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AQUEOUS LEAF EXTRACT OF *COLA ACUMINATA* (MALVACEAE) ALLEVIATES SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN WISTAR RATS BY MODULATING CHOLINERGIC NEUROTRANSMISSION.

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ABSTRACT

This study investigated the effects of aqueous leaf extract of *Cola acuminata* (ALECA) on cholinergic neurotransmission in scopolamine-induced rat model of memory impairment. A total of thirty-five male Wistar rats (178 12.32 g) were allotted into 7 groups (I–VII) at random as follows: I: control, received 5ml/kg body weight (body wt) of distilled water, II: received scopolamine alone (3mg/kg body wt), III: received Donepezil (2mg/kg body wt) plus scopolamine (3mg/kg body wt), IV: received *Cola acuminata* (50mg/kg body wt) plus scopolamine (3mg/kg body wt), V: received *Cola acuminata*, (100mg/kg body wt) plus scopolamine (3mg/kg body wt), VI: received *Cola acuminata* (300mg/kg body wt) plus scopolamine (3mg/kg body wt), VII: received *Cola acuminata* (400mg/kg body wt) plus scopolamine (3mg/kg body wt) for seven days. ALECA and distilled water were orally administered daily while scopolamine was injected intraperitoneally daily for seven days.

Behavioural test were conducted using Morris water maze, light and dark box to assess learning and memory. Acetylcholinesterase (AChE) activity in the brain was assayed using Ellman's method. Scopolamine caused a significant impairment in memory through decreased probing frequency and increase in escape latency time (memory index) in Light and Dark Box and Morris Water Maze respectively. Treatment with different doses of ALECA prevented cognitive impairment induced by scopolamine through increased probing frequency and decrease in escape latency time. However, this effect is not dose dependent and it compares favourably with the donepezil group (reference drug)

The mechanism of action of ALECA was also investigated through estimation of brain Acetylcholine esterase. We found that the brain's acetylcholinesterase activity significantly increased in response to scopolamine. While a significant ($p < 0.05$) decline in acetylcholinesterase activity was seen after treatment with ALECA., and this effect compares favourably with donepezil group. The effect of ALECA is also not dose dependent.

This study concludes that ALECA treatment could improve memory function in scopolamine-induced model of dementia through the inhibition of acetylcholine esterase activity.

Keywords: *Cola acuminata*; (*Malvaceae*) Acetylcholinesterase; Scopolamine; Morris water maze; Memory; Donepezil

INTRODUCTION

Dementia is a chronic medical condition characterized by gradual decline in mental as well as intellectual abilities which interfere with social and occupational activity of the individual (1). It is a clinical condition associated with general learning disability, and a reduction in functional ability from a previous state. It is also linked to behavioral, functional, and mental issues. (2). In addition to loss of memory, there is decreased motivation, language problem and emotional instability (3). Dementia is commonly found among the elderly and people with advance age are most susceptible. The commonest form of dementia found in 2/3rd of affected people is Alzheimer's disease other type of dementia include vascular, lewy bodies, frontotemporal, and Huntingtons, among others (4-6). About 50 million people are affected worldwide by dementia with a projected prevalence of more than 150 million by the year 2050 (7). In Africa, the number of cases of dementia ranges from 2.3% to 20% with an incidence rate of 13.3 per1000 people (8). In Nigeria, the prevalence of dementia was estimated to be about 4.9% with women having a higher prevalence than men (9). According to a study carried out in Ibadan, South-West Nigeria, the prevalence of dementia rose by more than 400% among the elderly over a span of twenty years (9).

It has been established that the cholinergic system is crucial for memory and learning. (10-12). Additionally, the cerebral cortex and hippocampus exhibit elevated acetylcholinesterase activity and a loss of cholinergic neurons, which are observed in dementia. In fact, acetyl cholinesterase (AChE) inhibitors like donepezil, rivastigmine, and galantamine were developed to treat Alzheimer's disease because of decreased cholinergic activities in the brains of patients with this type of dementia (13).

