Research Article

Ameliorative effect of hydroalcoholic extract of *Caryota urens* (Arecaceae) on streptozotocin-induced Alzheimer's model in mice

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ABSTRACT

Alzheimer's disease (AD) is a progressive brain disorder that gradually impairs memory and ability to learn, reasoning, judgment, communication, and daily activities. The present study is to evaluate the memory enhancement and cognitive effect of *Caryota urens* on Alzheimer's induced mice using various memory retention experiments such as Y maze, Morris water maze, and Passive avoidance. *In vitro* evaluation of neurotransmitters was done on isolated brain tissue homogenates. AD was induced by intracerebroventricular injection of streptozotocin to mice. The selected dose 200 mg/kg and 400 mg/kg of *C. urens* showed significant action in memory and learning processes. From the results, it can be concluded that *C. urens* has promising effect in memory enhancement.

KEY WORDS: Alzheimer's disease, *Caryota urens,* Cognitive effects

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease and one of the major causes of age-related dementia. Clinical characterization of the AD includes progressive loss of memory, cognitive impairment, and personality changes.[1] Major neuropathological lesions associated with AD are extracellular deposits of amyloid beta (Aβ) peptides in the form of senile plaques, which liberated from the amyloid precursor protein.[2] There will be an intracellular accumulation of neurofibrillary tangles composed of hyperphosphorylated tau protein, the loss of synapses, synaptic function, mitochondrial abnormalities, inflammatory responses, and neuronal loss.[3] Epidemiological studies revealed that, in 2016, there were 26.6 million cases of the AD in the world, and it was predicted that the worldwide occurrence of the AD will grow four-fold to 106.8 million by the year 2050.[4]

Although there is no cure for AD, Food and Drug Administration so far approved five drugs for the management of AD. Approved AD drugs are limited within two categories: Cholinesterase inhibitors (donepezil, galantamine, and rivastigmine) and memantine (a N-Methyl-D-aspartate receptor antagonist).[5] They can temporarily alleviate symptoms, or slow down their progression in some people. Side effects reported with these drugs are nausea, vomiting, weight loss, dizziness, headache, and anorexia by cholinesterase inhibitors. Side effects of memantine include headache, hypertension, and constipation.

Streptozotocin (STZ) administration through intracerebroventricular or intraperitoneal injection produces reduced cognition and increased cerebral aggregation of Aβ fragments, total tau protein, and Aβ deposits, also produce neuroinflammation, oxidative stress, and biochemical alterations, which is considered to be a valid experimental model of the early pathophysiological changes in neurodegenerative disease.[6]

The study is to evaluate the memory enhancement and cognitive effect of *Caryota urens* on Alzheimer's induced mice using memory retention experiments such as Y-Maze, Morris water maze, and Passive avoidance. *In vitro* evaluation of neurotransmitters

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and estimation were done on isolated brain tissue homogenates.

MATERIALS AND METHODS

Collection, Authentication, and Preparation of Hydroalcoholic Extract of *C. urens* (HAECU)

The fresh flowering bud of the plant of *C. urens* was collected and washed with running water. It was shade dried at room temperature, and that dried plant material was made into coarse powder. The powder was extracted with ethanol (70%) and water (30%) in Soxhlet extractor. The plant was identified and authenticated by Dr. D. Aravind, Assistant Professor, specialization in medicinal plants, Department of Botany, National Institute Of Siddha, Ministry of AYUSH (Government of India), and Chennai - 47. The HAECU was subjected to preliminary phytochemical screening for phytoconstituents.

Acute Toxicity Studies

The acute toxicity was done using OECD guidelines 423.[7] Adult female Swiss albino mice weighing 20–30 g were used for the study. The starting dose of 2000 mg/kg body weight p.o of HAECU was used. Body weight of mice before and after administration was noted, and any changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous system, motor activity, and behavior pattern were observed, and also sign of tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma were noted. The signs of toxicity were noted.

Experimental Animals

Swiss albino mice weighing 22–30 g were used for this study. The inbreed animals were procured from the animal house of C. L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai - 97. They were housed six mice/cage under standard laboratory conditions at a temperature 22 ± 2°C with 12:12 h light and dark circle. The animals were provided with standard animal feed, water *ad libitum*. The animals were adapted to laboratory conditions 1 week before initiation of experiments. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals. The study was approved by Institutional Animal Ethical Committee, IAEC Reference No: IAEC/XLVII02/CLBMCP/2015.

Experimental Design

The animals were divided randomly into five groups of six animals each.

