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# Cognitive and Neuroprotective Effects of *Vernonia amygdalina* in Scopolamine-induced Memory impaired Rats

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**Summary:** Cognitive impairment is largely associated with functional and structural loss in the brain of Alzheimer's disease (AD) models, and scopolamine has been successfully used to mimic these deficits in rodents. The cost and side effects of drugs presently used for the treatment of AD-related cognitive impairment have prompted research into alternative products, especially natural ones with high antioxidant capacity since oxidative stress is a major pathophysiology associated with AD. The current study evaluated the cognitive and neuroprotective effects of *Vernonia amygdalina* (*VA*) on scopolamine-induced cognitive impairment in rats. Thirty-five male rats were randomly divided into seven groups (n = 5). Group 1 served as the control and received distilled water. Groups 2 and 3 received *Vernonia amygdalina*, *VA* (50 and 100 mg/kg, respectively) per orally (p.o.). Group 4 received 1 mg/kg scopolamine SC (i.p.). Groups 5, 6, and 7 received pretreatment with either *VA* 50 mg/kg, *VA* 100 mg/kg, or Donepezil, DP (1 mg/kg), and then in combination with SC (1 mg/kg). The animals were subjected to memory tasks using the Morris water maze (MWM) and novel object recognition tasks (NORT). They were sacrificed on day 14, after which their brains were isolated for biochemical and histological studies. The study showed that during MWM and NORT, spatial and non-spatial recognition memories, respectively impaired in the SC group compared to the control group, were reversed in the *VA* pretreatment groups. Scopolamine injection caused significant decreases in superoxide dismutase and catalase levels and an increase in malonaldehyde (MDA) levels in group 4 compared with the control group. Pretreatments with either *VA* or DP, however, caused a significant increase in the SOD and catalase levels and a decrease in the MDA level compared with the SC group. Histological studies revealed that *VA* was more potent in protecting the brain against SC-induced neurodegeneration and morphological alterations in the hippocampus and prefrontal cortex. The findings of this study suggest that *VA* attenuates scopolamine-induced cognitive deficits via inhibition of oxidative stress and neuronal degeneration and enhancement of cognition in the brains of rats.

**Keywords:** *Cognitive deficits, Alzheimer disease, Vernonia amygdalina, Scopolamine, Memory, Oxidative stress.*

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## INTRODUCTION

Cognitive functions are multiple mental attributes that enable humans and associated organisms to effectively interact with their environment, carry out daily tasks, and adapt to different survival strategies. Amongst these are learning and memory which predominantly have been reported to show a decline in efficiency as a person ages, and in disease conditions like Alzheimer's disease (AD). AD is an age-related, progressive neurodegenerative disorder of the central nervous system, the most common form of dementia characterized by cognitive dysfunction, altered personality, loss of executive function, and ultimately death (Xingxuan *et al.*, 2008; Merlo *et al.*, 2010). Investigations of the brains of AD patients show low levels of neuronal antioxidant defense enzymes, elevated levels of oxidative stress biomarkers, and increased levels of reactive oxygen species (ROS) and nitrogen species (Walton *et al.*, 2012). Preclinical and clinical studies have revealed extensive neuronal loss and synaptic changes in the basal forebrain, particularly the areas involved in learning and memory (Giacobini, 2003). Scopolamine, an $M_1$ muscarinic receptor subtype has been experimentally used to induce AD-related disorders in animals. Tota *et al.* (2012) reported that scopolamine impairs memory through mechanisms that increase acetylcholinesterase and induce oxidative stress in the brain. Drugs like donepezil and tacrine currently used to manage AD, are associated with short half-lives, poor bioavailability, and serious side effects, including hepatotoxicity (Rountree *et al.*, 2009). Thus, there is growing research interest in the use of alternative remedies, such as plants with medicinal, antioxidant, and therapeutic

potential, for the treatment of AD-related cognitive deficits. Dietary polyphenols have gained significant attention due to their rich antioxidant and therapeutic potential in reducing somatic cell and neuronal damage (Engelhart *et al.,* 2002). *Vernonia amygdalina* (*VA*) leaves, commonly known as bitter leaf, have been widely used for both therapeutic and nutritional purposes (Ojiako and Nwanjo, 2006). *VA* possesses strong polyphenolic, antioxidant, anxiolytic, anti-nociceptive, and anti-inflammatory (Iwalokun *et al.,* 2006; Onasanwo *et al.,* 2017) properties. It is an abundant source of linoleic and linolenic acids, and polyunsaturated fatty acids found to be protective against cardiovascular diseases (Tapsell, 2006). This study was therefore designed to investigate the cognitive and neuroprotective effects of *VA* leaf extracts in scopolamine-induced cognitive impairment in Wistar rats.

