

Alpha lipoic acid reverses scopolamine-induced spatial memory loss and pyramidal cell neurodegeneration in the prefrontal cortex of Wistar rats

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ABSTRACT

Neurodegenerative disorders are linked to oxidative tissue damage characterized by gradual loss of cognitive functions and neuronal cells. Alpha-lipoic acid (AHA) has a strong antioxidant property. Scopolamine is an anti-muscarinic agent used to study the mechanism of memory loss in an animal model. This study is aimed at evaluating the antioxidant role of alpha lipoic acid in reversing scopolamine induced memory loss and neurodegenerative process in the prefrontal cortex of Wistar rats. Twenty adult male Wistar rats used were divided into four groups (n = 5): Group 1 received vehicle (Control), Group 2 had scopolamine (1 mg/kg, i.p) for 4 days, Group 3 received AHA (200 mg/kg, p.o) for 10 days while Group 4 were pretreated with scopolamine (1 mg/kg, i.p) for 4 days followed by oral administration of 200 mg/kg of AHA for 10 days. The rats were subjected to Y-maze test to assess their spatial memory. The rats were euthanized, the prefrontal area was excised and fixed in 10% formol-calcium and processed for Haematoxylin and Eosin, Cresyl fast violet for Nissl Bodies (Ribosome), and Glial Fibrillary Acidic Protein (GFAP) stains. Scopolamine caused a significant decline in spatial working memory, prefrontal neuron cell loss, and increased proliferation of reactive astrocytes (astrogliosis) when compared with the control and AHA treated group. AHA process of reversing scopolamine-induced memory deficit, prefrontal neuron cell loss, and generation of reactive astrocytes (astrogliosis) is mediated by its antioxidant mediated positive modulation of astrocyte-neuronal interaction during neuroinflammation in response to oxidative tissue damage.

Introduction

Neurodegenerative diseases have chronic and progressive onset, characterized by progressive and selective neuronal death in specific brain regions (Irwin et al., 2016; Molz and Schröder, 2017). The most common neurodegenerative diseases include Alzheimer's disease (AD) characterized by progressive loss of memory, cognitive decline, and behavioral impairments (Di Domenico et al., 2015; De Domenico and Giudetti, 2017; Dos Santos et al., 2019; Chen et al., 2019; Memudu and Adanike, 2019). This health condition affects middle to old-aged individuals (Dos Santos et al., 2019; Irwin et al., 2016) and accounts for significant morbidity and mortality, globally (Bais and Kumari 2018). There was a prediction based on epidemiology study, that the number of people with AD will rise to 1.25 billion by 2050 (Dos Santos et al., 2019; Pohanka, 2014) hence the need to continue studies in a bid to unravel

therapeutics to combat it.

According to Boisvert et al. (2018), AD pathogenesis is linked with histo-cellular, anatomical, and neurochemical alterations in the brain that results in a decline in neuronal activity, loss of cortical neurons, loss of synaptic connection, and memory function. These pathological changes in Alzheimer's disease target regions of the prefrontal cortex and hippocampus, responsible for memory (Huang et al., 2016; Assefa et al., 2018). The pathogenesis of AD suggests that oxidative stress plays a key role in memory loss (Pohanka, 2014). Memory is a vital function of the brain (Farahmandfar et al., 2012) and learning precedes memory (Johansen et al., 2011). Memory decline is related to various etiological factors such as increasing age, production of free radicals, varied emotions, reduction in cholinergic activity, elevated oxidative stress, and neuroinflammatory reactions, which cause the occurrence of amnesia, dementia to more threatening conditions such as schizophrenia and

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Alzheimer's disease (Alikatte et al., 2012).

It has been estimated that fifty million people are currently living with memory impairment worldwide, and this is predicted to double in the next 20–30 years (Ravindranath and Sundarakumar, 2021; Hallam et al., 2022) this demographic study is attributed largely to the ageing population (Prince et al., 2015).

The cholinergic system plays a significant function in learning and memory; the loss of cholinergic neurons and reduced choline acetyltransferase activity in the cerebral cortex and hippocampus is a pathological feature in memory impairment (Gupta and Kulshreshtha, 2017).

Over the years, scopolamine has been used to study memory impairment in animal study (Chen et al., 2019; Memudu and Adanike, 2019; Memudu and Adewumi, 2021). Being a muscarinic receptor antagonist, it targets the cholinergic system and impairs memory by increasing oxidative stress (Saraf et al., 2011; Goverdhan et al., 2012; Jafariana et al., 2019; Nigam et al., 2019). Brain tissue is susceptible to oxidative stress due to its high consumption of oxygen especially the frontal cortex and hippocampus; as these regions are important for brain-specific functions such as cognition and memory, damage to these areas could have significant neurological effects (Karim et al., 2017). Our previous study on scopolamine induced oxidative damage in the hippocampal CA1 horns and dentate gyrus caused marked disruption of pyramidal cortical and hippocampal CA1 neurons characterized by pruning of the neurites, pyknosis, vacuolations of the neuropil and astrogliosis (Memudu and Adanike, 2019, Memudu and Adewumi, 2021), this implies its ability to disrupt normal neuron structure and activity (Nigam et al., 2019) not only in the fronto-hippocampal cortex but also in the cerebello-hippocampal axis for learning and movement (Memudu and Adewumi, 2021).