- Group I - Normal control mice received vehicle solution.
- Group II - AD mice (AD induced by intracerebroventricular [i.c.v.] injection) of STZ).
- Group III - AD mice treated with standard drug Donepezil (5 mg/kg, i.p.).
- Group IV - AD mice pretreated with HAECU (200 mg/kg, p.o).
- Group V - AD mice pretreated with HAECU (400 mg/kg, p.o).

Amnesia was induced by (i.c.v. injection) of STZ for the II, III, IV, and V groups animals on the 21st day of their extract treatment period, and the treatment was continued for further 9 days. Control animals were given 1% w/v of CMC orally using intragastric catheter. The last dose was given 60 min before behavioral testing and on the 30th-day scarification of animals was done for *in vitro* studies.[8]

Experimental dementia of AD in mice was induced by i.c.v. STZ. Mice were anesthetized with anesthetic ether and i.c.v. injections were made with a hypodermic needle of 0.4 mm external diameter attached to a 10 µl Hamilton microliter syringe. STZ was dissolved in freshly made artificial cerebrospinal fluid (25 mg/ml) solution.

Assessment of Memory and Retention

Morris water maze test[9,10,11]

The Morris water maze test is performed to evaluate spatial working and reference memory. The animals were placed into a large circular pool of water where it is divided into four qua`drants, in one of the quadrants the hidden platform is placed where it can escape. Morris water maze is a large circular tank made of black opaque polyvinyl chloride (1.8–2 m in diameter and 0.4–0.6 m high). The pool is filled up to a height of 30 cm with water maintained at around 25°C and rendered opaque by the addition of small quantity of milk. Each animal is subjected to 4 consecutive trials for 4 days (21–24) during which mice were allowed to escape on to the hidden platform and allowed to remain for 20 s. Escape latency (EL) time to locate the hidden platform in water maze is noted as an index of acquisition or learning if the animal is unable to locate the hidden platform within 120 s, it is gently guided by hand to the platform and allowed to remain there for 20 s. On the 29th day, 60 min after the last dose, platform is removed and time spent by each animal in target quadrant searching for the hidden platform is noted as an index of retrieval and noted.

Passive shock avoidance test[12]

Passive avoidance test used to examine the long-term memory. The instrument made up of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of plexiglass, featuring a grid floor (3 mm stainless steel rods set 8mm apart) with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. Electric shock (20V, A/C) was delivered to the grid floor. During the training session, each mouse

was gently placed on the wooden platform set in the center of the grid floor, when the mouse stepped down and placed all its paw on the grid floor, shocks were delivered for 15 s, and the step-down latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from the wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range of 2–15 s during the first were used for the second session and the retention test. The second session was carried out 90 min after the first test. During the second session, if the animals stepped down before 60 s, electric shocks were delivered once again for 15 s. During the second test, animals were removed from shock free zone, if they did not step down for a period of 60 s and subjected to retention test.

***Y maze test*[13]**

Immediate working memory performance was assessed by recording spontaneous alternation behavior in a single session in a Y maze made up of black painted wood. Each arm is 40 cm long, 12 cm high, 3 cm wide at the bottom and 10 cm wide at the top and converged into an equilateral triangular central area. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The series of arm entries was recorded visually. Entry was considered to be completed when the hind paws of the mouse had completely entered the arm. Alternation was defined as successive entries into the three different arms (A, B, and C) on overlapping triplet sets. Percentage alternation was calculated as the ratio of actual to possible alternation (defined as the total number of arm entries minus two), multiplied by 100 as shown % alternation={(No. of alternations)/(Total arm entries −2)} × 100.

After the treatment period, the mice were euthanized; brain tissue was extracted and subsequently assayed for various neurotransmitters (dopamine, serotonin, glutamate, and ach esterase).

Statistical Analysis

The statistical analysis was carried by one-way ANOVA followed by Dunnett's *t*-test. *P* values <0.05 (95% confidence limit) were considered statistically significant, using Software GraphPad Prism 7.5.

RESULTS

Acute Oral Toxicity Studies

The acute oral toxicity was done according to the OECD 423 (acute toxic class method) guidelines. There was no change in the body weight before and after treatment of the experiment, and no sign of toxicity was observed. Observations are shown in Table 1.

Effect of HAECU on Step Down Passive Shock Avoidance Test

The SDL of Group II animals was significantly decreased ($P < 0.001$) when compared with Group I animals. Treatment with HAECU (200 and 400 mg/kg) and standard drug showed a significant increase in SDL when compared with Group II. The increase in SDL indicates an increase in short-term memory. Results are given in Graph shown as Figure 1.

Effect of HAECU on Y Maze Task

The percentage of alteration was significantly decreased in Group II when compared with Group I animals significantly ($P < 0.001$). Treatment with HAECU (200 and 400 mg/kg) showed significantly increased in the percentage of alteration when compared to Group II animals. The standard drug also showed significantly increased in the percentage of alteration when compared Group II animals. Results are given in graph shown as Figure 2.

