number 557/02/c/CPCSEA) was taken prior to the experiments and in order to reduce the day variation, all protocols and the experiments were conducted from 9-to 14 h, in a special noise-free room with controlled illumination.

Drugs and Chemicals

The alcoholic extract of *B. hispida* was used as experimental extract, dissolved in 3% tween-80 (s.d.fine Chem.Ltd. Mumbai), and diazepam (Ranbaxy Laboratories Ltd, Mumbai, India) as standard anxiolytic drug was used.

Determination of acute toxicity (LD50)

The acute toxicity of alcoholic extract of fruits of *B. hispida* was determined by using female albino mice (20-30g) those maintained under standard husbandry conditions. The animals were fasted 3 hrs prior to the experiment, up and down procedure (OECD guideline No. 425) of CPCSEA was adopted for toxicity studies.¹⁸ Animals were administered

with single dose of extract and observed for its mortality during 48 hours study period (short term toxicity). Based on short-term profile of drug, the dose of the next animals was determined as per OECD guideline No.425. The LD50 of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA.

Locomotor Activity¹⁹

The locomotor activity was measured using an Actophotometer. The movement of the animal cuts off a beam of light falling on the photocell and a count was recorded and displayed digitally. The animals were divided into 5 groups consists of 6 animals. Group- I was treated with 3% tween-80 p.o. (control), Group-II was treated with diazepam 2mg/kg p.o. (standard) and Group-III, IV, V were treated with different doses of AEBH i.e. 100, 200 and 400mg/kg p.o. respectively. At zero min. spontaneous motor activity was recorded, after oral administration of the vehicle/standard /extract, each group of animals were individually placed in the actophotometer for 10min at the prefixed time interval i.e., 30min., 1hr and 2hr.

Elevated Plus Maze (EPM)²⁰

This test has been widely validated to measure anxiety in rodents. The plus-maze apparatus comprises of two open arms (16x5cm) and two closed arms (16x5x12cm) that extend from a common central platform (5x5cm). The entire maze is elevated to a height of 38cms above the ground level. The animals were divided into 5 groups consists of 8 animals. Group-I was treated with 3% tween-80 p.o. (control), Group-II was treated with diazepam 2mg/kg p.o. (standard) and Group-III, IV, V were treated with different doses of AEBH i.e. 100, 200 and 400mg/kg p.o. respectively. One hour after the oral administration vehicle / standard / extract, each mouse was placed at the center of the maze facing one of the open arm. Number of entries and the time spent in the open and closed arms was recorded during 5 min session. Entry into an arm was defined as the animal placing all four paws onto the arm. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution).

Light-Dark Transition test (LDT)²¹

The apparatus consisted of a two-compartment chamber (40cmx60cmx20cm), comprising a brightly illuminated area of 40cmx40 cm and a dark area of 40cmx20cm were separated by a wall with a round hole of 7cm diameter. The animals were divided into 5 groups consists of 8 animals. Group-I was treated with 3% tween-80 p.o. (control), Group-II was treated with diazepam 2mg/kg p.o. (standard) and Group-III, IV, V were treated with different doses of AEBH i.e. 100, 200 and 400mg/kg p.o. respectively. One hour after the oral administration of vehicle / standard / extract, each animal was placed at the center of the illuminated compartment, facing the dark area. The latency (time taken to enter the dark place), time spent in the light box, numbers of crossings and number of rearings in light box were recorded during 5 min session.²²

Table No: 1. Locomotor activity of Alcoholic extract of *B.hispida* in Actophotometer