Scopolamine, (muscarinic acetylcholine receptor blocker) causes cognitive impairment by blocking the muscarinic acetylcholine receptor site in the central nervous system, which leads to decreased cholinergic discharge that destroys hippocampus neurons and thus, impairs learning and memory. (14-16). Hence, scopolamine is, often, used extensively to test for potential anti-dementia drugs (17). Acetylcholine esterase (AChE) inhibitors are frequently prescribed medications for the management of dementia associated with neurodegenerative diseases. However, Due to their side effects and high cost, these medications are not often used. In addition, they do not provide a radical cure for neurodegenerative diseases, hence, it is worthwhile to look for new directions to manage the memory loss of patients with

neurodegenerative disorders. Recent research has concentrated on using medicinal plants as inexpensive, natural sources of AChE inhibitors with minimal side effects which could be utilized to treat this disease. *Cola acuminata* is a specie in the genus *Cola*, (family; *Malvaceae*, subfamily; *Sterculiaceae*). *Cola acuminata* is a tree of about 20 m height, medium in size. It bears fruits enclosing large nut (cola). The cola tree (evergreen) has oblong leathery dark green leaves, yellow flowers and star shaped fruits. It is commonly known as cola nut or bissi nut (18-19), and locally called *Obi-abata* (Yoruba). It is originally grown in West Africa but it is now cultivated in other tropical countries (19). The plant is known to enhance alertness and elevate mood (20). In addition, it is utilized by alternative medicine practitioners for treating people with memory loss (21). *Cola acuminata* leaf acts as a stimulant (22), it also used to prepare tonic for the management of conditions like cough, diarrhoea (23) and chest symptoms (24).

Cola acuminata plant has been reported to contain several secondary metabolites like Flavonoids, Tannins, Saponins, Alkaloids, Phenolic compounds, other substances present are fructose, kolatin, kolanin, theobromine (25). These secondary metabolites give medicinal plants their pharmacological potential, which results in positive health effects.

Ethnobotanical survey and other studies have reported anti-dementia effect of *Cola acuminata* seed (21, 26, 27). However, the leaf of *Cola acuminata* has not received adequate attention by researchers in this aspect. Hence, there is paucity of information on the memory enhancing effect of *Cola acuminata* leaf. Thus, the study assessed the neuro-protective effects of aqueous extract of *Cola acuminata* leaves in scopolamine-induced model of dementia in Wistar rats

MATERIALS AND METHODS

Collection of Plant Material

Cola accuminata plants are readily found in Ilupeju, a town in Oye local government area of Ekiti State, Nigeria, and this is where its leaves were obtained. They were identified and authenticated at the Plant Biology Department of University of Ilorin, Nigeria; with the voucher No. UILH/001/701/2021.

Drugs and Reagents

Scopolamine was manufactured by Tianyao Pharmaceutical Co. Ltd. Hubei, China. Torrent Pharma Ltd, United Kingdom produced Donepezil, and Ketamine was the product of Paksons Pharma Pvt Ltd India. All drugs were freshly made and administered based on body weight.

Laboratory Animals

A total of 35 male Wistar rats, weighing 178 x 12.32 g, were taken from the breeding colony of the Biochemistry Department of the University of Ilorin. They were then kept at the animal house of the Central Research Laboratory, in cages that measured 75 x 70 x 75 cm. The animals were given a standard rat diet from Ogooluwa Feeds Ltd. in Sango, Ilorin, as well as unlimited amounts of water. They underwent a natural photoperiod of approximately 12 hours of day light and 12 hours of darkness, temperature: 22°±3°Celsius; relative humidity of 50–55% while being maintained in standard laboratory conditions.