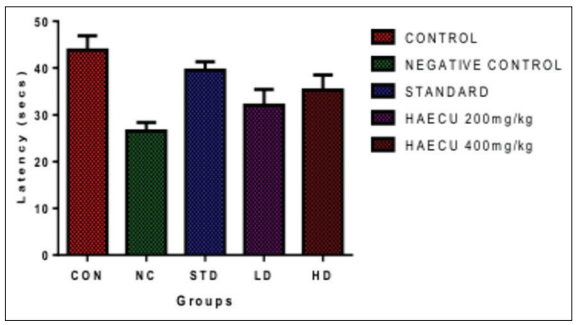

Figure 1: Effect of hydroalcoholic extract of *Caryota urens* in passive avoidance

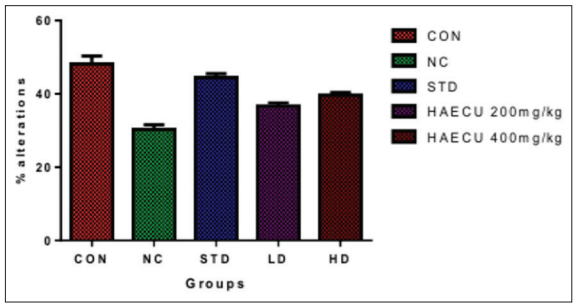

Figure 2: Effect of hydroalcoholic extract of *Caryota urens* in Y maze

Table 1: Effect of HAECU in acute toxicity study

Treatment	Dose	Weight of animal (g)		Signs of toxicity	Onset of toxicity	Reversible of irreversible	Duration
HAECU	2000 mg/kg	25	28	No signs of toxicity	Nil	Nil	14 days
HAECU	2000 mg/kg	20	22				
HAECU	2000 mg/kg	20	22				

HAECU: Hydroalcoholic extract of *Caryota urens*

Effect of HAECU on Morris Water Maze Task

The EL of Group II animals was significantly increased when compared with Group I animals. Treatment with HAECU (200 and 400 mg/kg) and standard drug showed a significant decrease in the EL onto the hidden platform when compared with Group II animals. The decrease in EL indicates memory retention and non-spatial working memory. Results are given in Table 2.

In Vitro Biochemical Estimations (Neurotransmitter Levels)

Effect of HAECU on dopamine

The brain dopamine level in Group II animals was significantly decreased ($P < 0.001$) when compared with Group I animals. Treatment with HAECU (200 and 400 mg/kg) and standard drug showed a significant increase in dopamine level on comparison with Group II animals. Results are given in Table 3.

Effect of HAECU on serotonin

The serotonin level in the brain of Group II animals was decreased significantly ($P < 0.001$) when compared with Group I animals. Treatment with HAECU (200 and 400 mg/kg) significantly increased the serotonin level when compared with Group II animals. The standard drug also showed a significantly increased level of serotonin when compared Group II animals. Results are given in Table 4.

Effect of HAECU on acetylcholinesterase

The level of AChE in Group II animals showed a significant increase when compared with Group I animals. Treatment with HAECU (200 and 400 mg/kg) showed a significant decrease in the AChE level when compared with Group II animals. Group III ($P < 0.001$) showed a significant reduction in AChE activity when compared with Group II animals. Results are given in Table 5.

DISCUSSION

The present study has revealed the ameliorative effect of HAECU on STZ-induced AD in mice using various behavioral parameters such as Passive avoidance task, Y maze task, and Morris water maze test. It was found that treatment with HAECU protects cognitive deficits in the STZ-induced AD.

Passive avoidance behavior based on negative reinforcement was used to examine the level of memory. An electric shock is as reinforcement during training sessions for 15 s in the SDL[14] was recorded. SDL is increased as a form of long-term memory where STZ-induced animals showed decreased SDL. Treatment with HAECU at test dosages showed improvement in long-term memory index of an increase in SDL.

Y maze task is one of the simplest versions of spontaneous alteration task which is used to measure spatial working memory. The ability to alternate requires that the mice know which arm they have already visited. The animals which were STZ induced had a reduced spontaneous alteration but animals treated with HAECU produced a significant increase in alteration which was comparable to the untreated control which, in turn, indicates the increased spatial working memory of the animals.