Treatment	Initial mean±SEM	No. of movements		
		30min mean±SEM	1hr mean±SEM	2hr mean±SEM
Control (3%Tween-80 p.o)	274.83±37.09	266.16±36.27	239.16±41.82	227.5±42.03
Standard.(Diazepam, 2mg/kg p.o.)	317.50±14.83	46.33 ^{***} ±14.4	25.66 ^{***} ±6.58	27.16 ^{***} ±4.70
AEBH (100mg/kg p.o.)	321.83±22.66	262.33 ^{ns} ±11.2	164 ^{ns} ±13.20	172.5 ^{ns} ±39.66
AEBH (200mg/kg p.o.)	317.66±24.82	256.16 ^{ns} ±25.2	177.83 ^{ns} ±14.6	224.16 ^{ns} ±17.55
AEBH (400mg/kg p.o.)	300.00±15.00	218.83 ^{ns} ±21.4	108.33 [*] ±6.9	153 ^{ns} ±17.24

n=6, Significance at P<0.05*, <0.01**, <0.001*** and ns-not significant

Table No: 2. Anxiolytic activity of Alcoholic extract of *B.hispida* on Elevated Plus Maze

Treatment	%Number of entries (Counts in 5min session)		%Time spent in 5min session	
	Open arm mean±SEM	Closed arm mean±SEM	Open arm mean±SEM	Closed arm mean±SEM
Control (3%Tween-80 p.o)	19.84±3.76	80.14±3.76	8.63±0.99	70.37±5.56
Standard.(Diazepam,2mg/kg p.o.)	69.16 ^{***} ±4.92	30.83±4.92	57.42 ^{**} ±6.43	33.86 [*] ±6.90
AEBH (100mg/kg p.o.)	26.46 ^{ns} ±4.81	73.52 ^s ±4.81	14.80 ^{ns} ±1.95	55.60 ^{ns} ±3.37
AEBH (200mg/kg p.o.)	44.68 ^{**} ±4.30	55.30 ^{**} ±2.5	26.98 ^{**} ±2.98	51.10 [*] ±1.96
AEBH(400mg/kg p.o.)	53.24 ^{***} ±2.51	46.74 ^{***} ±2.5	34.15 ^{**} ±3.12	30.81 [*] ±2.85

n=6, Significance at P<0.05*, <0.01**, <0.001*** and ns-not significant

Table No: 3. Anxiolytic activity of Alcoholic extract of *B.hispida* on Light-Dark Transition test

Treatment	Latency (sec) mean±SEM	No. Of Crossings mean±SEM	Time spent in light box (sec) mean±SEM	No. of rearings in L.box mean±SEM
Control (3%Tween-80 p.o)	14.87±1.42	6.75±0.49	89.37±6.08	15.37±0.94
Standard.(Diazepam,2mg/kg .o.)	32.12 ^{**} ±3.55	9.62 ^{**} ±0.49	170.12 ^{***} ±11.4	2.12 ^{***} ±0.44
AEBH (100mg/kg p.o.)	22.62 ^{ns} ±2.36	7.87 ^{ns} ±0.51	97.37 ^{ns} ±1.53	13.75 ^{ns} ±0.92
AEBH (200mg/kg p.o.)	32.0 ^{**} ±2.57	9.75 ^{**} ±0.55	160.75 ^{***} ±5.21	5.75 ^{***} ±0.92
AEBH(400mg/kg p.o.)	34.0 ^{***} ±3.54	8.87 [*] ±0.51	137.0 ^{**} ±15.25	6.87 ^{***} ±0.87

n=6, Significance at P<0.05*, <0.01**, <0.001*** and ns-not significant

Preliminary Phytochemical Tests^{23,24}

The preliminary phytochemical investigations were carried out with alcoholic extract of *B. hispida* for qualitative identification of phytochemical constituents present with the extract. Tests were carried out by following standard methods. All the chemicals and reagents used in the tests were of analytical grade.

Statistical Analysis

The results were subjected to statistical analysis by using one-way ANOVA followed by Tukey- Kramer test to assess the significance difference if any among the groups. P<0.05 will be considered as significant.

RESULTS

Acute Oral Toxicity Study: The alcoholic extract of fruits of *B. hispida* was administered orally to different groups of mice with different dose levels and found that even upto the dose of 2000mg/kg body weight, it has not produced any behavioural symptoms or mortality.

