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(Research Article)

Anxiolytic Behavioural Model for *Benincasa Hispida*

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ABSTRACT

Anxiety is unpleasant feeling of apprehension or fearful concern. The disability and health costs caused by anxiety disorders are comparable to those of other common medical conditions such as, diabetes, arthritis or hypertension. According to various literatures available on Benincasa hispida, the present study was undertaken for evaluation of anxiolytic activity of alcoholic extract of Benincasa hispida on various behavioural models. A different dose of extract was tested on models like OFT, Hole Board and Mirror chamber. The medium and high dose (200 and 400mg/kg b.w.) caused significant increase in central locomotion, while Numbers of rearings, Immobility time, Grooming time, Defecation and urination were not significantly reduced in OFT model. In Hole-board model, significant increase in latency to the 1st head dips, number of head dips and time spent in head dips was observed. But in mirror chamber model, reduced latency to enter the mirrored chamber and increased both the number of entries; as well as the time spent in the mirrored chamber was noted. From the results it was concluded that alcoholic extract of Benincasa hispida successfully avoid anxiety induced in behavioural animal model.

Key Words: *Benincasa hispida*, LD_{50}, Mirror Chamber, Anxiolytic activity, Hole- Board model

INTRODUCTION

Benincasa hispida (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 [1].Some scientific studies have been carried out to reveal its Anti-ulcer[2], anti-diarrhoel[3], anti-angiogenic[4], anti-inflammatory[5], anticancer[6], antiasthmatic[7], antioxidant and angiotensin converting enzyme inhibitor[8], analgesic[9], anorectic[10], nootropics[11], prevent the withdrawl symptoms of morphine addiction[12], hypoglycemia[13] and diuretic[14] activities. The major constituents of this fruits are triterpenoids, flavonoids, glycosides, sacchrides, proteins, carotenes, vitamins, minerals, ß-sitosterin and uronic acid[1,15,16,17,18]. In the light of above information, the present investigation was undertaken to evaluate the anxiolytic potential of alcoholic extract of *B.hispida* fruit.

MATERIALS AND METHODS

Plant materials and preparation of extracts[2,3]

The fresh fruits were collected during October-November from the local market of Raichur and were identified by botanist of our college. 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 55^0C) 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.

Drugs and chemicals

Diazepam [Ranbaxy Laboratories Ltd, Mumbai, India], Tween-80 [s.d.fine Chem Ltd. Mumbai], Alcohol [The Ugar Sugar Works Ltd. Ugar Khurd, Belgaum] and Distilled water [Mysore Petro Chemicals, Raichur, India]

Animals

Swiss albino mice of either sex (18-20 g) procured from Bio. Need, Bangalore were maintained for 7 days in the animal house of P.E.S College of Pharmacy, Bangalore under standard conditions: temperature (24 ± 10 C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. Groups of 6 mice (18-24) were used in all sets of experiments. All the experiments were conducted after obtaining permission from the Institutional Animal Ethics Committee (IAEC) of V.L. College of Pharmacy, Raichur.

Determination of LD_{50}

Female, nulliparous and non pregnant mice weighing 18-22 g were fasted for 3 hours before administration of extracts. The dosing of the animals was done as per OECD guidelines[19]. Number of animals died at the particular dose levels were recorded after 2days and 14 days of drug administration. LD_{50} values were calculated using AOT 425 software provided by Environmental Protection Agency, USA.

Assessment of Anxiolytic activity

Open field Model of Anxiety[20]

The open field apparatus was made up of plywood consists of 56 x 56 (l x b) cm. The entire apparatus was painted black and 6 mm thick white lines divided the floor in to 16 square of identical dimension. Open field was lightening by 40 W bulb focusing on to the field from the height of about 100 cm. The entire room, except the open field was kept dark during the experiment. One hour after the drug treatment, each animal were placed at one corner of the apparatus and the following behavioral aspects were noted in the next 5 min.:

Latency: Time taken by animal to leave square in which it was placed
Ambulation: Number of square passed by animal
Rearing: Number of times animal stood on its hind legs.

Hole-Board Apparatus[21]

The apparatus used in this model consists of wooden chamber (40x40x25 cm) with 16 holes (diameter 3 cm) on the floor, elevated from the ground so that the rats could peep through the holes. On the eighth day one hr after oral administration of the std/extracts in respective groups, each rats was placed in individually in the apparatus. During a five minutes test period the following parameters are taken:

Latency to the first head dips.
The number of head dips through the holes.
The total time spend with the head dips.
No. of rearings.
No. of defecation units.

