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Effect of Methanolic Extract of *Benincasa hispida* on Cognitive Function in Experimental Animals

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ABSTRACT

The management of cognitive disorders such as dementia and Alzheimer's disease has been a significant challenge, as no drugs with established efficacy are currently available. Some nootropic drugs, like piracetam and aniracetam, have been associated with severe adverse effects in elderly individuals. In this study, we investigated the potential nootropic properties of methanol fruit extract from *Benincasa hispida* using various behavioral models. *Benincasa hispida* was administered orally at doses of 200 and 400 mg/kg daily for a duration of five weeks. For comparison, donepezil was used as the positive control at a dose of 3 mg/kg. Notably, the group receiving *Benincasa hispida* at a dosage of 400 mg/kg exhibited a significantly memory enhancing effects in the Morris water maze test and passive avoidance test. Moreover, the memory-enhancing effect of *Benincasa hispida* at 400 mg/kg was found to be on par with that of donepezil at 3 mg/kg in the same test. These findings suggested that the methanolic extract of *Benincasa hispida* may serve as an effective memory-enhancing agent in the management of dementia, offering a promising avenue for further research and treatment development.

Keywords: *Benincasa hispida*, Alzheimer disease, morris water maze, passive avoidance test, memory, antioxidant activity.

1. INTRODUCTION

During the past decades, there has been a notable rise in the utilization of psychoactive drugs to manage conditions like anxiety, stress, and psychosomatic disorders. These drugs have demonstrated effectiveness in alleviating the symptoms associated with these conditions, offering some relief to individuals who struggle with them. However, there is a critical downside to the prolonged use of psychoactive drugs, such as tranquilizers and psychotropic medications.[1]

Extended use of these drugs can trigger a range of side effects affecting various body systems. This includes the autonomic nervous system, responsible for regulating functions like heart rate and digestion.[2] Disruption of these functions can lead to problems such as irregular heartbeats or digestive issues. Furthermore, the endocrine system, which controls hormone release, may become imbalanced due to these medications, potentially impacting mood, and overall well-being.[3,4] Allergic reactions, skin rashes, itching, or more severe responses, can also occur in some individuals. Users may experience neurological side effects, including issues with coordination, memory, or concentration. These side effects can be especially troublesome, as they can impair daily functioning.[5]

It's important to note that while psychoactive drugs can offer temporary relief from symptoms, they typically do not address the root causes of the conditions they are meant to treat. This means that individuals relying solely on these medications may

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find their symptoms returning or worsening once they discontinue their use. This is where the appeal of herbal medicine lies, as it offers a potential alternative with fewer side effects and a focus on addressing underlying causes for more sustained relief.[6,7]

Numerous herbal remedies show promise in managing chronic neurological conditions like anxiety, depression, headaches, and epilepsy, often resistant to conventional treatments.[8] *Benincasa hispida*, commonly known as winter melon, wax gourd, or ash gourd, is a fruit-bearing vine in the gourd family (Cucurbitaceae). It is grown for its edible fruit, which is used in various culinary dishes in many parts of the world, particularly in Asian cuisine. The fruit is large, often round, or oblong, with a smooth, waxy green skin and white or pale green flesh. It is a versatile ingredient in cooking and is utilized in both savory and sweet dishes.

Triterpenoids, found in the seeds and flesh, exhibit diverse potential health benefits, while flavonoids, known for their antioxidant properties, are present in the fruit. This versatile fruit is also a source of essential vitamins, including vitamin C, and minerals like calcium and phosphorus. Its high dietary fiber content supports digestive health and weight management. Additionally, saponins, proteins, amino acids, and carotenoids, contribute to the nutritional and potential therapeutic value of winter melon. It holds promise for its potential health benefits, including antioxidant and anti-inflammatory effects, blood sugar regulation, weight management, immune support, cardiovascular health, antibacterial properties, diuretic effects, neuroprotective effect, and potential anticancer activity.[9-11]

No studies have previously investigated the impact of *Benincasa hispida* fruit extract on memory and cognitive function in an animal model. This study aimed to elucidate the potential protective effects of *Benincasa hispida* fruit extract on memory and cognitive abilities in adult swiss albino mice.