AHA is synthesized in the mitochondria of animal and vegetable cells; it can be consumed exogenously through the consumption of dark green leafy vegetables and meats (Bittner et al., 2017; Dinicola et al., 2017; Tibullo et al., 2017), hence easily accessible as a natural supplement to improve brain health, hence a potent therapeutic option for neurodegenerative disorder (Triggiani et al., 2020) because of its anti-oxidant and anti-inflammatory properties (Molz and Schröder, 2017) as well as its ability to cross the blood-brain barrier (Galeshkalami et al., 2019).

Astrocytes are supporting non-neuronal cells that maintain synaptic activity through the regulation of glutamate homeostasis in the brain (Gollihue et al., 2020). According to Jin-Ting et al. (2020), the gap proteins connexins on astrocytes are involved in memory formation hence an increased expression of connexins results in the proliferation of reactive astrocytes, neuronal cell damage, and loss of cognitive function. The type of connexins mostly seen in astrocytes is connexin 43 (a gap junction protein in astrocytes) (Price et al., 2018; He et al., 2020). Furthermore, astrocyte is a significant biomarker for oxidative stress and antioxidants activity, hence any drug or supplement that acts on astrocytes via its anti-oxidant property is targeted to treat neurodegenerative diseases such as AD or memory impairment according to Gentile and D'Amato (2018) and Siracusa et al. (2019). AHA has been reported to reverse scopolamine induced neurodegeneration, impaired movement and memory via the cerebello-hippocampal (Memudu and Adewumi, 2021). In progression, this study was designed to investigate the changes in the prefrontal cortical neurons, following administration of AHA in reversing scopolamine-induced neurodegeneration and memory decline by accessing neuro-histochemical changes, astrocytes (anti-GFAP) expression and spontaneous alternation index using the Y-maze behavioral paradigm.

Materials and methods

Experimental animals

Twenty (20) Adult Male Wistar rats weighing between 150 g and 200 g were procured from National Veterinary Research Institute (NVRI)

Vom Jos, Plateau State, Nigeria and housed in polyacrylic cages in the Animal House of Bingham University, Karu, Nigeria. Five rats housed per cage were fed with rat pelleted feed (Vital Feeds Limited Nyanya, Nasarawa State, Nigeria), water available ad libitum and maintained in the standard laboratory and specific pathogen-free (SPF) conditions of 12:12 hr dark/light cycle, temperature $35 \pm 2^\circ\text{C}$ and $52 \pm 5\%$ relative humidity. Animals were acclimatized for at least 7 days to the laboratory conditions before behavioral experiments. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals; and Animal Care and Use in Mental Health; of the National Institute of Health (NRC, 2011), the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) and the experimental protocols approved by the Departmental Research, Animal Care and Use Ethics Committee. The behavioral procedures were carried out in specially equipped rooms in the animal facility between 08:00 a.m. and 12:00 p.m. to avoid experimental deviations due to diurnal variations.

Experimental materials

Alpha lipoic acid

This study used capsulated Alpha Lipoic Acid (Puritans' Pride, Inc, USA) procured from H-Medix Pharmacy, Abuja, Nigeria. The study dose was taken as 200 mg/kg orally according to Ikuta et al. (2016), it is rapidly absorbed within 10 min (in rats) after oral administration.

Scopolamine (hyoscine butylbromide)

Scopolamine interferes with memory and cognitive function in human beings and experimental animals by blocking muscarinic receptors (Golechha et al., 2012) along with the impairment of long-term potentiation and the central cholinergic system (Alikatte et al., 2012).

Scopolamine hyoscine (Hyoscine Butylbromide B.P 10 mg, manufactured by YanzhouXierKangtai Pharmaceutical Co., Ltd, Shandong, China) used in this study was procured from H-Medix, Abuja, Nigeria. The study dose administered to induced memory impairment was 1 mg/kg body weight i.p (intraperitoneally) [1 mg/kg, i.p], according to Dobhal et al. (2014), Budzynska et al. (2015), and Nigam et al. (2019). In this study, scopolamine was administered for 4 days according to Chen et al. (2019) to establish a scopolamine-invoke memory deficit (Budzynska et al., 2015) characterized by hyperactivity, anxiety-like behaviors, and learning impairment in rats (Jafariana et al., 2019). This study was done for two weeks (14 days).

