



Neurobehavioural Enhancement in Scopolamine Hydrobromide-Induced Alzheimer Type Cognitive Dysfunction in Rats Following Administration of Ethanol Seed Extract of *Telfairia occidentalis* (Hook.f.) Cucurbitaceae

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ABSTRACT

Alzheimer's disease is a severe, chronic neurodegenerative disease that accounts for about 80% cases of dementia. The disease is characterized by loss of cognitive function, primary memory, judgement and reasoning, movement coordination and pattern recognition. The study assessed neurobehavioural enhancement of scopolamine-induced Alzheimer type cognitive dysfunction in female and male rats, following administration of ethanol extract of *Telfairia occidentalis* seeds. With ethical approval from the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria (FAREC-FBMS 042ANA3719), thirty Wistar rats weighing between 180-200 g were used for the study and grouped into five. Alzheimer's type cognitive dysfunction was induced in groups II through V before the extract and drug administration followed by Morris water maze test. The result revealed enhanced learning and memory ($p < 0.05$) compared to the positive (group II) treated with scopolamine only which may be attributed to the phytochemical components of the ethanol extract of *Telfairia occidentalis* seeds that may have caused development of more synapses leading to learning and memory improvement. In conclusion, ethanol extract of *Telfairia occidentalis* seeds enhanced learning and memory in scopolamine hydrobromide-induced Alzheimer type cognitive dysfunction in rats.

Keywords: Alzheimer type cognitive dysfunction, learning and memory, Scopolamine hydrobromide, *Telfairia occidentalis*, Wistar rats.

Introduction

Cognitive dysfunction including Alzheimer's disease (AD) and other neurodegenerative disorders are major health problem which are debilitating in nature.^{1,2} The global prevalence of dementia in adults aged >60 years ranged from 5 to 7% with AD as the most common type followed by vascular dementia.^{3,4} There are scarce and conflicting reports on the prevalence of dementia with far-reaching implications on the public health policies in the sub-Saharan Africa.^{3,5,6} In Nigeria, incidence of dementia and Alzheimer's in the Yoruba Africans is two to three times less, compared to the African Americans.⁷ However, cases of dementia increased by 400% over the past 20 years such as from 1995-2015.⁸ Research also revealed gender difference on the incidence of AD in which two thirds of patients with AD are women, raising the intriguing suggestions that there are biological mechanisms underlying the higher incidence of AD in women.⁹ Neuroprotection refers to strategies and mechanisms able to defend the central nervous system against neuronal injury due to acute stroke or trauma including chronic neurodegenerative disorders.¹⁰

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Recently, the use of herbal plants such as *Telfairia occidentalis* (pumpkin) as an alternative in the prevention of AD has become paramount to many scientists.¹¹ *Telfairia occidentalis* seeds are flat and dark green; some of which are enveloped in a yellow-white shell; though, some produce seeds without husk. The nutritional value of pumpkin seed is based on its high protein contents and high percentage of oil which is common in our locality and grows in most part of Nigeria.¹² Since AD affects predominantly the cerebral cortex and hippocampus, *Telfairia occidentalis* containing neuroprotective, antioxidants, antidiabetic, antibacterial, anti-inflammatory and antifungal effects may help to reduce or ameliorate the disease.^{13,14} Moreover, neurodegenerative disease is attributed to oxidative stress induced by the generation of free radicals causing cellular damage by modifying macromolecules such as DNA, carbohydrates, proteins and lipids.^{15,16} The endogenous (secretion of glutathione by neuronal cells) and exogenous antioxidants from *Telfairia occidentalis* help to neutralize excess free radicals, protect cells against toxic effects and also, contribute to disease prevention.¹⁷ The free radical scavenging property of *Telfairia occidentalis*, attributed to the presence of high amount of polyphenols (flavonoids and vitamin C) may provide safer and enhanced alternative,^{18,19} hence, the rationale to investigate the neurobehavioral effect of ethanol extract of *Telfairia occidentalis* seeds in scopolamine hydrobromide-induced Alzheimer type cognitive dysfunction in rats in the present study.

Materials and Methods

Breeding of animals

Thirty adult female and male Wistar rats weighing between 180-200 g were obtained from the University of Calabar animal farm and kept in

animal room in the Department of Anatomical Sciences for two weeks under standard conditions of temperature (27°C – 30°C) for acclimatization. The animals were fed with rat chow bought from the Agro Feed Mill Nigeria Limited, Calabar and allowed access to drinking water. After acclimatization, the experimental rats were randomly grouped into five, each containing six rats designated as I, II, III, IV and V.

Ethical approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Nigeria (Approval number: FAREC-FBMS 042ANA3719) in line with the principles of laboratory animal care (NIH publication NO. 85-23, revised 1985) as well as specific national laws applied.

