Evaluation of the Memory and Learning Improving Effects of *Benincasa hispida* Seeds in Mice

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ABSTRACT

Background: Management of cognitive disorders like dementia and Alzheimer's disease has been challenging since no potential drug is available with proved efficacy. Some nootropic drugs like piracetam, aniracetam and cholinesterase inhibitors such as Donepezil® have found to exhibit severe toxic effects in elderly. **Aim:** In the present study, we assessed the nootropic potential of aqueous and methanol extract of *Benincasa hispida* on various behavioural models. **Method:** The young animals treated with aqueous and methanol extract (200 and 400 mg kg^{-1}) showed dose-dependent reduction in transfer latency at both 9th and 10th day by elevated plus maze, morris water maze and object recognition task. **Conclusion:** From the results, it was concluded that methanolic extract of *Benincasa hispida* can be a useful memory restorative agent in the treatment of dementia.

Key words: *Benincasa hispida*, anixolytic activity, memory enhancer, morris water maze

INTRODUCTION

Memory impairment is commonly seen by physicians in multiple disciplines including neurology, psychiatry, medicine and surgery[1]. Memory loss is often the most disabling feature of many disorders, impairing the normal daily activities of the patients and profoundly affecting their families. The key features of these dreaded disorders are memory impairments, deterioration of language, visuo-spatial, motor, sensory abnormalities, gait disturbance and seizures. There are around 30 million patients suffering from Alzheimer's Disease (AD) which is the major cause of dementia, all over the world[2]. In India, AD patients are estimated to be around 3 million[3]. Cognition is that operation of mind by means of which, we become aware of our surroundings, objects and thoughts. Cognitive disorders such as delirium, dementia and amnesic disorders are common in elderly individuals. Memory is vulnerable to a variety of pathologic processes including neurodegenerative diseases, strokes, tumors, head trauma, hypoxia, cardiac surgery, malnutrition, attention deficit disorder, depression, anxiety, the side effects of medication and normal ageing[4]. Presently, there are no satisfactory diagnostic procedures and therapeutic regimens available for the management of these cognitive disorders. Despite the severity and high prevalence of these diseases,

Allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, neurobiologists all over the world are looking for new directions and alternative strategies for managing cognitive disorders. The most common cause of dementia in the elderly is probably Alzheimer's Disease (AD), a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language[5]. Nootropic agents like, Piracetam and Cholinesterase inhibitors like, Donepezil are commonly used for improving memory, mood and behavior. However, the resulting adverse effects of these drugs such as diarrhea, insomnia, nausea, bronchitis, loose stools, muscular cramps and other known side effects[6] have made their use limited and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders. The present studies deal with the evaluation of memory enhancement activity of ethanol and aqueous extract of *Benincasa hispida* seeds.

MATERIALS AND METHODS

Plant material: The plant *Benincasa hispida* (Thumb.) Cogn. was collected from Hisar in the month of August 2011 and identified by Dr. H.B. Singh, Head, Raw Materials, Herbarium and Museum Division, National Institute of Science Communication and Information Resources (NISCIR), New Delhi, vide reference No.

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NISCAIR/RHMD/Consult/-2011-12/1881/181 and voucher specimen retained in the Pharmacognosy section of Department for the future reference. Seeds were shade dried at $35 \pm 5°C$ and pulverized and used for carrying the experimental work procedures pertaining biological evaluation.

Methanol extract: The air-dried crude drug (500 g) was pulverized and extracted with methanol using soxhlet apparatus for 16 h. Methanol removal carried out under pressure afforded a semi solid mass with a yield of 13.6% w/w. The extract was further used for the evaluation of memory enhancing activity.

Aqueous extract: The 500 g of coarse dried powder of seeds of plant *Benincasa hispida* (Thumb.) Cogn. was extracted with distilled water by cold maceration method for 72 h and filtered through muslin cloth. The filtrate was then concentrated to get thick extract.