Experimental design

Rats were divided into four (4) groups of five animals (n = 5) each: as shown in Table 1 below.

Table 1

Experimental animal grouping and treatment procedure, Scopolamine was administered for four (4) days while AHA was given for ten (10) days.

Group (n = 5)	Treatments	Duration (Days)
A – Control	Normal saline+ Water and Rat Pellets	14 days
B – Scopolamine Induced memory Impaired model	Water and Rat Pellets + 1 mg/kg Scopolamine injection intraperitoneally (i.p)	4 days
C – Alpha Lipoic Acid (AHA / α -LA)	Water and Rat Pellets + 200 mg/kg oral Alpha Lipoic Acid	10 days
D – Alpha Lipoic Acid Treated Memory Impaired group	Water and Rat Pellets + Pretreatment with 1 mg/kg i.p Scopolamine for 4 days + post-treatment with 200 mg/kg oral Alpha Lipoic Acid for 10 days	14 days

Behavioral testing

During this experiment, experimental animals were handled in compliance with “Methods and welfare considerations in behavioral research with animals-National Institutes of Mental Health and Guidelines for the care and use of mammals in neuroscience and behavioral research (National Research Council, 2011).

Y-maze test

Y-maze paradigm is one of the most convenient assays for rapid and reliable assessment of effects of drugs on memory in rodents (Aydin et al., 2016). Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (75 cm long, 25 cm high, and 15 cm wide) and an equilateral triangular central area (Aydin et al., 2016) with an angle of 120 degrees between the arms. The animals were allowed to explore the maze for 8 min before the 5 min exploration test duration. An arm entry was counted when the hind paws of the rat were completely within the arm while a spontaneous alternation behavior was defined as entry into all three arms on consecutive choices (Ionita et al., 2017). The pattern of arm entries was recorded. A correct alternation is scored when the animal successfully explored each of the three arms of the maze per triad of exploration (e.g ABC, CAB, BCA), Once the two arms were explored per triad, it is recorded as an incomplete alternation (e.g ABA, ACA, BAB...etc). The percentage of correct alternations of each rat is scored as a ratio of the number of correct alternations to the total alternation multiplied by 100 (Ionita et al., 2017).

The number of maximum spontaneous alternation behavior was then the total number of arms entered minus two and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) \times 100. The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was tested. Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory (Aydin et al., 2016; Ionita et al., 2017). The Y-Maze test was conducted one week before drug administration and during the experiments.

Experimental animal euthanasia and brain tissue collection for preservation

Twenty –four hours after the last drug administration and behavioral assessment, the final body weights were taken and the rats were randomly decapitated via cervical dislocation (Carbone et al., 2012; Moody et al., 2014; Magalhães et al., 2019). The whole brain was gently excised and wet weight taken using an analytical weighing balance (OHAUS Pioneer™, India). The brain tissue was carefully excised and fixed 10% formol calcium for histological, histochemical, and immunohistochemical tissue processing. Coronal sections of the prefrontal cortex (PFC) was obtained stereotaxically according to the guide using brain atlas (Paxinos and Watson, 2007).

Prefrontal cortex tissue processing for histological, histochemical, and immunohistochemistry

The coronal section of the PFC tissue was processed using an automated tissue processor (LEICA) according to the histological routine process described by Bancroft and Gamble (2008). Tissues were embedded using paraffin wax, molded tissue blocks were serially sectioned using Leica rotatory Microtome set at 5 μ m. Sections of the prefrontal cortex tissue were mounted on glass slides using DPX. Tissues were then stained using Haematoxylin and Eosin (H and E) to display tissue histology, histochemistry for protein (ribosome) using Cresyl Fast Violet (CFV) stain, and immunohistochemistry of astrocytes using glial fibrillary acidic protein (GFAP) stain (GFAP).

Histological staining

prefrontal cortex was stained using Haematoxylin and eosin (H and E) stain to demonstrate the general histological appearance of the layer V pyramidal neurons and neuropil using the stepwise procedure described by Bancroft and Gamble, (2008) and Akinrinade et al. (2015).

Histochemical staining for Nissl or chromatophilic bodies

The labeled PFC slides sections were arranged in staining slide rack for Cresyl Fast Violet staining done according to the procedure described by Bancroft and Gamble (2008) and Memudu et al. (2020).