Plant extract preparation

Fresh *Telfairia occidentalis* seeds were obtained from the Watt market, Calabar, Cross River State, Nigeria. The fresh *Telfairia occidentalis* seeds were identified, authenticated and registered with voucher number: HERB/BOT/UCC/322 in the Department of Botany, University of Calabar, Calabar. The plant seeds were removed from the shell, washed to free debris, chopped into smaller pieces and air-dried in the laboratory. The dried samples were blended into powdered form and 100 g of the powdered seeds soaked in 80% ethanol which was later agitated using blender with model number Bravo3JARS Mixer grinder and kept to cool (0-8°C) for 48 hours. The mixture was then filtered using chess cloth and Whatman No.1 filter paper. The filtrate was concentrated in a vacuum at temperature of 40-45°C to obtain the crude paste, kept in a cool dry place for use during administration.

Induction of Alzheimer's type cognitive dysfunction

Alzheimer's type cognitive dysfunction was induced to the adult female and male rats in groups II, III, IV and V through intraperitoneal injection of 1.0 mg/kg body weight of scopolamine hydrobromide (SHB) for 7 days.

Determination of LD₅₀

LD₅₀ of the ethanol extract of *Telfairia occidentalis* seeds was established to be >7000 mg/kg according to the Lorke's method. The dosage was determined using 12.5% (875 mg/kg) and 25% (1750 mg/kg) of 7000 mg/kg body weight of ethanol extract of *Telfairia occidentalis* seeds.

Plant extract and Donepezil administration

Group I served as the negative control and received animal feed and water *ad libitum*; group II served as the positive control and received 1.0 mg/kg body weight of SHB only; group III received 1.0 mg/kg body weight of SHB and 875 mg/kg body weight of ethanol extract of *Telfairia occidentalis* seeds; group IV received 1.0 mg/kg body weight of SHB and 1750 mg/kg body weight of ethanol extract of *Telfairia occidentalis* seeds while group V received 1.0 mg/kg body weight of SHB and 1.0 mg/kg body weight of Donepezil.

Morris water maze test

Twenty-four hours after the last administration, the experimental animals were subjected to Morris water maze (MWM) test in order to assess spatial learning and memory.²⁰ The rats were trained to use extra maze visual cues to locate an escape platform hidden just below the surface of opaque water.²¹ The ability to do this depends on learning and remembering locations. The hidden platform version of MWM is a test of spatial memory sensitive to hippocampal damage while the visible platform version of MWM is a non-hippocampal task, disrupted by dorsal striatum lesion.²² The testing in Morris water maze lasted for seven days. The animals were trained for six days; the first three days were for acquisition training with the invisible escape platform (North-West quadrant). Day 4 to 6 were the reversal training with the invisible escape platform (South-East quadrant). On the seventh day, a probe trial was conducted with no escape platform.

Statistical analysis

Data obtained were analysed using the Analysis of Variance (ANOVA) with values expressed mean ± standard error of mean (SEM) at $p < 0.05$.

Results and Discussion

Figure 1 shows values of swim latency (sec) for the three (3) days acquisition training. These are: 41.40 ± 4.34, 16.47 ± 4.26 and 23.01 ± 3.84 (group I); 55.11 ± 4.94, 38.63 ± 5.25 and 41.72 ± 3.11 (group II); 26.97 ± 2.30, 26.11 ± 1.87 and 13.93 ± 2.29 (group III); 36.51 ± 3.44, 31.64 ± 3.03 and 26.08 ± 2.70 (group IV); 41.99 ± 4.12, 22.21 ± 5.06 and 23.56 ± 6.30 (group V). The swim latency for groups III and IV acquisition training for day 1 was significantly lower compared to the groups I and II. Group V was significantly not different compared to the control group I but significantly different compared to group II ($p < 0.05$). The swim latency during the acquisition training period in the different experimental groups showed that day 2 acquisition training in all the experimental groups were significantly lower when compared to the day 1 acquisition training ($p < 0.05$). During the day 2 acquisition training, the swim latency of group II was significantly higher compared to the control group and other experimental groups, indicating memory deficit in the positive control group II; groups III, IV and V were significantly higher compared with the negative control group I. During day 3-acquisition training, the swim latency of group II was significantly higher compared to the control group I and groups III, IV and V. Groups III, IV and V were not significantly different compared to group I. However, group III was significantly lower compared to the other groups ($p < 0.05$).