Mirror Chamber[22]

The apparatus consist of a mirrored cube open on one side that was placed inside a square wooden box. The mirrored cube measuring 13cm on a side was constructed of 5 pieces of mirrored glass with one mirrored side and an opposite side painted dark brown. The 3 mirrored side panes, a top pane and the floor pane faced the interior of the cube. The container box was 40X40X30.5 cm. The mirrored cube was placed in the centre of the wooden container to form a 5 cm corridor that completely surrounded the mirror chamber. A mirror was also placed on the container wall so that it faces the single open side of the mirrored chamber. The other 3 walls of the container were painted dark brown. On the eighth day one hr after oral administration of the std/extracts in respective groups, the mice were placed in the chamber of mirrors at fixed corners and the following parameters were noted for 5 min.:

Latency to enter the chamber: the time in seconds for first entry into the chamber of mirrors.

Number of entries in 5 min (Criteria for entry: 4 paws placed on the floor panel of the mirrored chamber).

Total time in seconds spent in the chamber during the 5 min test period.

RESULTS AND DISCUSSON

Open field: In the open field Diazepam (5 mg/kg), AEBH(200 and 400 mg/kg), significantly ($P < 0.01$) increased total time spent in central compartment while ALECH (300 mg/kg), significantly ($P < 0.05$) increased total time spent in central compartment, no. of square crossed by animal. Different doses of AEBH when administered orally daily once for 7 days, a significant effect on locomotion was observed with medium and high doses and significant increase in central locomotion was recorded with medium and higher doses but not with low dose. Numbers of rearings, Immobility time, grooming time, Defecation and urination were not significantly reduced with all the doses (Table-1).

Hole- Board model: different doses of AEBH when administered orally daily once for 7 days, high and medium doses (200 and 400mg/kg) but not low dose (100mg/kg) has shown significant increase in latency to the 1st head dips, number of head dips and time spent in head dips. A significant increase in number of rearing was observed in both medium and high doses as compared to control group. Defecation units have significantly reduced with high dose but not with low and medium doses as compared to control group. Standard drug diazepam (2mg/kg) had exhibited significant anxiolytic activity (Table-2). Increased exploratory behavior characterized by an increase in the number as well as duration of head dips is an indication of anxiolytic activity.

Mirrored Chamber Test in mice: Different doses when administered orally daily once for 7 days, The average latency to enter the mirrored chamber by the Vehicle-treated mice was 139.83 ±23.697 .The vehicle-treated mice entered the mirrored chamber less frequently (1.5 ± 0.34) and spent; a mean total time of 30.33 ±10.452 s in the chamber during the 5-min test period. Diazepam (2mg/kg) treatment significantly reduced the latency to enter the mirrored chamber and increased both the number of entries; as well as the time spent in the mirrored chamber, AEBH (200 and 400 mg/kg) produced a marked and dose dependent decrease in the latency to enter, and increased the number of entries and time spent in the mirrored chamber as compared to vehicle-treated controls (Table-3).

CONCLUSION

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 disorder.[23]

In the OFT, the confrontation with the situation induces anxiety behavior in rodents is triggered by two factors, i.e., individual testing as the animal was separated from its social group and agoraphobia, as the arena is very large, relative to the animals breeding or the natural environment. In such situations rodents show thigmotaxic behavior identified by spontaneous preference to the periphery of the apparatus and reduced ambulation[25].Rodents demonstrate anxiety, fear and curiosity when placed in a new environment, and an overall assessment of behavior could be determined through the observation of freezing, grooming (fear), rearing, head dips (curiosity) and the number of fecal boli[24] . Anxiolytic treatment decreases this anxiety induced inhibition of exploratory behavior. Medium and high doses (200 and 400mg/kg) of AEBH had shown more profound effects on total locomotion, central locomotion and grooming. Even though all the three extract showed no significant effect on rearings, but the immobility time has drastically reduced as observed dose dependently and AEBH didn't altered above parameters significantly. All doses of AEBH didn't significantly alter the defecation and urination.

Hole-board model indicated that head-dipping behaviour was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behaviour[25].The AEBH medium and high doses (200 and 400mg/kg) shows increase in number, latency and duration of head dipping and the number of rearing in the hole board test. It could be argued that the increased head-dipping in rats is merely an artifact of the hyperactivity induced by the drug.