Immunohistochemical staining of the prefrontal cortex for astrocytes using glial fibrillary acidic protein (GFAP)

Astrocytes anti-GFAP protein in the formalin-fixed paraffin-embedded rat brain tissue sections were examined using glial IHC protocol according to Bancroft and Gamble, 2008 method. GFAP is commonly used as an astrocyte marker. GFAP localizes intermediate filaments and stains well in astrocyte cellular processes. The following primary antibodies were used Novocastra-mouse monoclonal: GFAP-antibody Leica Microsystems-Novocastra™, United Kingdom (1:100 dilutions), and the secondary antibody (Novocastra biotinylated secondary antibodies; biotinylated donkey anti-goat IgG, 1:200). The peroxidase-coupling was done using the avidin-biotin complex (ABC Kit, Vector Laboratories, Burlingame, CA). The immunoreaction product was visualized with 3, 3'-diaminobenzidine (DAB, Dako) for chromogen development. The counterstain was done using Mayer's Haematoxylin for two minutes. Slides were then air-dried and covered with cover glass using Distrene Plasticizer Xylene (DPX) mounting media as described by Bancroft and Gamble (2008) method.

Tissue photomicrography

Photomicrographs of slides obtained from histological, histochemical, and immunohistochemical staining of prefrontal cortices were captured using LEICA binocular microscope which was connected to a 5.0-megapixel Amscope camera (Amscope Inc., Irvine, CA, USA) and the images were captured at x400 magnification and stored using the joint photographic expert group (JPEG) format for analysis.

Statistical analysis

Data were analyzed using Graphpad Prism 8.4.3 (686) statistical tool. Statistical test of significance was done using one-way ANOVA with Tukey's multiple comparison test. Statistical Significance (*) was set at $p < 0.05$. Data were expressed as mean \pm standard deviation.

Results

AHA improves working and spatial memory

The order of arm entered is taken to ascertain spontaneous alternation behavior (SAB) and spatial working memory. This study scores complete alternation (A, B, C- B, A, C- B, A, C). The control group showed a significant increase in SAB as compared with the memory-impaired (B) group ($p < ***0.0006$), AHA treated memory-impaired (D) group ($p < ** 0.0044$). However, the memory-impaired model (B) demonstrated a reduction in SAB as compared with the control, AHA ($***p < 0.0001$), and AHA treated memory-impaired group ($** p < 0.0027$). This study showed that AHA treatment had a significant increase in SAB as compared with the AHA treated memory-impaired group ($*p < 0.0442$) as represented in Fig. 1.

Histological and histochemical changes in prefrontal cortex

The histological demonstration of the histo-morphological

Mean Test Spontaneous Alternation test (SAB) %

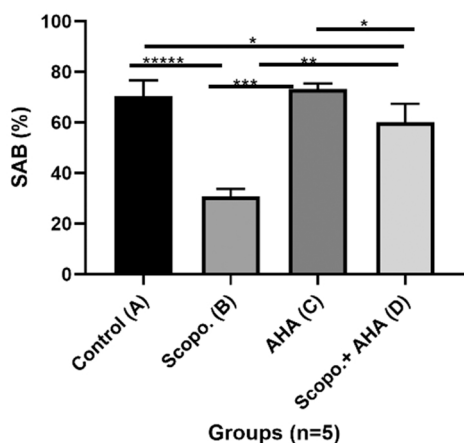


Fig. 1. Graphical representation of Mean Pretest and Test Spontaneous Alternation (%) of experimental animals used in this study. There was a significant difference @ $p < 0.05$ for Control (A) vs. Scopo. (B) ($p < ***0.0006$), Control (A) vs. Scopo. + AHA (D) ($p < ** 0.0044$), Scopo. (B) vs. AHA (C) ($***p < 0.0001$), Scopo. (B) vs. Scopo. + AHA (D) ($** p < 0.0027$) and AHA (C) vs. Scopo. + AHA (D) ($*p < 0.0442$) using Tukey's multiple comparisons test. F- statistics show significance difference using Tukey's multiple comparisons tests. Data analysed using one-way ANOVA and expressed as Mean \pm Standard Deviation (Mean \pm SD). Statistical Significance is taken at $P < 0.05$ (*) using the Tukey Post hoc test. Legend: A= Control, B= Scopolamine induced Memory impaired Model C= Alpha Lipoic Acid, D=Alpha Lipoic Acid Treated Memory Impaired group.

appearance of the PFC as shown in (Fig. 2) displayed that the control and AHA all have a normal appearance of the neurons and neuropil with large neurons having dendritic and axonal projection from its large basophilic stained cytoplasm of the pyramidal cell body as compared with the memory-impaired group characterized with presence of pyknotic or clusters of degenerated pyramidal cells and loss of neurites projections. The AHA treated (D) memory-impaired PFC showed regenerating neurons or protected neurons with intact cytology with few un-clustered degenerated pyramidal cells.

AHA attenuates chromatolysis in neuronal cells of the prefrontal cortex

The PFC was stained with CFV stain used to demonstrate the Nissl granulations that represent the histochemistry of ribosomal activity in

the neurons. The PFC of the control group showed well-arranged Nissl positive neurons similar to AHA (C) treated group (Fig. 3). The PFC of the memory impaired (B) or scopolamine treated rat showed clusters of chromatolytic neurons. The AHA treated (D) group shows few regenerating Nissl positive neurons in the PFC as compared with the scopolamine study group (B). This illustrates that AHA aid protection of basophilic content of the neuron's cytoplasm (ribosomal RNA) from chromatolysis.