Figure 2 shows values of swim latency (sec) for three (3) days reversal training which include: 29.18 ± 2.67, 13.64 ± 2.28, and 7.43 ± 0.75 (group I), 40.49 ± 8.01, 26.26 ± 5.04 and 15.53 ± 2.48 (group II); 28.80 ± 5.38, 16.30 ± 2.85 and 12.68 ± 2.40 (group III); 32.42 ± 4.75, 18.16 ± 3.38 and 15.20 ± 2.70 (group IV); 35.83 ± 5.50, 24.51 ± 5.28 and 20.40 ± 3.47 (group V). Day 4, 5 and 6 were tested for reversal training. Similar to the results of the acquisition tests, the swim latency was higher in group II treated with scopolamine hydrobromide compared to the normal control; group I (Figure 2). Group IV was significantly higher compared to the control group I and lower compared to positive control group II ($p < 0.05$). The swim latency during the reversal training period in the different experimental groups showed that day 5 (Figure 2) was significantly lower compared to day 4 reversal training period ($p < 0.05$). However, group V showed significant difference compared to the treated groups on the 5th day of reversal training, indicating learning deficit ($p < 0.05$) (Figure 2).

Figure 3 shows values of swim latency in the different groups during probe trial on day 7 of MWM test. The probe trial in North-West (NW) and South-East (SE) quadrants are 8.10 ± 1.37 and 18.29 ± 0.69 (group I); 04.2 ± 1.04 and 11.21 ± 1.45 (group II); 11.76 ± 3.01 and 14.78 ± 2.26 (group III); 6.00 ± 0.62 and 17.10 ± 0.91 (group IV); 10.10 ± 0.43 and 14.35 ± 0.82 (group V). Result in Figure 3 revealed that group II was significantly reduced in the SE and NW quadrants compared to groups I, III, IV and V, indicating memory deficit. Groups III and V were significantly reduced compared to group I in the SE quadrant, while in the NW quadrant, group IV was significantly lower compared to group I with groups III and V indicating a significant increase compared to group I which revealed memory enhancement ($p < 0.05$).

In the present study, an invisible platform was kept at a predetermined quadrant (NW) during day 1, 2 and 3 of the acquisition training. The time taken to reach the platform indicates spatial learning and memory. The MWM test result (Figures 1 and 2) showed general increase in escape latency of the scopolamine hydrobromide (SHB) treated group during the acquisition and reversal training compared to the negative control. This indicates learning impairment induced by SHB which support similar literatures on scopolamine inducing memory impairment assessed using MWM.²³⁻²⁵ The result of donepezil significantly reversed this effect by decreasing the escape latency time, attributed to the nootropic properties of donepezil in the present study. Patients with Alzheimer's have profound deficits in cognitive and social functions mediated in part by a decline in cholinergic function. Acetylcholinesterase inhibitors (AChEI) are the most commonly prescribed treatment for the cognitive deficits in patients with AD.²⁶ Donepezil is an AChEI and possibly improved scopolamine-induced impairment by inhibiting acetylcholinesterase activity and improving cholinergic neurotransmission in the brain.

In this study, the experimental rats in groups III and IV treated with the ethanol extract of *Telfairia occidentalis* seeds showed reduced escape (swim) latency time. This inferred that the ethanol extract of *Telfairia occidentalis* seeds reversed SHB-induced cognitive impairment. This result is in tandem with similar work, which established that scopolamine induces memory impairment associated with the attenuation of cholinergic neurotransmission as well as increased processes connected with oxidative stress in the brain.²⁷ Meanwhile, the mechanism of action of these plants in mitigating the deleterious effect of scopolamine may be through its antioxidant properties or acting as acetylcholinesterase inhibitor.

The probe trial in MWM used in the present study, determines whether the animals remembered the hidden platform in the target quadrants. Result (Figure 3) showed that the SHB treated group II indicate the least time spent on the target quadrants, demonstrating lack of memory. The probe trial constitutes evidence for spatial memory as the experimental rats with hippocampal lesion do poorly in probe test in the group treated with SHB alone, during the probe trial day of the present study. This findings correlate with similar works.^{28,29}

In the present study, group I spent most time in the SE quadrant followed by groups III, IV and V compared with group II. Group II spent less time in the NW quadrant compared with group I while group III and V spent more time in the NW quadrant. This result support similar work in which atrophied, karyorrhectic and disrupted plasma membrane of pyramidal cells in the hippocampus were ameliorated following administration of the aqueous *Telfairia occidentalis* seeds in scopolamine-induced Alzheimer type cognitive dysfunction in rats.³⁰ In addition, the treated groups (III, IV and V) that demonstrated intact spatial working memory may be due to the antioxidant contents in the ethanol extract of *Telfairia occidentalis* seeds. This may however, be dose-dependent as enhancement of neurobehaviour was influenced in the experimental rats used in the present study.