Mirror Chamber Model: Behavioral models of anxiety rely on the introduction of a stimulus to create a novel state within the organism. The nature of state, and the inference of anxiety, is assessed by the response of the subject to that stimulus in the presence and absences of drugs known to be efficacious anxiolytic in man. Many animal species exhibit approach-avoidance response upon placement of a mirror within their environment. In order to identify new behavioral measures with qualitatively different responses, a mirrored chamber apparatus was developed for which mice shown an extended latency to enter. Novel stimulation evokes both exploration and anxiety and thereby generates an approach avoidance conflict behavior. The response to an apparent animal reflected in the mirror might also be a source of anxiety[22].

There is a significant reduction in latency to enter into the mirror chamber seen with medium and high dose (200 and 400mg/kg) and there is increase in the number of entries and time spent in the mirrored chamber, but not with low dose (100mg/kg). But the effect of low dose (400mg/kg) was found to be insignificant when compared with control. Diazepam (2mg/kg) treatment significantly reduced the latency to enter the mirrored chamber and increased both the number of entries; as well as the time spent in the mirrored chamber.

Table- 1: Anxiolytic effect of AEBH Open field test

Treatment	Total locomotion	Central locomotion	No. of rearings	No .of grooming	Immobility time	No. of emboli	Urination
Control	72.17± 12.35	5.17 ± 1.078	19.00± 5.099	7.83± 0.4773	22.79± 1.259	1.67± 0.4944	0.667± 0.210
Diazepam 5mg/kg p.o.	137.33± 15.09	29.0**± 1.438	2.667*± 1.667	*2.33***± *0.8433*	8.00**± 1.438	1.50 ^{ns}± 0.4282	0.167^{ns} ± 0.1667
AEBH 100mg/kg p.o.	83.67 ± 6.302	8.50^{ns}± 1.057	6.333^{ns}± 3.509	4.667^{ns}± 1.585	14.23^{ns}± 4.095	1.67^{ns}± 0.4944	0.50^{ns}± 0.3416
AEBH 200mg/kg p.o.	124.83 ± 13.546	18.17 ** ± 2.330	20.0^{ns}± 4.017	3.000** ± 0.7303	13.318* ± 1.331	1.33^{ns}± 0.4216	0.50^{ns}± 0.2236
AEBH 400mg/kg p.o.	128.67± 14.50	16.17**± 0.95	12.400ns ±1.749	3.17**± 0.9804	6.298** ± 2.444	1.00^{ns}± 0.5164	0.33^{ns}± 0.2108

n = 6, Significant at ns=not significant, P<0.05* and <0.01**

Table-2: Anxiolytic effect of AEBH with Hole Board Model in rats

Treatment	No of head dips	Time spent in head dips	Latency to first head dips	No. of rearings	No. of defecation
Control	5.833 ±1.470	10.333 ±2.290	38.333 ± 5.321	6.500 ±0.9916	3.667 ±0.6146
Diazepam 5mg/kg p.o.	14.833** ±0.7032	42.833** ±3.851	12.833** ± 0.7923	22.000** ±3.044	0.8333* ±0.4773
AEBH 100mg/kg p.o.	9.000^{ns} ±3.327	19.000^{ns} ±5.825	29.500^{ns} ±2.705	15.000^{ns} ±1.807	2.667^{ns} ±0.8433
AEBH 200mg/kg p.o.	13.000* ± 1.000	23.333* ±0.9545	20.833** ±1.493	17.833* ±3.719	1.667^{ns} ±0.5578
AEBH 400mg/kg p.o.	14.000* ± 1.238	23.833* ±1.327	14.500** ±2.172	19.833** ±2.688	1.167* ±0.6540

n = 6, Significant at ns=not significant, P<0.05* and <0.01**

Table-3:Anxiolytic effect of AEBH with Mirror chamber model in mice

Treatment	Latency to enter in mirror Chamber (Sec/5min)	No of Entries in Mirror Chamber	Time spent In mirror Chamber (Sec/5min)
Control	139.83±23.697	1.500±0.3416	30.333±10.452
Diazepam 5mg/kg p.o.	2.500**±1.310	7.000**±0.7303	133.17**±10.619
AEBH 100mg/kg p.o.	82.167ns±35.283	2.000ns±0.6325	65.333ns±16.697
AEBH 200mg/kg p.o.	46.667*±22.311	3.833*±0.7032	101.33**±3.353
AEBH 400mg/kg p.o.	20.333**±11.783	5.000**±0.4472	113.83**±3.525

n = 6, Significant at ns=not significant, P<0.05* and <0.01**

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