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Research Article

Crinum zeylanicum memory enhancing effect is mediated via central cholinergic transmission system

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ABSTRACT

Background: *Crinum zeylanicum* is widely used in the ethno-therapeutic management of folk management of epilepsy, pain, neuropsychiatric, and dementing disorders in Nigeria. The current study was carried out to evaluate the possible mechanism of the memory enhancing the effect of *C. zeylanicum* extract and alkaloidal rich fraction in Wistar rats.
Methods: The effect of *Crinum zeylanicum* bulb extract (250, 500, and 1000 mg/kg body weight orally), alkaloidal rich fraction (10, 20, and 40 mg/kg body weight p.o.), normal saline (10 ml/kg orally), or Eserine (0.3 mg/kg body weight i.p.) on spatial memory in rats was evaluated using the Y-maze. The blood samples obtained from rats in all treatment groups were evaluated for cholinesterase activities using modified Michelle electrometric method.
Results: The extract and the alkaloid significantly ($p<0.05$) and dose-dependently increased spontaneous alternation behavior of rats in Y-maze. The extract produced 20.00%, 35.55%, and 52.00% inhibition of cholinesterase activity in the blood at 250, 500, and 1000 mg/kg body weight, respectively. The alkaloid produced 56.67%, 62.67%, and 68.67% inhibition of cholinesterase activity in blood at 10, 20, and 40 mg/kg body weight (p.o.). Eserine a standard cholinesterase inhibitor at 0.3 mg/kg body weight produced a significant increase in spontaneous alternation behavior and produced 73.33% inhibition of blood cholinesterase activity. Data obtained from the study showed that the enhanced spontaneous alternation behavior observed in rats treated with the extract, and the alkaloid may be due to facilitation of cholinergic transmission resulting from inhibition of cholinesterase activity.
Conclusion: The extract, as well as its partially purified alkaloid, possesses potential that may be employed for therapeutic management of Alzheimer's disease.

Keywords: Azheimer's disease, Cholinesterase activity, Blood, Brain homogenate, Y-maze

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease of this century and the most prevalent cause of dementia among the elderly.[1,2] The disorder is characterized by the presence of extra-neuronal amyloid and tau deposits, dysfunction in cholinergic transmission typified by a progressive decline in levels

of acetylcholine (ACh) resulting memory impairment and behavioral deficits.[3] One of the most remarkable biochemical changes in AD patients is the reduction of ACh levels in the hippocampus and cortex of the brain. That is why the most common used symptomatic treatment of mild AD is through enhancement of cholinergic function through inhibition of acetylcholinesterase (AChE).[4,5] However, the currently used cholinesterase inhibitors tacrine, donepezil, galanthamine, and rivastigmine usefulness is limited by their non-selectivity, limited efficacy, poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges, and hepatotoxicity.[6,7] Therefore, there is a need for developing new compounds with multiple potencies and minimal side effect profiles.

Medicinal plants have been the primary source of medicines and are rich sources of secondary metabolites and oils that are important in therapeutics. The most important advantage of them is their low price and availability worldwide beside their safety.[8] *Crinum zeylanicum* use as ethno-therapeutic agent in the management of convulsion, insomnia, anxiety, and amnesia has been validated by Adeniyi et al.,[8] Tijani et al.,[9] and Yahaya Tijani et al.[10] The pharmacological evidence for therapeutic benefit of its use in management of cognitive deficit has been reported by Tijani et al.[11] This study was therefore carried out to evaluate the involvement of cholinergic neurotransmission in the memory enhancing effects of the extract and alkaloid of *C. zeylanicum*.

METHODS

Drugs and chemicals

Sodium barbital (BDH), Eserine Sigma Chemical Co., (St. Louis, Missouri, USA), sodium chloride (BDH).

Plant material

The plant *C. zeylanicum* was collected by Mallam Muazzam Ibrahim an ethnobotanist, authenticated by Mrs. Jemilat Ibrahim a taxonomist all of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja where a voucher sample (NIPRD/H/6258) was prepared and deposited.

Extraction of plant material

The bulb of *C. zeylanicum* was crushed and air-dried at room temperature. 100 g of the dry plant material was macerated in 70% methanol for 48 hrs. The resulting mixture was filtered using muslin cloth followed by Whatman filter paper (No. 1). The aliquots obtained was dried on water bath and stored at − 4°C until required for use.