Immunohistochemical expression of astrocytes using GFAP

Astrocyte's expression was displayed immuno-histochemically by the brown coloration of its astrocytic process having the GFAP intermediate filament protein (anti-GFAP protein). This study showed a mild astrocytic expression in PFC of the control group, with the scopolamine (B) treated group demonstrating notable expression of the anti-GFAP protein in the PFC (Fig. 4). However, AHA treated memory-impaired tissue shows a reduction in anti-GFAP protein expression as compared with the scopolamine model which is almost similar to the astrocytic expression in the control group.

Discussion

In this present study, we used scopolamine to investigate the effects of alpha-lipoic acid on the histo-morphological changes in the PFC area of the brain involved in memory. The findings obtained from this study show that AHA reversed histological, histochemical, and immunohistochemical changes in the prefrontal cortex induced by scopolamine.

Y maze test is an established model for testing spatial memory function in animal models to evaluate the cognitive and anti-amnesic effects of some drugs or supplements (Goverdhan et al., 2012; Vivar, 2015). In this present study, scopolamine caused a reduction in spontaneous alternations, which correlates with Jafariana et al. (2019) study. The reduction is caused as a result of the loss of neuron function and synaptic connectivity (Mahboob et al., 2016) linked to scopolamine-induced oxidative tissue damage. Memory impaired features of scopolamine in this present study demonstrate its use as an experimental model for dementia (Zehnpfennig et al., 2015; Bittner et al., 2015).

This study showed that 200 mg/kg of AHA was effective in reversing scopolamine-induced memory deficits demonstrated by a significant increase spontaneous alternation behavior when compared with the scopolamine-induced memory-impaired rats and this supports report

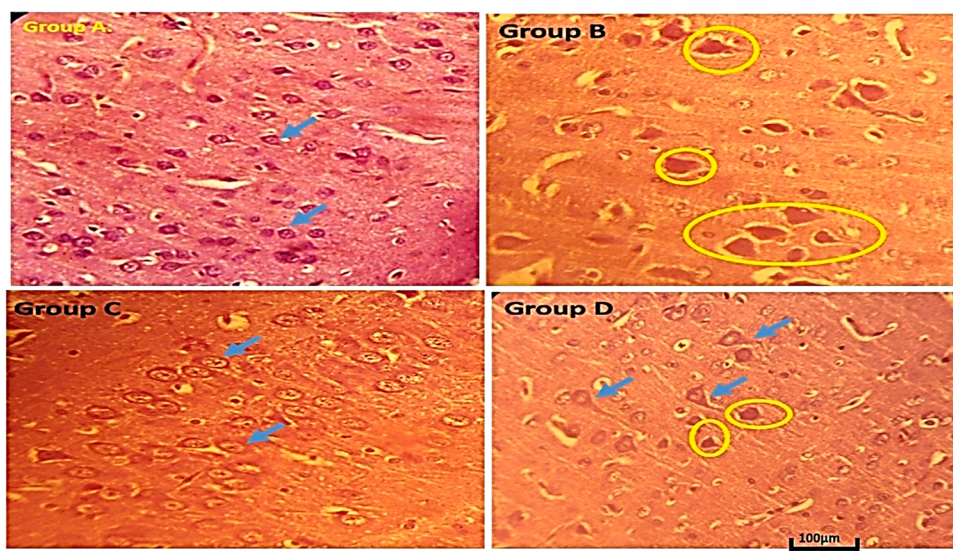


Fig. 2. Representative photomicrograph of the prefrontal cortex pyramidal cells of adult male Wistar rats stained with Haematoxylin and Eosin (H and E) stain captured using objective lens of $\times 10$. Legend: A= Control, B= Scopolamine induced Memory impaired Model C= Alpha Lipoic Acid, D=Alpha Lipoic Acid Treated Memory Impaired group; Blue arrows= normal pyramidal neurons; yellow circle = necrotic/ pyknotic cells. The histo-morphology of the extrapyramidal layer of the PFC of the control and AHA treated animals showed neurons with dendritic projections from its large basophilic stained pyramidal perikaryon with the absence of pyknotic or red neurons as seen in Scopolamine treated group. Comparing the histological appearance of Group D shows better neuron morphology as compared with group B even though there are few chromatolytic cells, the histo-morphological appearance of Group D is quite similar to the control and AHA treated animals. (Scale bar 100 μ m).