2. MATERIAL & METHODS

2.1 Chemicals and Drugs

Donepezil hydrochloride (DH) was obtained from Actavis Limited in India. Ascorbic acid, sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were sourced from standard suppliers.

2.2 Collection and Extraction of Plant Material

The *Benincasa hispida* fruits were procured from Ghatkesar village in Telangana, India, and their authenticity was verified by the Botanical Survey of India. The literature indicated that the ethanolic extract contains the majority of the active phytochemical ingredients.[9] Therefore, ethanol was chosen as the preferred extraction solvent. The fruits were air-dried and then coarsely ground. A sequential extraction method was employed, and the powdered material was subjected to ethanol extraction using a Soxhlet apparatus. Following this, the extracts were concentrated under reduced pressure and maintained at a temperature within the range of 8–10°C throughout the entire study duration.[12]

2.3 Antioxidant Activity

The antioxidant activity was determined by total antioxidant capacity assay. The total antioxidant capacity assay is a spectroscopic technique used to quantitatively assess the antioxidant capacity by forming a phosphomolybdenum complex. This method relies on the reduction of Mo (VI) to Mo (V) by the analyte in the sample, resulting in the creation of a green phosphate Mo (V) complex under acidic conditions. Various concentrations of standard and test sample solutions were mixed with 1 ml of a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The test tubes were sealed, incubated in a boiling water bath at 95°C for 90 minutes, and then allowed to cool to room temperature. The reference solution, used as a blank, consisted of 1 ml of the reagent solution and 0.1 ml of distilled water. The absorbance of the aqueous solutions was measured at 695 nm against the blank using a UV spectrophotometer.[13]

2.4 Experimental Animals

Swiss male albino mice weighing between 20 to 25 grams were utilized in the study. These mice were housed in a controlled environment with a temperature of 25°C ± 2°C and a relative humidity of 45-55%, following a standard 12-hour light to 12-hour dark cycle. They had unrestricted access to both food and water, with food being withheld for 6 hours before drug administration. The Institutional Animal Ethics

Committee (IAEC) approved the experimental protocol, which was conducted in accordance with IAEC's established guidelines.

The mice were divided into 6 groups with 6 mice in each group. To prevent potential interactions between aluminum and other substances, $AlCl_3$ was administered 30 minutes prior to donepezil hydrochloride (DH) and the *Benincasa hispida* methanolic extract (BHME).[14] After 35 days, the mice were then exposed to the Morris water maze test and passive avoidance test.

- Group 1 (Vehicle Control): Water p.o. for 35 days
- Group 2 (Negative Control): $AlCl_3$ 50 mg/kg/day p.o. for35 days
- Group 3 (Positive Control): Donepezil hydrochloride (DH) 3 mg/kg/day, p.o. + $AlCl_3$ 50 mg/kg/day p.o. for 35 days
- Group 4 (Treatment Control I): BHME 200 mg/kg/day, p.o. + $AlCl_3$ 50 mg/kg/day p.o. for 35 days
- Group 5 (Treatment Control II): BHME 400 mg/kg/day, p.o. + $AlCl_3$ 50 mg/kg/day p.o. for 35 days

2.5 Assessment of Memory and Cognitive Function

2.5.1 Morris Water Maze Test

The Morris Water Maze (MWM) task was conducted spanning a period of six days. The water maze is a large circular metallic pool with a black interior, measuring 110 cm in diameter and 40 cm in height. The water, maintained at a temperature of 20 ± 1°C, was filled to a depth of 30 cm. The water maze area was partitioned into four equal quadrants, and a transparent escape platform, measuring 10 cm in diameter and 25 cm in height, was positioned in the south-west quadrant. The entire experiment was under constant surveillance via an overhead camera fixed directly above the pool. Each mouse was gently held by the base of its tail and introduced into the water, and the time it took for the mouse to locate the platform (escape latency) was recorded. Each mouse underwent four trials per day.