Animals and chemicals used: The healthy albino mice of either sex, weighing 20-25 g, were used for the evaluation of memory enhancing activity. They were procured from Agronomy Department of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. The animals were housed under standard environmental conditions of temperature and humidity ($25 \pm 0.50°C$) and (12 h light/dark cycle) were utilized for the studies. The animals were fed with standard pellet diet and water *ad libitum*. The Institutional animal ethical committee (Guru Jambheshwar University of Science and Technology, Haryana, India) reference no.Ph/2010/250, dated 19/3/2010 approved the experimental protocol and care of laboratory animals were taken as per the guidelines of CPCSEA, Ministry of Forest and Environment, Government of India (Registration number 0436).

A total of 120 young male mice were employed in the present study. Each group comprised of 6 mice:

- **Group I:** Control group for young mice. Food was administered orally for ten consecutive days. TL was recorded after 90 min of food administration on 9th day and retention was examined after 24 h (i.e., on 10th day)
- **Group II:** Positive control for young mice. Piracetam (400 mg kg^{-1} i.p.) was injected to young mice for 10 consecutive days. TL was recorded after 60 min of injection on 9th day and retention was examined after 24 h (i.e., on 10th day)
- **Group III-VI:** Aqueous and methanol extract (200-400 mg kg^{-1}, respectively) were administered orally along with diet for 10 consecutive days. TL was recorded after 90 min of food administration on 9th day and retention was examined after 24 h (i.e., on 10th day)
- **Group VII:** Scopolamine (0.4 mg kg^{-1} i.p.) was injected on 9th day to young mice and TL was recorded 45 min after injection. Retention was examined after 24 h (i.e., on 10th day)

Chemical used in the present study were Scopolamine (Hi-Media laboratories Pvt. Ltd., Mumbai, India) Piracetam, NootropilUCB India Pvt. Ltd., Vapi, India), normal saline, aqueous and methanolic extract of *Benincasa hispida* (Thumb.) Cogn. and Gum acacia. Solution of scopolamine and Piracetam was prepared in normal saline and injected intraperitoneal. All semisolid extracts of seeds of *Benincasa hispida* (Thumb.) Cogn. were suspended in gum acacia (2%) and prepared in distilled water. Piracetam and scopolamine were diluted in normal saline.

Behavioral methods for testing learning, memory and anti-anxiety activity
Elevated plus maze (EPM): Elevated plus maize served as the exteroceptive behavioral model to evaluate memory in mice. The procedure, technique and end point for testing memory was followed as per the parameters described by the investigators working in the area of psychopharmacology[7,8]. The elevated plus maze for mice consisted of two open arms (16×5 cm^2) and two covered arms ($16 \times 5 \times 12$ cm^3) extended from a central platform (5×5 cm^2) and the maze was elevated to the height of 25 cm from the floor[9]. On the first day (i.e., ninth day of drug treatment), each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency was defined as the time (in sec) taken by the animal to move from the open arm in to one of the covered arms with all its four legs. TL was recorded on the first day (training session) for each animal. The animal was allowed to explore the maze for another 2 min and then returned to its home cage. Retention of this learned Task (memory) was examined 24 h after the first day trial (i.e., tenth day, 24 h after last dose). Significant reduction in TL value indicated improvement in memory.

Object recognition task (ORT): Object recognition task is a convenient model used to evaluate the object recognition memory in mice. The observation area consisted of a circular open field, 480 mm in diameter and the wall height 400 mm. Two different sets of objects (a cone, a ball, a cylinder etc.) made of aluminum were used. All objects were available in triplicate. They could not be displaced by the mouse nor could the mouse climb onto or hide under the objects. The objects had no natural significance and they were never associated with any kind of reinforcer. Two objects were presented in

the first trial (T1) and a third one in the recognition trial (T2) to prevent odor cues. Objects were cleaned with tap water and detergents after each trial. During two consecutive days, the mice were habituated to the apparatus and the testing procedure. They were allowed to explore the empty apparatus twice for 3 min each day (one morning and one afternoon session). Animal was placed into the apparatus, equidistant from the two objects, facing the wall in front of the experimenter. Maximum duration of exploration was allowed 3 min. Animals were trained in pairs of two trials that were separated by a retention interval of 1 h. During the T1 the apparatus contained two identical objects, "A1" and "A2". These objects were placed in a symmetrical position about 120 mm (with reference to the centre of the object) away from the wall. During T2 the apparatus contained two different objects, a copy of the familiar one "A" from T1 and a novel object "B". Mean time (in sec) exploring the familiar object A and novel object B during T2 were measured. Discrimination Index (DI) is the difference between the exploration time of novel object and familiar object. Increase in DI indicates enhanced recognition memory. ORT allows the assessment of acquisition, consolidation or retrieval of object information separately[10].