## MATERIALS AND METHODS

**Experimental animals:** Male Wistar rats (80-120 g) obtained from the central animal house of the University of Ibadan, were used for this study. The rats were housed in plastic cages at room temperature and given free access to commercial food pellets and water *ad libitum.* They were acclimatized for two weeks before commencement of experiments. The experimental procedures were carried out in accordance with the guidelines for animal research as detailed in the guidelines for the Care and Use of Laboratory Animals by the National Research Council of the National Academy of Sciences, 2011.

**Plant and chemicals:** *Vernonia amygdalina*, Scopolamine hydrobromide (Sigma–Aldrich.co., St. Louis, U.S.A), and Donepezil hydrochloride (torrent pharma, UK Ltd.) were used in the study.

**Plant Extraction using Soxhlet technique:** Fresh mature leaves of *VA* were obtained and verified at the University of Ibadan. The leaves were air-dried and blended into powering form using an electric blender. Its weight (2600 g) was determined using an electric weighing balance and then soaked in 80% (v/v) methanol for 72 hours at a temperature of 40°C. The extract was filtered using a cheese material, and thereafter with Whatman No 1 filter paper. The filtrate was concentrated to dryness using a rotary evaporator.

**Experimental design and treatments:** The rats were divided into 7 groups (n = 5). Group 1 received distilled water (per orally, *p.o*) alone throughout the experiment, from day 1-14 and served as the Control (CT). Group 2 and 3 received *Vernonia amygdalina*, *VA* (50 and 100 mg/kg respectively) alone *(p.o)* for 14 days. Group 4 received distilled water *(p.o)* from day 1-7 as well as 1 mg/kg scopolamine SC (*i.p*) from day 8-14. Groups 5, 6 and 7 were pretreated with *Vernonia amygdalina* (SC+*VA* 50 mg/kg), (SC+*VA* 100 mg/kg), and Donepezil (SC+DP 1 mg/kg) respectively, *p.o* for 7 days, and then in combination with scopolamine (1 mg/kg) (*i.p*) one hour later from day 8-14.

**Behavioral assessments**

**Novel object recognition task (NORT):** On the experimental day, animals underwent two sessions, each consisting of a single trial. In the initial session, referred to as the acquisition trial (trial 1), animals were placed in an arena with two identical objects for 5 minutes. Any rat that did not engage with the objects for at least 20 seconds within the 5-minute period were excluded from the experiments. Exploration was defined as the animal bringing its nose within 1 cm of the object while looking at, sniffing, or touching it. In the second session, known as the retrieval session (trial 2), which occurred 24 hours after trial 1, one of the objects used in the first trial was replaced by a new, unfamiliar object (the novel object). Animals were once again placed in the arena for 5 minutes, during which their total exploration time for each were recorded and the time spent actively exploring the familiar (F) or novel (N) object in trial 2. Olfactory cues were eliminated in between trials by cleaning the test box with 70% of ethanol. Percentage exploratory preference which served as index non-spatial recognition memory was evaluated using the formular: $[(N)/(N+F)] \times 100$ (Bevins and Besheer, 2006).

**Morris water maze (MWM):** The MWM apparatus used in this study was made of a circular GP water tank (150 cm diameter and 74 cm height) filled with water (25 ± 2 °C) to a depth of 30 cm. The pool was divided into four hypothetical quadrants, designated as: East, West, North, and South. Throughout the trial, a white round platform of diameter 10 cm was placed 2 cm below the surface of the water in a constant position; in the middle of the southwest quadrant. The water was made opaque by adding a food coloring (peak milk) in order to prevent the animal from noting the depth of the water. Animals were placed 1 inch above the water, positioned to face the wall of the pool. In each trial rats were given a maximum of 60 s cut-off time to locate the hidden platform. This was recorded using a stopwatch, and then averaged for each training session. In the event that the animal was unable to locate the hidden platform within 60 s, it was gently guided to it, and allowed to stay there for 30 s. Each rat was subjected to a daily session of 2 trials for 3 consecutive days, while the animals were allowed to rest on the 4$^{th}$ day. On day the 5$^{th}$ day, the platform height was further lowered below the water level, and the rats underwent a spatial *probing* trial in which they were given 60 s to search for the platform. Escape latency time (ELT) which was the time taken to locate the hidden platform in the water maze was used as an index of spatial learning and memory (Morris, 1984; Ishola *et al.,* 2013).