On the first day, the platform was made visible by keeping the water level 1 cm below the platform. On days 2 through 5, the platform was submerged below the water surface. Day 6 marked the probe trial, during which the platform was entirely removed, and the time spent by the mouse in the target quadrant (south-west) was noted. Each animal had 90 seconds to locate the platform. If a mouse failed to do so within this time frame, it was gently guided to the platform. Upon reaching the platform, the mouse remained there for 5 seconds, after which it was dried with a towel and returned to its cage.[15]

2.5.2 Passive Avoidance Test

A passive avoidance test was conducted using a shuttle box apparatus, comprising one illuminated chamber and one dark chamber separated by a grid door. Electrical shocks were administered through a separate stimulator to the grid floor of the shuttle box. This test was carried out for each mouse over four consecutive days.

On the first and second days, each mouse was introduced and allowed to acclimate to the apparatus for 60 seconds. On the third day, an acquisition trial was conducted. The animals were initially placed in the illuminated compartment, and the door separating the two chambers was opened 20 seconds later. The initial latency (T1), or the time it took for a mouse to enter the dark compartment, was measured. When a mouse entered the dark compartment, the door was closed, and an electric foot-shock (1 mA for 1 second) was administered through stainless steel rods using a constant current shock generator. All animals entered the dark compartment within the 60-second cutoff latency during the training session and received foot-shock. On the fourth day, the step-through latency (T2) for the animals was recorded using the same procedure, but this time without the delivery of a foot-shock.[16]

2.6 Statistical Analysis

The results were presented as the mean ± SEM (Standard Error of the Mean). The data was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test within GraphPad Prism (version 7.03).

3. RESULTS

3.1 Antioxidant Activity

The ethanolic extract derived from *Benincasa hispida* fruits exhibited an explicit capacity to scavenge free radicals, as evident from the phosphomolybdenum assay. The antioxidant compounds present in

Benincasa hispida fruits extract effectively facilitated the reduction of Mo (VI) to Mo (V) and the formation of a green phosphate Mo (V) complex under acidic conditions. The scavenging activity demonstrated a direct correlation with the dose. The highest scavenging activity of the extract was recorded at a concentration of 100 µg/ml, which closely approximated the efficacy of the standard drug, ascorbic acid (Figure 1).

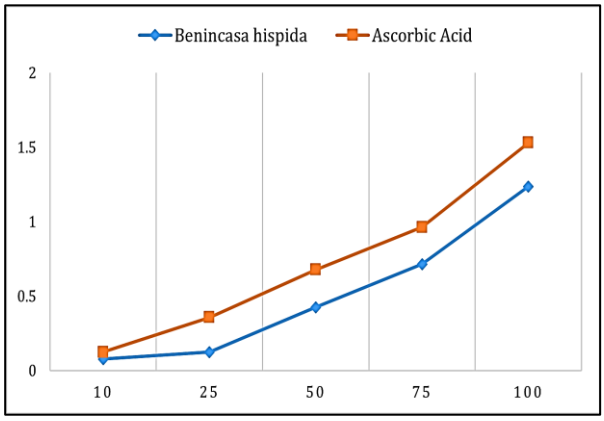

Fig. 1: Assessing antioxidant activity of *Benincasa hispida* through the total antioxidant capacity assay

3.2 Effect of BHME on Morris Water Maze Test

The MWM behavioural test was employed to evaluate escape latencies (EL) and time spent in the target quadrant (TSTQ), both measured in seconds. The EL results for all groups are presented in Table 1. There were notable differences in EL among the groups over the course of five days ($P < 0.0001$). Furthermore, a significant distinction in EL was observed between the BHME 400 mg/kg group and the $AlCl_3$ group ($P < 0.0001$), with mean ELs of 27.35 ± 2.69 s and 61.34 ± 1.68 s, respectively. Similarly, a significant contrast was noted between the DH group and the $AlCl_3$ group ($P < 0.0001$). No significant difference in EL was observed between the BHME group at 400 mg/kg and the DH 3 mg/kg group ($P < 0.0060$). Notably, the EL for the $AlCl_3$ group (61.34 ± 1.68 s) was significantly higher than that of the vehicle control group (water, 37.46 ± 2.82 s). The DH 3 mg/kg group exhibited the shortest EL (21.34 ± 1.56 s), followed by the BHME at 400 mg/kg group (27.35 ± 2.69 s). In a comparison between the two BHME dosage groups, the BHME at 400 mg/kg group displayed a shorter EL than the BHME at 200 mg/kg group.