Exploration is defined as directing the nose to the object at a distance of not more than 2 cm and/or touching the object with muzzle. Sitting on the object is not considered as exploratory behavior.

Morris water maze: Morris water maze for mice consisted of a circular pool (60 cm in diameter, 25 cm in height) filled to a depth of 20 cm with water maintained at 25°C. The water was made opaque with non-toxic white coloured dye. The dark was divided in to four equal quadrant with the help of 2 threads fixed at right angles to each other on the rim of the pool. A submerged platform (with top surface 6×6 cm and painted in white) was placed in sided target quadrants (Q4 in present study) of this pool 1 cm below surface of water. The position of platform was kept unaltered throughout the training period. Each animal was subjected to four consecutive trials each day with the gap of 5 min. for four consecutive days (stating from the 6th day of drug administration to 9th day), during which they were allowed to escape the hidden platform and to remain there for 20 sec. During the training session, the mouse was gently placed in the water between quadrants facing the wall of the pool with drop location changing for each trial and allowed 120 sec to submerged platform. If the mouse failed to find the platform with in 120 sec, it was guided gently to the platform and allowed to remain there for 20 sec. Each animal was subjected to training trial for four consecutive days, the starting point was

changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remained constant throughout the training period.

On 5th day (i.e., 10th day of drug administration), the platform was removed and mouse was placed in any of 3 quadrant and allowed to explore the target quadrant for 300 sec. Mean time spent in all quadrant Q1 Q2 Q3 was recorded. The mean time spent in the target quadrant in search of missing platform mean noted as index of retrieval or memory. The observer should stood at the same position. Care was taken not to disturb the relative location of WM with respect to other object in the laboratory[11].

RESULTS

Effect of *Benincasa hispida* (Thumb.) cogn. seed powder on transfer latency using elevated plus maze: Transfer Latency (TL) of 9th day and 10th day of drug treatment reflected improvement of both learning and memory. The young animals treated with aqueous and methanol extract (200 and 400 mg kg^{-1}) showed dose-dependent reduction in TL of both 9th and 10th day, indicating significant improvement in memory, when compared with control group. Scopolamine (0.4 mg kg^{-1} i.p.) injected before training significantly increased ($p < 0.01$) the TL of 9th and 10th day indicating impairment in both learning and memory (amnesia), respectively. The mice treated with extracts for 10 consecutive days reversed successfully the amnesia induced by Scopolamine. Piracetam (used as the positive control) at the dose of 400 mg kg^{-1}, i.p. improved memory ($p < 0.01$) and reversed the amnesia induced by Scopolamine (Table 1).

Effect of *Benincasa hispida* (Thumb.) cogn. seed powder on transfer latency using object recognition task: There was dose dependent significant increase in 10th day discrimination index for the aqueous and methanol extract of *Benincasa hispida* (200-400 mg kg^{-1}) treated groups in comparison to control group. Aqueous and methanol extracts (200-400 mg kg^{-1}) administered for 10 days reversed memory deficits due to Scopolamine induced amnesia. The results were comparable to Piracetam (400 mg kg^{-1} i.p.), a standard memory enhancer agent (Table 2).