Table 2: Effect of HAECU in Morris water maze

Groups	EL (s)
Control	12.00±0.96
Negative control	36.00±2.20[a]*
Standard	12.83±1.44[b]***
HAECU 200 mg/kg	19.17±1.57[b]***
HAECU 400 mg/kg	15.33±1.25[b]***

Values are represented in mean±SEM, n=6. Comparison: [a]Group I versus Group II, [b]Group II versus Group III and Group IV. Statistical significance test for comparison was done by one-way ANOVA followed by Dunnett's t-test. Ns: Non significant, *P<0.05, **P<0.01, *P<0.001. EL: Escape latency, HAECU: Hydroalcoholic extract of *Caryota urens*, SEM: Standard error mean

Table 3: Effect of HAECU in dopamine

Groups	Ng/mg wet tissue
Control	432±13.45
Negative control	303.0±4.51[a]***
Stndard	385.6±3.80[b]***
HAECU 200 mg/kg	352.9±2.44[b]**
HAECU 400 mg/kg	367.5±4.60[b]***

Values are represented in mean±SEM, n=6. Comparison: [a]Group I versus Group II, [b]Group II versus Group III and Group IV Statistical significance test for comparison was done by one-way ANOVA followed by Dunnett's t-test. Ns: Nonsignificant, *P<0.05, **P<0.01, *P<0.001. HAECU: Hydroalcoholic extract of *Caryota urens*, SEM: Standard error mean

Table 4: Effect of HAECU in serotonin

Groups	Ng/mg wet tissue
Control	247.33±5.86
Negative control	177.48±3.15[a]***
Standard	238.56±5.46[b]***
HAECU 200 mg/kg	203.52±2.726[b]**
HAECU 400 mg/kg	220.00±2.926[b]***

Values are represented in mean±SEM, n=6. Comparison: [a]Group I versus Group II, [b]Group II versus Group III and Group IV statistical significance test for comparison was done by one-way ANOVA followed by Dunnett's t-test. Ns: Nonsignificant, *P<0.05, **P<0.01, *P<0.001. HAECU: Hydroalcoholic extract of *Caryota urens*, SEM: Standard error mean

Table 5: Effect of HAECU in acetylcholinesterase

Groups	Ng/mg wet tissue
Control	24.53±0.67
Negative control	32.40±0.9[a]***
Standard	18.31±0.81[b]***
HAECU 200 mg/kg	22.43±0.50[b]***
HAECU 400 mg/kg	20.25±0.72[b]***

Values are represented in mean±SEM, n=6. Comparison: [a]Group I versus Group II, [b]Group II versus Group III and Group IV. Statistical significance test for comparison was done by one-way ANOVA followed by Dunnett's t-test. Ns: Nonsignificant, *P<0.05, **P <0.01, *P<0.001. HAECU: Hydroalcoholic extract of *Caryota urens*, SEM: Standard error mean

Morris water maze task is specific for spatial memory. The essential feature of this technique is that mice are placed in a large circular pool of water and can escape into a hidden platform.[15] STZ-induced animals exhibited an increased time for EL indicating loss of visual cues to escape into the platform such a diminished cognition was reversed by the administration of the HAECU at 200 mg/kg and 400 mg/kg dosage levels and exhibited EL, indicating the well-developed spatial memory in spite of the STZ-induced AD.

Both nicotinic and muscarinic cholinergic receptors are involved in cognitive and memory functions, and several studies have suggested their roles in dementia. Marked cholinergic deficit is a hallmark of the pathogenesis of AD[16] and various drugs including AchE inhibitors have designed to target this deficit, initially, the cholinergic deficit was thought to be a muscarinic nature, but recent study shows a specific loss of nicotinic acetylcholine receptors and marked loss of cholinergic neurons. There was a significant reduction in the level of acetylcholine esterase in the animals treated with HAECU and increased Ach which regulates the impaired memory by STZ induction.

Serotonin is the critical neurotransmitter modulating short-term habituation and in asphyxia. In general, hippocampal depletions affects habituation in the open field, but rodents also commonly exhibit decreased locomotor and exploratory activity.

The formation of new memories is thought to require the hippocampus and adjacent medial temporal lobe, but the final storage of memories is widely distributed by the neocortical network. Lesion studies have suggested that there is a wide distribution of neocortical memory traces encoded in the strength of synaptic connections among neurons across large areas of the neocortex. Serotonergic effects have also been detected and on the region implicated in memory storage are richly innervated by the serotonergic system.[17]

CONCLUSION

The present study showed the action of *C. urens* STZ-induced AD on mice model. The selected dose 200 mg/kg and 400 mg/kg of *C. urens* improved the memory and learning processes in mice, but higher dose 400 mg/kg showed significant action than lower dose 200 mg/kg. From the results, it can be concluded that *C. urens* has got a significant effect in memory enhancement. Further molecular level studies are required for the identification and confirmation of phytoconstitutents which are responsible for its CNS action.

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