Preparation of Aqueous Extract of *Cola acuminata* Leaves

Aqueous leaf extract of *Cola acuminata* (ALECA) was prepared with little modification of the procedure described by Tiwari *et al* (28). Fresh leaves of *Cola acuminata* were air dried for fourteen days. The dried leaves were then milled with a QASA blender and grinder (Model: QBL2048, Cheerfegly industrial co Ltd. Taiwan). The powdered leaf (190g) was macerated in 2000 mls of distilled water over

a 24-hour period, followed by filtration with

wt orally) and scopolamine (3mg/kg body wt

Groups (n=5)	Dosage administered to each group
I: Control	Vehicle (5 ml/kg body wt distilled water) oral
II: Scopolamine only treated	Scopolamine (3 mg/kg body wt) ip.
III: Donepezil treated	Donepezil (2mg/kg body wt) oral + Scopolamine (3mg/kg body wt) intraperitoneally (ip).
IV: ALECA + scopolamine group	<i>Cola acuminata</i> (50mg/kg body wt) oral + Scopolamine (3 mg/kg body wt) ip.
V: ALECA + scopolamine group	<i>Cola acuminata</i> (100mg/kg body wt) oral + scopolamine(3mg/kg body wt) ip.
VI: ALECA + scopolamine group	<i>Cola acuminata</i> (300mg/kg body wt) oral + Scopolamine (3mg/kg body wt) ip.
VII: ALECA + scopolamine group	<i>Cola acuminata</i> (400mg/kg body wt) oral + Scopolamine(3mg/kg body wt) ip.

N^o1 whatman filter paper (125mm Ø cat No 1001 125). it was then freeze-dried (Freeze dryer Model: HXLG10-50DG, Hunan Kaida Scientific Instruments Co. Ltd.) which yielded 25.2g (13.3%) of a dark green extract. The extract was refrigerated at 4°C throughout the 7 days of the investigation. Thirty-five (35) male rats were randomly allotted into seven (7) groups, n=5 (I-VII). As shown in table 1, Group I was the Control, received 5 ml/kg body weight (body wt) of distilled water, Group II: Scopolamine only, received 3mg/kg body wt. intraperitoneally, Group III: received donepezil (2mg/kg body

Table 1: Animal Grouping and Administration

intraperitoneally) daily. Groups IV, V, VI and VII received oral administration of 50 mg/kg body wt, 100 mg/kg body wt, 300 mg/kg body wt and 400 mg/kg body wt of *Cola acuminata* aqueous leaf extract respectively (29,30), followed by intraperitoneal administration of scopolamine (3mg/kg body wt) (31,32).

Prior to the start of the experiment, the rats had a two-week acclimatization period.

This experiment was carried out under the NIH guidelines with respect to laboratory animal care and use (33). Administration of the treatment regimens was done in the morning (between the hours of 08:00 and 10:00), and lasted for 7 consecutive days (32). On the final day of treatment, the Morris water maze (MWM) and modified light and dark box (MLDB) were employed to evaluate spatial memory function one hour after scopolamine injection.

The qualitative and quantitative phytochemical screening of aqueous leaf extract of *Cola acuminata* (ALECA) were done using the methods of Mohammad and co. (34), Singh and co. (35), Sakar and co. (36), Ayodeji and colleagues (37), and Zairi and colleagues (38), to detect the presence and quantity of Saponins, Alkaloids, Flavonoids, Steroids, Triterpenoids, Glycosides, Tannins, Reducing sugar, and Phenols.

Phytochemical Screening of Aqueous Leaf Extract of *Cola acuminata*

Behavioral Tests

Morris Water-Maze Test

The Morris water maze (39) is commonly used to test for spatial memory. It is a circular open pool, about 70 cm deep and 200 cm in diameter. The pool contains water up to 60 cm of its height. A hidden platform with a top surface of approximately 15 cm was submerged at a depth of 1.5 cm beneath the water surface and maintained at the same point throughout the experiment. The submerged platform was made invisible by adding milk to the water to make it opaque, the platform was able to blend almost completely into the background. Rats were initially trained to locate the platform. The maximum cut off duration for swimming was set at 60 seconds. When the rat locates the invisible platform (by standing on the platform), the timer was stopped and the rat was removed, the time taken was noted, but if the rat could not locate the platform in the specified time, the rat was directed onto the platform. The rats were taken out of the water at the conclusion of the experiment, towel-dried, and put back in their original cages. During the acquisition session, the amount of time it took to discover the platform (escape latency time, ELT), was taken as a learning indicator. and recorded with the aid of a video system and later analyzed. Before drug administration (treatment), each animal underwent four acquisition trials daily for 5 successive days. On day 7 of treatment, the rats were re-exposed to the maze (to test for their spatial and long-term memory functions), and a video camera was positioned on top of the middle of the pool to capture the image of the animal as it swims for the measurements of the ELT. The time it takes the animal to locate the hidden platform (ELT) was noted as an index of memory.