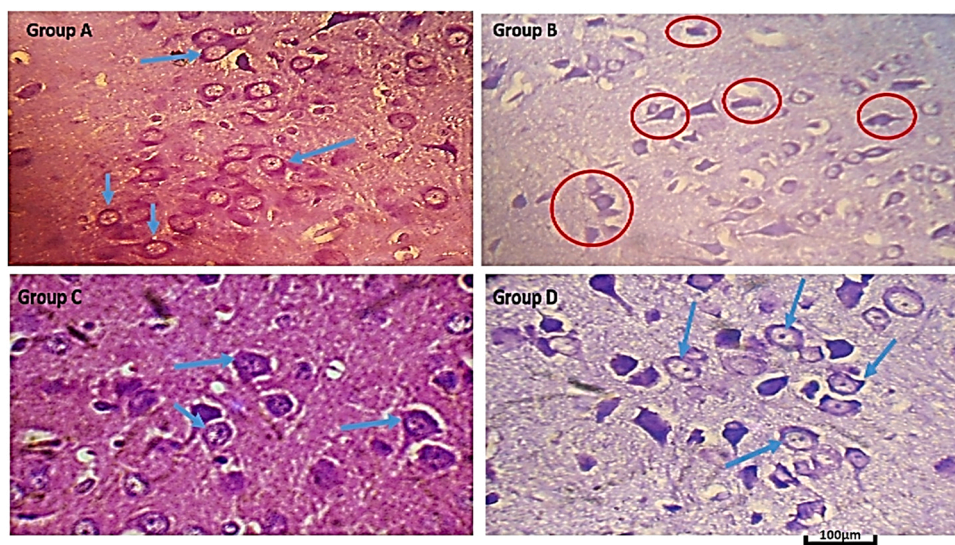


Fig. 3. Representative photomicrograph of the prefrontal cortex pyramidal cell layer of adult male Wistar rats stained with Cresyl Fast Violet (CFV) stain captured using objective lens of x40. Scale bar 100 µm. Legend: A= Control, B= Scopolamine induced Memory impaired Model C= Alpha Lipoic Acid, D=Alpha Lipoic Acid Treated Memory impaired group. Blue arrows= Normal Pyramidal neurons; red circle = necrotic/ pyknotic cells. This histochemical demonstration of Nissl / Chromatophilic bodies representing the ribosomes in the perikaryon of the prefrontal cortical pyramidal neurons in the control and AHA treated rats show the presence undistorted neuron cell bodies and granulation of the chromatophilic bodies in the basophilic neurons cell bodies as shown in the blue arrows. Scopolamine treated shows a palely stained homogenous cytoplasm indicating poorly stained or lack of chromatophilic bodies implicating neuronal degeneration and loss of cytoplasmic organelles integrity (Scale bar 100 µm).

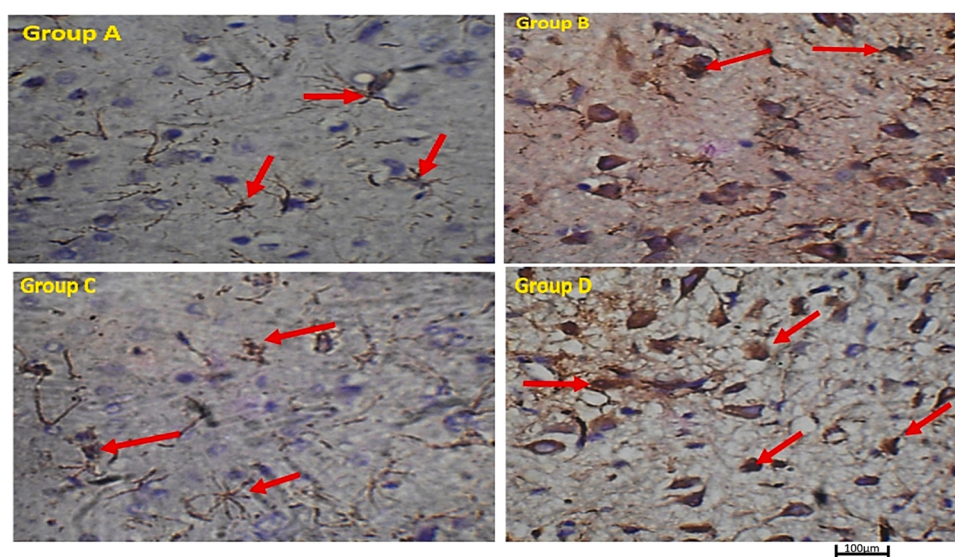


Fig. 4. Representative photomicrograph of the prefrontal cortex pyramidal cell layer (A) of adult male Wistar rats stained for reactive astrocytes stained by anti-GFAP-immunoperoxidase technique with hematoxylin nuclear stain used to counterstain, captured using objective lens of x40. Scale bar 100 µm. Legend: A= Control, B= Scopolamine induced Memory impaired Model C= Alpha Lipoic Acid, D=Alpha Lipoic Acid Treated Memory impaired group; Red arrows= anti-GFAP protein expression for astrocytes. The control and AHA treated shows a mild expression of anti-GFAP protein for astrocytes while Memory impaired group (B) express astrocytic protein GFAP well as compared to the moderate expression in AHA treated Memory impaired rats. (Scale bar 100 µm).