On the sixth day, the probe trial was conducted to record the time each mouse spent in the target quadrant, with results presented in Table 1. The $AlCl_3$ group had the shortest duration spent in the target quadrant (5.61 ± 1.57 s), while the longest was observed in the DH 3 mg/kg group (25.33 ± 1.58 s). No significant difference in the time spent in the target quadrant was noted between the DH 3 mg/kg and BHME 400 mg/kg groups (23.24 ± 1.31 s). The BHME 400 mg/kg-treated group showed a significantly longer time spent in the target quadrant compared to the $AlCl_3$-treated group ($P < 0.0001$).

3.3 Effect of BHME on Passive Avoidance Test

As depicted in Table 2, the average initial latency (T1) demonstrated a statistically significant decrease in the $AlCl_3$ group when compared to the BHME 400 mg/kg group ($P < 0.0001$). However, there was no notable difference between the control group and the $AlCl_3$ group in terms of T1. Conversely, the T2 latency significantly increased in the BHME 400 mg/kg group when compared to the $AlCl_3$ group ($P < 0.0001$). Groups of intact animals receiving BHME extracts at 400 mg/kg displayed significantly higher step-through latency compared to the $AlCl_3$ group ($P < 0.0001$). Additionally, the results revealed no significant difference in step-through latency time between the control group and the $AlCl_3$ groups.

4. DISCUSSION

The current study investigated the effect of a methanolic extract of *Benincasa hispida* fruit in the aluminum mouse model for Alzheimer's disease using the Morris water maze test and passive avoidance test for assessing memory function.

Memory and its intricate processes play a pivotal role in understanding and addressing dementia, a collective term for cognitive disorders marked by the progressive loss of memory and cognitive function.[17] Alzheimer's disease (AD), the most prevalent form of dementia, is characterized by the accumulation of amyloid-beta plaques and tau tangles in the brain, which disrupt neuronal communication and lead to memory impairments. Other forms of dementia, such as vascular dementia and frontotemporal dementia, also result in memory deficits but stem from different pathological processes. Research into dementia seeks to unravel the complex mechanisms that underlie memory impairment and cognitive decline in these conditions.[18]

Table 1: The impact of BHME on the Morris water maze test

S. No.	Group	Escape Latencies Time (s)	Time Spent in Quadrant (s)
1	Vehicle Control	37.46 ± 2.82	8.34 ± 1.34
2	Negative Control	61.34 ± 1.68	5.61 ± 1.57
3	Positive Control	21.34 ± 1.56**	25.33 ± 1.58
4	Treatment Control I	33.21 ± 1.24	21.83 ± 1.23
5	Treatment Control II	27.35 ± 2.69*	23.24 ± 1.31*#

*The data were presented as mean ± SEM (n=6). Statistical analysis was conducted using a one-way ANOVA, followed by Dunnett's Multiple Comparison test. Escape latency time: *versus $AlCl_3$, P < 0.0001, **versus $AlCl_3$, P < 0.0001. Time spent in the quadrant: * versus donepezil, P = 0.9210, ## versus $AlCl_3$-treated group, P < 0.0001.*

Table 2: The initial latency (T1) and step-through (T2) latency time in the passive avoidance response

S. No.	Group	Initial Latency (T1)	Step-through Latency (T2)
1	Vehicle Control	6.74 ± 1.23	18.32 ± 1.52
2	Negative Control	4.35 ± 1.67	15.74 ± 8.14
3	Positive Control	22.64 ± 1.52**	50.09 ± 1.64**
4	Treatment Control I	16.38 ± 1.72*	38.36 ± 2.45*
5	Treatment Control II	18.01 ± 6.41**	43.82 ± 6.41**

*The data were presented as mean ± SEM (n=6). Statistical analysis was conducted using a one-way ANOVA, followed by Dunnett's Multiple Comparison test. *versus $AlCl_3$, P < 0.0001, **versus $AlCl_3$, P < 0.0001.*