Effect of *Benincasa hispida* (Thumb.) cogn. seed powder on transfer latency using morris water maze: Time spent in the region that previously contained the platform was recorded as Time Spent in Target Quadrant (TSTQ). Seed extracts aqueous (200-400 mg kg^{-1}) and methanolic (200-400 mg kg^{-1}) were administered orally for 10 days. Methanolic and aqueous extract was significantly increased TSTQ,

Table 1: Effect of *Benincasa hispida* (Thumb.) Cogn. seed extract on Transfer Latency (TL) of mice using Elevated Plus Maze (EPM) model

Group	Treatment	Dose	9th day TL (sec)	10th day TL (sec)
I	Control	2 mL	24.50 ± 1.38	18.97 ± 0.722
II	Piracetam	400 mg kg^{-1} i.p.	14.33 ± 0.95	8.60 ± 0.48
III	Methanol extract	200 mg kg^{-1} (orally)	17.64 ± 1.20	12.23 ± 0.85
IV	Methanol extract	400 mg kg^{-1} (orally)	16.85 ± 1.38	12.37 ± 1.36
V	Aqueous extract	200 mg kg^{-1} (orally)	20.66 ± 1.03	13.92 ± 1.33
VI	Aqueous extract	400 mg kg^{-1} (orally)	19.41 ± 1.17	12.37 ± 1.36
VII	Scopolamine	0.4 mg kg^{-1} i.p.	42.56 ± 1.44	37.92 ± 1.31

Table 2: Effect of *Benincasa hispida* (Thumb.) Cogn. seed extract on Transfer Latency (TL) of mice using Object Recognition Task (ORT) model

Group	Treatment	Dose	10th day DT (sec)
I	Control	2 mL	09.78 ± 0.38
II	Piracetam	400 mg kg^{-1} i.p.	14.01 ± 0.76
III	Methanol extract	200 mg kg^{-1} (orally)	12.30 ± 0.66
IV	Methanol extract	400 mg kg^{-1} (orally)	13.01 ± 0.78
V	Aqueous extract	200 mg kg^{-1} (orally)	11.08 ± 0.51
VI	Aqueous extract	400 mg kg^{-1} (orally)	12.20 ± 0.42
VII	Scopolamine	0.4 mg kg^{-1} i.p.	05.74 ± 0.46

Table 3: Effect of *Benincasa hispida* (Thumb.) Cogn. seed extract on Transfer Latency (TL) of mice using Morris Water Maize (MWM) model

Group	Treatment	Dose	10th day TSTQ (sec)
I	Control	2 mL	31.76 ± 0.60
II	Piracetam	400 mg kg^{-1} i.p.	55.94 ± 0.78
III	Methanol extract	200 mg kg^{-1} (orally)	41.51 ± 0.65
IV	Methanol extract	400 mg kg^{-1} (orally)	51.33 ± 0.78
V	Aqueous extract	200 mg kg^{-1} (orally)	41.51 ± 0.65
VI	Aqueous extract	400 mg kg^{-1} (orally)	44.86 ± 0.64
VII	Scopolamine	0.4 mg kg^{-1} i.p.	20.46 ± 0.46

indicating significant improvement in learning. Methanolic extract was more significant than aqueous extract. Piracetam (400 mg kg^{-1}) was used as positive control and significantly increased TSTQ on 10th day (Table 3).

DISCUSSION

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. Nootropics are a class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory[12,13]. Piracetam, the first representation of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. Repeated injections of piracetam had improved learning abilities and memory capacities of laboratory animals[14]. The data shown by experimental results underlined the importance of *B. hispida* methanol as well as aqueous extract in preventing memory loss. New neurons are continuously being added to certain areas of the brain, such as hippocampus and olfactory bulb in animals[15,16,17] as well as humans. There is a possibility that methanol extract of *B. hispida* for long periods not only arrests the neurodegenerative processes (responsible for dementia) but also stimulated the process of neurogenesis The elevated plus maze is a well established animal model for testing anxiolytic drugs[18]. Methanol extract of *B. hispida* fruit has been proved to be effective anixolytic agent in one study[19]. The results indicates the reduction in Transfer Latency on 9th and 10th day in elevated plus maze, while Time Spent in Target Quadrant was increased to a significant level in Moris Water Maze model indicating methanol extract more potent than that of aqueous extract by improving condition of amensia induced by Scopolamine.

Considering the lack and need of drugs with proven effectiveness in improving learning and memory, the specific memory improving effects of *B. hispida* reported here is of enormous interest and deserves further investigations using more experimental paradigms for further confirmation of memory improving potential of *B. hispida* in the treatment of various cognitive disorders.

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