Extraction of alkaloid

The alkaloid was extracted as earlier described by Tijani et al.[11] Finely powdered dried bulb of *C. zeylanicum* bulb (200 g) was moistened with water. The mixture of the plant material and water was treated with lime and ammonia solution, filtered and the filtrate extracted with chloroform to obtain aqueous phase and an organic phase. The aqueous phase was collected and treated with ammonia, followed by chloroform. The chloroform portion was evaporated at room temperature of 25°C to leave behind the crude alkaloid.

Preparation of buffer

The barbital-phosphate buffer solution was prepared from 1.24 g sodium barbital (BDH), 0.63 g potassium dihydrogen phosphate (Merck, Germany), and 35.07 g sodium chloride (BDH) dissolved in 1 L of distilled water as described by Pohanka.[12] The pH of the buffer was adjusted to 8.1 with 1 N HCl.

Animals

Male albino rat (18-22 g) obtained from the Animal Facility Centre of NIPRD, Abuja, Nigeria were used in the study. The rat were fed standard laboratory diet, given water *ad libitum* and maintained under laboratory conditions of temperature (22°C±1°C), relative humidity (14%±1%) and 12 hrs light and 12 hrs dark cycle. All behavioral experiments were performed between 700 and 1100 hrs daily to avoid interference of circadian rhythm on the outcome of the study. The use and handling of experimental animals was in accordance with the "NIH Guide for the Care and Use of Laboratory Animals."

Pharmacological assessment of memory on Y-maze

The method earlier described by Tijani et al.[9] was used for the assessment of short term memory in rats. Male rats randomly assigned into eight treatment groups of six rats each were given *C. zeylanicum* extract (250, 500, and 1000 mg/kg, oral), alkaloid (10, 20, and 40 mg/kg p.o), normal saline (10 ml/kg p.o), and Eserine (0.3 mg/kg body weight i.p) daily for 7 days. 1 hr after the last dose on the 7th day, each rat was singly placed within one arm (M) of the maze, and the arm entry sequence (e.g., MNO, OMN, where letters indicate arm codes) and the number of arm entries were recorded manually for each mouse over 8 mins period. An entry was defined as placing all four paws within the boundaries of the arm. Alternation was determined from successive entries into the three arms on overlapping triplet sets in which three different arms are entered. The percentage alternation for each mouse was determined as the ratio of actual to possible alternations (defined as the total number of arm entries minus 2), multiplied by 100 as shown by the following equation:[9,13]

% Alternation = [(Number of alternations)/(Total arm entries−2)] × 100

Assessment of cholinesterase activity

The Michelle electrometric method modified by Mohammad et al.,[14] was adopted for the study of acetylcholinesterase inhibitory effects of the extract and alkaloid obtained from *C. zeylanicum*. After a trial in Y-maze, rats from all the groups were sacrificed by cervical dislocation. The thorax of each rat was surgically opened, and blood was drawn from the heart with heparinized needles and syringes into the ethylenediaminetetraacetic bottle until required for the assay.

The reaction mixture in a 10 ml beaker contained 3 ml of distilled water, 0.2 ml of blood, and 3 ml of pH 8.1 barbital-phosphate buffer and its pH (pH 1) was measured with glass electrode using pH meter. Then 0.1 ml of 7.5% aqueous acetylthiocholine solution was added, and the reaction mixture was incubated at 37°C in a water bath for 20 mins. The blank contained phosphate buffer, acetylthiocholine but had no blood aliquot. After incubation, the pH of reaction mixture (pH 2) was measured, and the enzyme activity was calculated as follows:

ChE activity (ΔpH/20 mins) = (pH1-pH2)-ΔpH of blank

Statistical analysis

All data were expressed as the mean±standard error of mean. Statistical analysis was carried out using one-way analysis of variance. Any significant difference between means was assessed by Student's t-test at 95% level of significance.

RESULTS

Effect of treatment on Y-maze task

C. zeylanicum bulb extract and its alkaloidal rich fraction significantly ($p<0.05$) increased spontaneous alternation behavior (Table 1) when compared to control in a dose-dependent manner. Eserine produced significant ($p<0.05$) increase in alternation behavior of rats when compared to the controls.

Table 1: Effect of crude extract, alkaloidal-rich fraction and eserine on total arm entry and percentage alternation in Y-maze.

Treatment	Total arm entry Mean±SEM	% alternation behavior
Normal saline		
10 ml/kg	3.50±0.01	21.50
Crude extract		
250 mg/kg	7.32±0.24*	34.00
500 mg/kg	12.32±0.83*	47.00
1000 mg/kg	16.56±1.65*	53.00
Alkaloidal fraction		
10 mg/kg	14.72±1.65**	43.00
20 mg/kg	23.91±3.16**	49.00
40 mg/kg	28.20±1.80**	59.00
Eserine		
0.3 mg/kg	29.48±0.87**	64.00

*Significantly different from control at $p<0.05$, N=6. SEM: Standard error of mean, ****Significantly different at $p<0.01$

Table 2: Effect of crude extract, alkaloidal-rich fraction and eserine on cholinesterase inhibition in whole blood of rats.