The accumulation of β-amyloid protein (AβP) is a key factor in AD. Research indicates that aluminum can enhance the formation of AβP. Studies in mice show that orally administered aluminum increases AβP levels. This makes the aluminum mouse model a relevant system for studying AD's pathogenesis. In this study, mice exposed to aluminum displayed impaired memory, showed longer escape latencies & less time in the target quadrant during the Morris water maze test. This underscores the potential role of aluminum in AD-related cognitive decline.[19]

The Morris water maze test is a widely appreciated animal model for the evaluation of spatial learning and memory. Its face validity makes it particularly relevant as it closely mimics the real-world challenges of spatial navigation, making it a valuable tool for studying cognitive processes. The Morris water maze allows for comparability across different studies and laboratories.[20]

In the Morris Water Maze (MWM) test, a reduced escape latency (EL) over the course of 5 days and an increase in the time spent in the target quadrant on the 6th day show improved memory performance. Notably, treatment with BHME at a dose of 400 mg/kg demonstrated a significant enhancement in memory function when compared to the negative control group ($AlCl_3$) and the vehicle control group.

Furthermore, the BHME 400 mg/kg group exhibited memory-enhancing effects on par with the positive control group treated with DH, an approved medication for AD. Although the DH 3 mg/kg group displayed the shortest EL, the difference was not statistically significant when compared to the BHME 400 mg/kg group. This suggests that BHME at 400 mg/kg was equally effective as DH at 3 mg/kg in improving memory function. The significantly reduced EL in the BHME 400 mg/kg treated group, as opposed to the control group, indicates that *Benincasa hispida* extract ameliorated memory function in the MWM test. It is noteworthy that there was also a dose-dependent reduction in EL in BHME 200 mg/kg treatment groups.

During the probe trial on the 6th day, the platform in the maze was removed. The duration each mouse spent in the target quadrant was recorded, with longer times indicating improved memory function. Similar outcomes were observed during this probe trial. The group treated with DH spent the most extended time in the target quadrant. However, no significant difference was noted in the time spent in the target quadrant between the DH 3 mg/kg and the BHME 400 mg/kg groups. Mice treated with BHME at 400 mg/kg spent significantly more time in the target quadrant compared to the $AlCl_3$ group. The

results from EL and time spent in the target quadrant suggest that BHME mitigated aluminum-induced spatial memory impairment, enhancing memory function in the process. In the passive avoidance test, it was observed that there was no difference between positive control and BHME treated animals indicating good latency periods.

The DPPH free radical scavenging assay was performed to assess the antioxidant activity of the crude extract of BHME. Although a dose-dependent increase of free radical scavenging activity was seen with an increased dose of BHME, the activity was significantly less than that of ascorbic acid.

Alzheimer's disease is a multifaceted condition with various contributing factors, including exposure to toxic metals like aluminum, reduced cholinergic transmission, the presence of amyloid proteins, and neurodegeneration due to free radicals.[21] Epidemiological and laboratory studies provide evidence that the consumption of foods rich in antioxidants can play a role in slowing down the progression of AD. These positive outcomes are linked to the presence of antioxidant compounds in these dietary components.[22]

Addressing AD-related pathologies is challenging, but there's potential for treatment with *Benincasa hispida* extract (BHME). This study has revealed that BHME exhibits memory enhancing activity, which can help mitigate some of the cognitive impairments associated with AD as evident from its antioxidant activity, which is essential in countering the damaging effects of free radicals implicated in neurodegeneration.

5. CONCLUSION

The methanolic extract of *Benincasa hispida*, exhibited a significant memory-enhancing effect in the present study comparable to the results achieved with donepezil. Consequently, it can be inferred that high doses of *Benincasa hispida* are effective in mitigating memory deficits induced by aluminum chloride in mice, as evidenced by their performance in both the Morris water maze and passive avoidance test. This suggests the potential utility of *Benincasa hispida* in the treatment of Alzheimer's disease and related cognitive disorders. In summary, the study effectively illustrates the capacity of *Benincasa hispida* methanolic extract to significantly ameliorate spatial and passive avoidance memory impairments resulting from $AlCl_3$, signifying its promising therapeutic potential in neurological conditions like AD. However, further research is required to expand upon and corroborate these findings.

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