made by [de Araújo et al. \(2017\)](#), [Tzvetanova et al. \(2018\)](#) and [Memudu and Adewumi \(2021\)](#) on AHA neuroprotective potential in improving cognitive function and attenuation of neuro-degeneration. Furthermore, the sequence of arms entered used to measure spontaneous alternation and working memory ([Kaur et al., 2015](#)) shows that AHA treatment can reverse the scopolamine-induced decline in SAB; as shown by the significant increase in SAB as represented in [Fig. 4](#).

The histo-morphological changes in the PFC were demonstrated using H and E stain, it was observed that scopolamine-induced severe neuro-degeneration in PFC characterized by notable loss of cortical neurons via oxidative tissue damage of the neuron cell-mediated by scopolamine, and this correlates with scientific reports made by [Yuan and Shan \(2014\)](#); [Huang et al. \(2016\)](#), and [Assefa et al., \(2018\)](#) on scopolamine's ability to induce neuronal cell death mediated by its potential to mediate oxidative stress in the cholinergic pyramidal neurons ([Saraf et al., 2011](#)) and also in the pyramidal neurons in the hippocampus and purkinje neurons of the cerebellum as reported by [Memudu and Adewumi \(2021\)](#). The prefrontal cortical neurons appeared thinned/ pruned with sparse / less dense neuropil in the scopolamine treated group as compared with the control group. This

characteristic shows that scopolamine-induced neurodegeneration affects synaptic density and hence reduces synaptogenesis. This has been reported by [KaddourTaïr et al. \(2016\)](#) and [Memudu et al. \(2020\)](#) that neurodegeneration is associated with reduced neuronal cell process density that affects the brain volume. [KaddourTaïr et al. \(2016\)](#) study on aluminum-induced neurodegeneration reported the histo-pathological characterization as loss of neurites thickness which influence synaptogenesis and the strength of synaptogenesis contributes to the density of the neuropil ([KaddourTaïr et al., 2016](#)). In this study, scopolamine mediated memory loss is characterized by thinning of axons and dendrites, loss of cortical neurons which results in marked decline in synaptic density a characteristic of neuro-degeneration and memory impairment, this correlates with reports made by [Mahboob et al. \(2016\)](#); [Huang et al. \(2016\)](#) and [Assefa et al., \(2018\)](#).

The pathophysiological analysis of AD suggests that oxidative stress plays a key role in memory decline ([Pohanka, 2014](#)). The brain tissue most especially PFC involve in cognition and memory; requires high consumption of oxygen so they are particularly vulnerable to oxidative stress, damaging the neurons and leading to significant neurological effects as demonstrated in this study ([Karim et al., 2017](#); [Magalingam](#)

et al., 2018; Nigam et al., 2019; Dos Santos et al., 2019).

AHA neuroprotective function is exhibited through its antioxidant property which implies mobbing off free radicals (Lee et al., 2020) generated by scopolamine mediated oxidative tissue damage (Aykaç, et al., 2021) that can invoke the loss of cortical neurons, reduction of synaptogenesis (Dan et al., 2021), thinning/ pruning dendritic arborization and induce astrocytes reactivity (Gonzalez-Perez et al., 2002). However, the histopathological results of the PFC of AHA treated memory impaired model demonstrates an improved neuropil and neuronal cell integrity due to its protection of synaptic density/ integrity of neuronal synapses (Wu et al., 2021; Aykaç et al., 2021).

The histochemistry of chromatophilic bodies which represents basophilic granules of the ribosomes (RER) aggregation, which is required for protein synthesis/ neurons protein or amino acids which is the building block for neurotransmitter synthesis was ascertain to see changes due to scopolamine and AHA administration.

In this study, the prefrontal cortex of scopolamine-treated group was mildly positive for Nissl bodies because of the massive neurodegenerative changes characterized by transient chromatolysis and pyknotic pyramidal cells. This explains scopolamine-mediated oxidative tissue damage caused by chromatolysis of the chromatophilic bodies, making the neurons' cellular components homogenous in appearance (Mahboob et al., 2016). However, AHA treated group reversed this histo-pathogenesis by inhibiting free radical generation that activates neuronal cell death as reported by Zhao et al. (2016a,b). AHA has the potential to cross the blood-brain barrier to exhibit its potent neuro-protective antioxidant function (Galeshkalami, et al., 2019), thereby causing a reversal of oxidative stress-induced chromatolysis of the Nissl body in the PFC as reported in this present study. AHA inhibits chromatolysis in the presence of neurodegeneration-inducing compounds by preventing Nissl bodies degradation (Dixit et al., 2015; Tanbek et al., 2022). Similarly reaction was reported in our previous study which focused on the cerebello-hippocampal cortical neuron (Memudu and Adewumi, 2021) whereby AHA prevented degradation or chromatolysis of chromatophilic contents in pyramidal and Purkinje neurons.