Locomotor Activity

The low and medium doses of AEBH (100 and 200mg/kg) did not produce any significant reduction in locomotor activity, even at higher dose (400mg/kg), the extract failed to produce any significant reduction in locomotor activity except in 1h, as compared to control group. However, the diazepam treated group revealed a statistically significant decrease in locomotor activity as compared to control group. (Table 1)

Elevated Plus Maze

AEBH of medium and high doses (200 and 400mg/kg) but not lower dose (100mg/kg) significantly increased the % of open arm entries and % time spent in open arm, compared with the control group. As the standard (diazepam 2mg/kg) had significantly increased the % of open arm entries and % time spent in open arm and confirmed the anxiolytic activity of diazepam reported previously. All parameters expressed as a percentage (Eg. % open arm entries = 100×no.of open arm entries/ Total no. of entries). (Table 2)

Light-Dark Transition test (LDT)

The medium and high doses of AEBH (200 and 400mg/kg) but not lower dose (100mg/kg) had significantly increased the latency, time spent and number of entries into the light compartment and decreased the number of rearings in light compartment as compared to control group. (Table 3)

Preliminary Phytochemical Screening

The alcoholic extract of fruits of *B.hispida* was subjected to preliminary phytochemical investigation and found to contain carbohydrates, triterpenoids, glycosides, flavonoids and sterols.

DISCUSSION

Decrease in locomotor activity reveals depressant effect on CNS that may be due to the increase in the concentration of GABA in brain^{32,26,27}. In the locomotor study, AEBH extract produced a reduction in locomotor activity suggesting a sedative effect, although the diazepam sedative effect was more severe. However AEBH inhibited locomotor activity to a lesser extent than diazepam and thus has a better profile for an anxiolytic agent. There is considerable interest in the development of new anxiolytics without any sedative effect and no effect on locomotion.²⁸

The elevated plus maze is a well established animal model for testing anxiolytic drugs.^{26,29} A standard anxiolytic drug diazepam used clinically is also employed in behavioural pharmacology as a reference compound for inducing anxiolytic like effects.³⁰ Although original validation of the EPM was performed in rats, it has also been found to be selectively sensitive to the effects of anxiolytic and anxiogenic drugs in mice.³¹ AEBH of medium and high doses of AEBH (200 and 400mg/kg) had significantly increased the time spent and number of entries into the open arms, moreover these increases were not accompanied by statistically significant changes in motor activity as indicated by the number of total arm entries.

It has been suggested that some animal models based on spontaneous behavior ethologically based models like the light-dark test may be more sensitive to the behavioural responses than conditioned paradigms^{32,33}. The light-dark test may be useful to predict the anxiolytic like activity of drug in mice. It has the advantages of being quick and easy to use, without the prior training of animals, food and water deprivation is unnecessary and natural stimuli are used. Transitions have been reported to be an index of activity exploration because of habituation over time and the time spent in each compartment to be a reflection of aversion^{30,34}. AEBH of medium and high doses (200 and 400mg/kg) had significantly increased the time spent in light compartment, number of crossings and latency and decreased the number of rearings in light compartment, indicating that, both doses had produced significant anxiolytic effect but lower dose (100mg/kg) of AEBH had not exhibited any significant anxiolytic effect.

Several lines of evidence show that natural and synthetic flavonoids are potent anxiolytic agents without sedative, myorelaxant or amnesic effects. It is known the participation of GABA in these effects.³⁵ Phytochemical investigation showed the presence of triterpenoids, flavonoids, glycosides, and sterols in the AEBH. Flavonoids and triterpenoids are already reported for their anxiolytic activity. Hence these phytoconstituents might be responsible for the anxiolytic effect of AEBH. However, the exact mechanism(s) and the active compound(s) involved in these effects need to be clarified in future studies.

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