Treatment	ΔpH/20 mins	% inhibition
Normal saline		
10 ml/kg	1.50±0.18	-
Crude extract		
250 mg/kg	1.20±0.09	20.00
500 mg/kg	0.97±0.04*	35.33
1000 mg/kg	0.72±0.06*	52.00
Alkaloidal fraction		
10 mg/kg	0.65±0.07*	56.67
20 mg/kg	0.56±0.01**	62.67
40 mg/kg	0.47±0.04**	68.67
Eserine		
0.3 mg/kg	0.40±0.07**	73.33

*Significantly different from control at $p<0.05$, N=6, **Significantly different at $p<0.01$

DISCUSSION

The results obtained from this study showed that extract of *C. zeylanicum* bulb and the alkaloid obtained from it increased spontaneous alternation behavior but decreased blood cholinesterase activity in rat.

AD is associated with a progressive cognitive decline, leading to dementia, due to degeneration of the cholinergic nervous system.[15] ACh is a neurotransmitter which functions in the transmission of nerve impulses across synaptic clefts within the central nervous system.[16] Animal and human studies suggest that disruption of the cholinergic nervous system is a major factor in the early state of AD.[17,18] Enhancement of cholinergic transmission in the brain especially in the hippocampal region is an important potential therapeutic target in the treatment of AD.[19] Cholinergic transmission enhancement can be achieved through inhibition of synaptic ACh degradation by cholinesterase inhibitors.[20] The resultant effects of cholinergic dysfunction in AD include memory and cognitive deficit as well as impairment of behavior.[21,22] Because of the ubiquity of cholinesterase, its activity cannot be used as the sole indicator of a cholinergic system in the absence of additional supporting evidence.[23] Therefore,

the assessment of effects of extract and its alkaloid rich fraction on learning and memory of treated rats was carried out in Y-maze. The choice of Y-maze for the study was based on the positive correlation between spontaneous alternation behavior and spatial learning and memory.[22] Oral administration of the extract and the alkaloid to rats resulted in increased spontaneous alternation behavior. This observation suggests that both the extract and its alkaloidal rich fraction crossed the blood brain barrier and possibly inhibits cholinesterase activity in selected areas of the brain consistent with observed inhibition of blood cholinesterase activity. Any agent capable of producing an increase in the percentage of spontaneous alternation behavior of rodents possesses nootropic effect or memory enhancing the effect. Agents that produce a decrease in spontaneous alternation behavior of rodents act as amnesic agent. The increase in spontaneous alternation behavior produced by *C. zeylanicum* extract and alkaloid isolated from it suggests memory enhancing the effect.

Cognitive dysfunction has been shown to be associated with impaired cholinergic transmission and the facilitation of central cholinergic transmission results in improved memory. Moreover, selective loss of cholinergic neurons in certain parts of the brain appeared to be associated with senile dementia.[24] The degeneration and dysfunction of cortical cholinergic neurons are closely associated with cognitive dysfunction.[25] Thus, any agent which enhances cholinergic function can be used for the treatment of dementia. This is based on the assertion that increasing the availability of ACh at ACh receptors in the brain, results in better neuron to neuron transport that improves cognitive functions. Cholinergic nerves, however, can be found in both the central and peripheral nervous systems and different body tissues.[26] The extract and the alkaloid rich fraction of *C. zeylanicum* bulb improved the cholinergic function in treated rats through increased spontaneous alternation behavior and inhibition of cholinesterase. Eserine (0.3 mg/kg, i.p) injected for 7 consecutive days improved memory of rats. The memory enhancing effects of Eserine has been well reported in the literature.[27,28] Eserine, a cholinesterase inhibitor, can improve memory in normal subjects,[27] as well as in patients with dementia.[28] The extract and the alkaloid rich fraction produced enhanced memory without cholinomimetic side effect characterized by motor disturbance (Table 2). The cholinesterase inhibitory effect of the extract and its isolated alkaloid is consistent with reports on other amaryllidaceae alkaloids including alkaloids from *Crinum jagus* and *Zephyranthes carinata.*[29]

CONCLUSION

C. zeylanicum bulb extract and its alkaloid rich fraction showed memory enhancing activity in rats probably by inhibiting AChE activity.

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