Immunohistochemistry of astrocytes activity which plays a fundamental role in neuron functions and dysfunctions is targeted as biomarkers in new therapeutics or drug development for Alzheimer's disease management (Gandy et al., 2014; Finsterwald et al., 2015; Gentile and D'Amato, 2018). In this study, astrocytes reactivity was studied because reports mentioned its response to oxidative tissue damage in a reaction characterized by gliosis which is a neuro-inflammatory response (Cabezas et al., 2014; Moslemnezhad et al., 2016).

Mitochondria is a cellular organelle initiating processes of neurodegeneration (Mancuso et al., 2006; Ramesh et al., 2018). A report hinted that, mitochondria and astrocyte work together to maintain neuron homeostasis, damage to the mitochondria makes the astrocytes increase matrix production due to calcium or ROS release, consequently activating caspases and causing progressive neuronal cell death (Palop and Mucke, 2016). From Palop and Mucke (2016) and Ramesh et al. (2018) hints, we deduced the mechanistic interplay between scopolamine and astrocytes in neuronal cell damage as well as memory loss. This present study demonstrated this in scopolamine-induced necrosis, neuronal cell death, and chromatophil /Nissl bodies' aggregation which simultaneously activates reactive astrocytes proliferation (gliosis) in response to aforementioned neuro-inflammation as described by Gouras et al. (2015) and Reiss et al. (2018). Astrocyte proliferation has been linked to cognitive impairment as it leads to the production of a huge number of inhibitory neurotransmitters such as GABA which is released to inhibit excitatory neurotransmission in PFC (Haim et al., 2015; Boddum et al., 2016).

Haim et al. (2015) made a report on the negative side effects of reactive astrocytes (gliosis) during neuro-inflammatory response as the alteration of glutamate homeostasis that results in a reduction in

glutamate uptake (causing excitotoxicity in the neurons), altered brain energy metabolism, altered K^+ and Ca^{2+} ion homeostasis which increases glutamate, GABA, cytokines, and inflammatory mediators release (Rudy et al., 2015). AHA treated PFC has a mild expression of astrocytes (anti-GFAP) protein in this study. This support reports that AHA can promote the inhibition of inflammatory processes thereby down-regulating the process of cognitive impairment (Dos Santos et al., 2019; Molz and Schröder, 2017) via its ability to induce neuroprotection through synergistic interaction with astrocytes (Molz and Schröder, 2017). Dos Santos et al., 2019 reported possible AHA mechanisms of neuroprotection linked with mitochondrial disorders and other neurodegenerative disorders. Another mechanism is the ability of AHA to activate the synthesis of endogenous antioxidants thereby elevating the intracellular levels of glutathione and ubiquinone (Roberts and Moreau, 2015; Seifar et al., 2019) which helps to reduce the migration of T lymphocytes, monocytes, and other pro-inflammatory cytokines into the brain (Fiedler et al., 2018; Zhang et al., 2018), thereby declining the presence of inflammatory proteins (Seifar, et al., 2019) which results in the inhibition of reactive astrocytes in AHA treated scopolamine-induced memory-impairment in this study.

This present study describes the role of AHA induced antioxidant activity coupled with the role of astrocytes in repairing / protecting neurons, synaptogenesis from scopolamine invoked oxidative stress-mediated loss of neurites/synapses in the PFC as described by Gollihue et al. (2020) hence this study has provided scientific evidence that AHA targets astrocytes hence it is good therapeutics in managing neurodegenerative diseases associated with cognitive loss (Harada et al., 2016). Hence, AHA neuroprotective mechanism against scopolamine-induced memory impairment can be summarized based on its ability to inhibit or downplay inflammatory processes which correlate with protected neuron cytology, presence of Nissl positive neurons in PFC, and an improved spontaneous alternation behavior.

Conclusion

Alpha-Lipoic acid demonstrates its ability to reverse scopolamine-induced memory impairment and neurodegeneration by reversing neuronal cell loss, attenuation of reactive astrocytes (astroglia) generation resulting in neuron-repair, improved synaptogenesis, increased Nissl (ribosome) synthesis that provides amino acid for neurotransmitters synthesis, improved spontaneous alternation behavior and spatial working memory in AD model. Therefore, alpha-Lipoic acid could serve as a potential therapeutic supplement to help treat AD.

Ethical Approval statement

The experimental protocols approved by the Departmental Research, Animal Care and Use Ethics Committee.

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Conflicts of Interest

We declare no conflict of interest.

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