Light and Dark Box

The light and dark box, a tool often used to assess mood, was modified to test for spatial memory (40). The tool has two compartments: a bright area and a dark

section. One of the compartments is painted white (the bright portion), and the other is painted black, giving the maze its name (light and dark box). The employed light and dark box measured 50 cm by 30 cm by 100 cm, and the two sections were connected by an 8 cm² aperture. The rats had three days of training, consisting of three trials lasting five minutes each. Only the animals that had memorized the opening path (number of times the rat probe the aperture) between the light and dark compartments at the end of training were employed in the experiment. The opening path from the dark box was then closed with a black plank to assess the rat memory, through re-introduction of the rat into the maze to explore for 5 minutes, frequent probing of the blocked exit showed memory formation. On the 7th day, an hour after the final treatments, the rats were reintroduced to the modified light and dark box and were given five minutes to explore the maze. This was captured with an overhead camera (Logtech Webcam, 5MP) and afterwards evaluated by a blinded researcher. The number of times (frequency) each rat probed the blocked aperture was recorded as a measure of how well their spatial memory was preserved or improved.

Sample Collection

Following behavioral evaluations on the seventh day of administration, ketamine was administered intraperitoneally at a dose of 100 mg/kg body wt. to the rats in order to put them to sleep. The brain tissue was isolated and weighed. Phosphate buffer solution (0.1 M, pH 7.4) was used to homogenize the brain tissue. Prior to biochemical analysis, the obtained homogenate was centrifuged for 10 minutes at 1000 x g. then the supernatant was separated and kept at -20⁰ C.

Biochemical Analysis

Determination of Acetylcholine esterase (AChE) Level

The cholinergic marker, acetylcholinesterase, was determined using acetylcholinesterase

assay kit. Which is based on Ellman's method (41). Briefly, The phosphate buffer (2.7 ml) and Ellman's reagent (0.1 ml of 5, 5-dithiobis 2-nitrobenzoate, DTNB) were added to the supernatant (from the homogenate) and incubated for 5 minutes. This is followed by the addition of 0.1ml of newly made acetylthiocholine iodide (pH 8), the absorbance was measured at 412nm.

Statistical Analysis

Data were presented as mean $\pm$ standard error of the mean (SEM). one way analysis of variance (ANOVA) was used as test of significance, then Newman-keuls multiple comparisons test (post hoc test,) using Graph - Pad (Prism 5) statistical software produced by Lab Archives Computer software, Carlsbad, USA.

Statistically significant differences were accepted at $p < 0.05$, $n=5$.

RESULTS

Table 2, shows the results of the qualitative and quantitative screening of the aqueous leaf extract of *Cola acuminata* (ALECA). The ALECA contains saponins, flavonoids, alkaloids, triterpenoids, steroids, glycosides while tannins, phenol and reducing sugar were absent. Similarly, the quantitative analysis of the plant extract revealed that the leaf has a high amount of alkaloids followed by flavonoids and small amount of saponins. The protective effects of ALECA on scopolamine-induced dementia was investigated after 7 days of treatment. The result showed that (Table 3) Scopolamine administration caused spatial memory impairment as demonstrated by a significant increase in escape latency time (ELT) in rats treated with scopolamine only for 7 days in comparison with the sham control. Combination of either of 50mg/kg, 100mg/kg, 300mg/kg, or 400mg/kg b.w (i.e. different doses) of ALECA and scopolamine prevented cognitive impairment elicited in scopolamine alone group as shown by decreased escape latency time, this effect is

similar to that obtained with rats treated with donepezil (the positive control) and the control groups (the sham control). Furthermore, the ALECA has no dose-dependent effects on memory performance (i.e. no significant difference in memory function among the intervention groups)