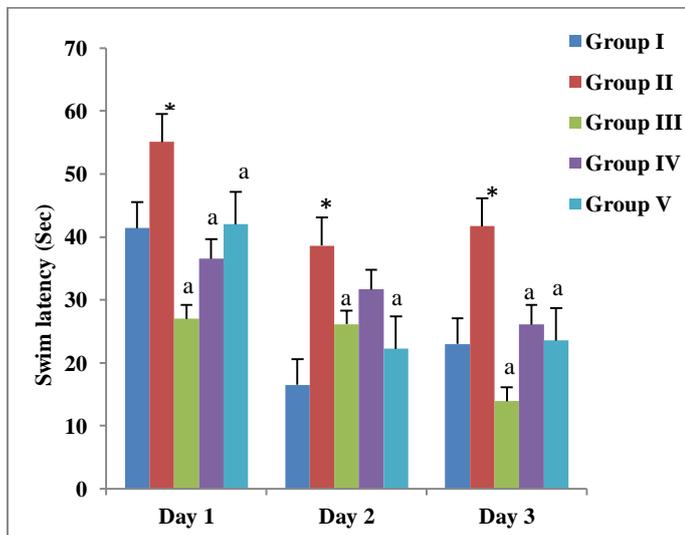


Figure 1: Comparison of swim latency in the different experimental groups during acquisition training on day 1, 2 and 3 of Morris water maze test.

Values are expressed as mean \pm SEM, n = 6. * = significantly different control at $P < 0.05$, a = significantly different scopolamine hydrobromide at $P < 0.05$, b = significantly different Donepezil at $P < 0.05$.

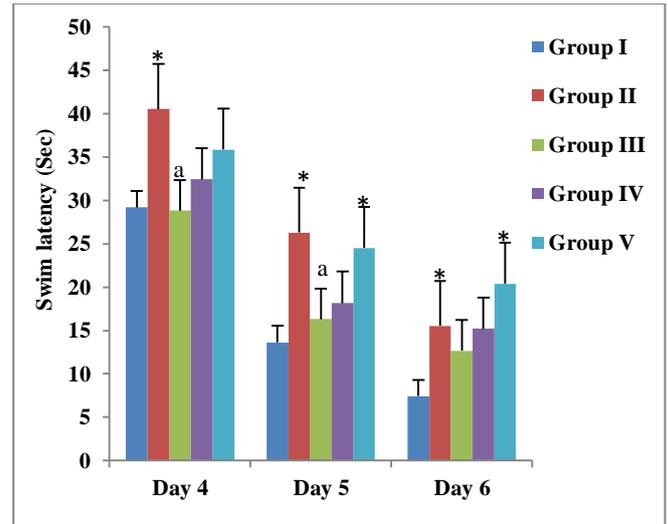


Figure 2: Comparison of swim latency during reversal training on days 4, 5 and 6 of Morris water maze test.

Values are expressed as mean \pm SEM, n = 6. * = significantly different control at $P < 0.05$, a = significantly different scopolamine hydrobromide at $P < 0.05$, b = significantly different Donepezil at $P < 0.05$.

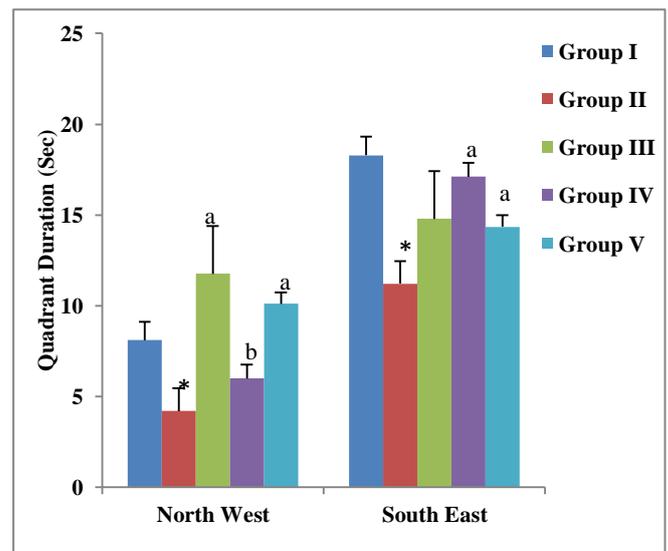


Figure 3: Comparison of swim latency in the different groups during probe trial on day 7 of Morris water maze test.

Values are expressed as mean \pm SEM, n = 6. * = significantly different control at $P < 0.05$, a = significantly different scopolamine hydrobromide at $P < 0.05$, b = significantly different Donepezil at $P < 0.05$.

Conclusion

In conclusion, the present study showed that the ethanol extract of *Telfairia occidentalis* seeds enhanced learning and memory in scopolamine hydrobromide-induced Alzheimer type cognitive dysfunction in rats.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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