Table 3 revealed that Scopolamine led to a decline in the probing frequency $p < 0.05$ (i.e. frequency of the rat visits to the escape aperture) in rats treated with scopolamine only for 7 days in comparison with the control. In contrast the combination of either of 50 mg/kg b.w, 100mg/kg, 300mg/kg, or 400mg/kg b.w of ALECA and scopolamine led to an increase ($p < 0.05$) in probing frequency compared to rats treated with scopolamine only This effect of ALECA is not dose dependent and is similar to the data obtained in the rats treated with donepezil (the positive control) group ($p < 0.05$).

The effect of ALECA on the scopolamine-induced rise in acetylcholine esterase activity was investigated in rats. As depicted in table 3, scopolamine caused a significant rise in the activity of acetylcholinesterase in rat brain treated with scopolamine alone in compared to the control. Conversely, combining different doses of ALECA with scopolamine prevented scopolamine-mediated increase in acetylcholinesterase activity in rats' brain, comparable to the figures obtained in the rats treated with donepezil (positive control) group. In addition, ALECA has no dose dependent effects on acetylcholine esterase activity in rats' brain

DISCUSSION

The preliminary phytochemical screening revealed that aqueous leaf extract of *Cola acuminata* contains some secondary metabolites such as Alkaloids, Flavonoids, Terpenoids, glycosides, Saponins that may be responsible for its pharmacological effects. However, some of these metabolites are only found in trace amounts, while others are present in large concentrations. The result of

the phytochemical screening in this study is in agreement with previous studies (42,43), although, previous studies reported the presence of Tannins and Phenolic compounds while these compounds were found to be absent in our findings. The disparity between their reports and ours may be attributed to difference in the environment where the plants were grown, season (time of collection of the plant), method of extraction and determination of the secondary metabolites. Studies have demonstrated that medicinal plants with neuroprotective benefits are frequently rich in alkaloids, terpenoids, flavonoids, saponins, and phenolic acids (44-49). These secondary metabolites' neuroprotective properties are manifested through different mechanisms. Alkaloids, flavonoids, terpenoids, and glycosides are known to act by inhibiting the activity of the enzyme acetylcholine esterase, which will reduce the intracellular breakdown of acetylcholine. Additionally, these metabolites have the ability to reduce inflammatory factors, reduce the production of reactive oxygen species (ROS), or increase the activities of antioxidant enzymes. (46, 50-52). In the present study, administration of scopolamine alone caused cognitive dysfunction as revealed by decreased escape latency time similar to previous studies (40,53). This may be due to the fact that scopolamine, a muscarinic cholinergic receptor antagonist prevents the action of acetylcholine in the brain, leading to the inhibition of cholinergic transmission, hence memory impairment. Remarkably, treatment with different doses (50 mg/kg b.w., 100 mg/kg b.w., 300 mg/kg b.w., 400 mg/kg b.w) of ALECA prevented scopolamine induced cognitive deficit as shown by increased escape latency time comparable with that of the donepezil (reference drug) group. Similarly, the increase in the frequency of visiting the blocked escape route (modified light and dark box test) by rats treated with ALECA (50mg/kg b.w., 100mg/kg b.w.,

300mg/kg b.w. and 400mg/kg b.w.) in a manner similar to the reference drug (donepezil) indicates an improvement in cognitive functions and memory restorative effect of ALECA. The memory restorative effect of ALECA may be attributed to its secondary metabolites like alkaloids, flavonoids, glycosides, triterpenoids and others, because studies have documented neuroprotective properties of these metabolites (45-50, 54-57). These secondary metabolites may exhibit their neuroprotective effects through several mechanisms. It is known that alkaloids, flavonoids, terpenoids, glycosides may act through inhibition of acetylcholine esterase activity which will lead to reduction in intracellular degradation of acetylcholine thereby improve memory, or may act through reduction of inflammatory factors, or reduction in reactive oxygen species (ROS) generation and enhancement of antioxidant enzymes activity (46,50-52). The secondary metabolites may also act by antagonising GABA_A receptor binding resulting in cholinergic upregulation, leading to memory improvement (58). In addition, several conventional drugs like galantamine and rivastigmine used in the management of dementia (Alzheimer's disease) are alkaloids derivatives (59).

In the brain of both humans and animals, the central cholinergic system is crucial for learning and memory. The reduction in brain acetylcholine level, due to the loss of brain cholinergic neurons have been reported to be one of the causes of deteriorating memory in older people. (32,60). Research has shown that activity of acetylcholinesterase in the brain increases following scopolamine injection (32,61). Increase activity of acetylcholinesterase in the brain will result in increased degradation of acetylcholine into acetate ion and choline, thereby dwindle cholinergic neurotransmission leading to memory impairment. In this present study, scopolamine caused increased acetylcholine esterase activity after 7 days of treatment.

However, treatment with different dose of ALECA offered a neuroprotective role on the scopolamine induced increase in acetylcholine esterase comparable with that of donepezil. This ameliorative effects suggests that the extracts acted by inhibiting increase in acetylcholine esterase activity which in turn lead to a rise in brain acetylcholine level and increase in cholinergic neurotransmission hence improve cognition. In this study decrease in acetylcholine esterase activity in the brain correlates with improved memory as evidenced by reduction in escape latency time and higher probing frequency in the extract treated groups.

CONCLUSION

In conclusion, ALECA is rich in phytochemical compounds and exhibited neuroprotective effects through inhibition of acetylcholinesterase activity.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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Table 2: Result of Phytochemical Evaluation of ALECA

Compounds	Observation	Quantity (mg/100g)
Saponins	Present	0.00098±0.00
Alkaloids	Present	183.2±0.12
Flavonoids	Present	28.67±0.10
Steroids	Present	9.47±0.06
Triterpenoids	Present	0.72±0.00
Glycosides	Present	0.58±0.00
Proteins	Present	2.10%
Phenol	Absent	----
Tannins	Absent	---
Reducing sugar	Absent	----

Values are mean ± SEM of 4 replica

Table 3: Effects of Aqueous Leaf Extract of *Cola acuminata* (ALECA) on escape latency time, probing frequency and Acetylcholine esterase activity in scopolamine-induced rat model of dementia.

Groups	Escape latency (s)	Probing Frequency	Acetylcholine esterase (U/ml)
I: Control	4.80 ± 0.37	3.60 ± 0.24	0.0279 ± 0.002
II: Scopolamine only treated	7.40 ± 1.12 ^a	0.40 ± 0.24 ^a	0.0479 ± 0.003 ^a
III: Donepezil treated +scopolamine	2.00 ± 0.45 ^b	4.00 ± 0.32 ^b	0.0337 ± 0.001 ^b
IV: 50 mg/kg b.w Aqueous leaf extract of <i>Cola acuminata</i> + scopolamine	2.80 ± 0.58 ^b	6.20 ± 1.77 ^b	0.0319 ± 0.004 ^b
V: 100 mg/kg b.w Aqueous leaf extract of <i>Cola acuminata</i> + scopolamine	2.20 ± 0.37 ^b	5.60 ± 1.69 ^b	0.0386 ± 0.02 ^b
VI: 300 mg/kg b.w Aqueous leaf extract of <i>Cola acuminata</i> + scopolamine	2.20 ± 0.20 ^b	5.20 ± 0.49 ^b	0.0349 ± 0.002 ^b
VII: 400 mg/kg b.w Aqueous leaf extract of <i>Cola acuminata</i> + scopolamine	2.80 ± 0.20 ^b	4.80 ± 0.66 ^b	0.0325 ± 0.003 ^b

Values are mean ± SEM of 5 rats per group

P value <0.05

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