**Preparation of Brain Homogenate:** On the 14$^{th}$ day of the protocol schedule, animals underwent cervical dislocation. Brains were removed and rinsed with ice-cold isotonic saline solution. Brain tissue samples were then homogenized with 10 times (w/v) ice-cold 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 min to obtain the supernatant. The supernatant was collected and separated into different portions that were kept refrigerated for the different biochemical assays.

**Biochemical estimations**

**Estimation of Protein concentration:** The protein concentration of brain homogenate was determined by the Biuret method described by Gornal *et al.,* (1949).

**Estimation of Malondialdehyde (MDA):** MDA, an end product of lipid peroxidation was measured by the method described by Varshney and Kale (1990), and expressed in units/mg protein.

**Estimation of Superoxide Dismutase (SOD) Activity:** SOD activity was estimated by the method described by Misra and Frodvich (1972) based on the inhibition of Epinephrine-Adrenochrome transition by the enzyme. The enzyme activity was expressed in terms of units/mg protein.

**Estimation of Nitrite Level:** Nitrite level which served as an indicator of nitric oxide (NO) production was estimated using the Greiss reaction as described by Green *et al.* (1982).

**Histological Studies**: Some of the animals were perfused with 0.01 M phosphate buffer solution (PBS, pH 7.4) followed by 4% paraformaldehyde (PFA) in PBS. Brains were removed and post-fixed in 4% formaldehyde for histological study. The tissues were observed and cut into small pieces not more than 4mm thick into pre-labeled cassettes. The brains were removed and placed in a new formaldehyde solution for 24 h to be fixed before being dehydrated using alcohol (70% for 24 h 80% for 1 h and 95% for 1 h absolute 1 and absolute 11, for 1 hr) then cleared in xylene and embedded in paraffin. Sections were cut with a microtome (Leica TP1020, Germany) at 5 µm thicknesses, mounted on glass slides, and stained with the routine haematoxylin and eosin (H&E) technique stain, and Cresyl fast violet (Nissl). Staining was done as described by Avwioro (2010). Examination of slides, photography, and morphometric studies were done at the Histopathology Department, University Teaching Hospital, Ibadan. Good sections from each group were signed and documented as photomicrographs using Olympus digital camera.

**Statistical Analysis**: All the data were expressed as mean ± standard error of mean, (n=5). Data were analyzed using one- or two-way ANOVA followed by Turkey post hoc test (where applicable) to determine the level of significance. Analysis was done using GraphPad prism software, version 7 (Diego, CA, USA).

## RESULTS

**Comparison of percentage exploratory preference (PEP) during NORT in rats:** One-way ANOVA showed significant differences in the PEP across the groups. Post hoc analysis showed that PEP significantly decreased in the SC group ($p<0.0001$) compared with the control group. However, *VA* inhibited the effects of scopolamine-induction in the pretreatment groups during NORT test (Fig. 1).

**Comparison of escape latency time (ELT) during acquisition trials in MWM:** One-way ANOVA showed that there were no significant differences in the ELT across the groups during MWM acquisition trials of day 10, 11, and 12. Post hoc analysis showed progressive decreases in ELT in all the groups, except in the group that received scopolamine injection alone (Fig. 2)

**Comparison of ELT during probe trial in MWM:** One-way ANOVA showed significant differences in the ELT to access spatial memory across the groups. Post hoc analysis revealed that ELT was significantly increased in the SC group which received scopolamine injection alone ($p<0.0001$) and in the SC+*VA* 50 mg/kg ($p<0.01$) compared with the control group. ELT was however significantly decreased SC+*VA* 50 mg/kg ($p<0.001$) and SC+DP 1 mg/kg ($p<0.01$) compared with the SC group (Fig. 3).

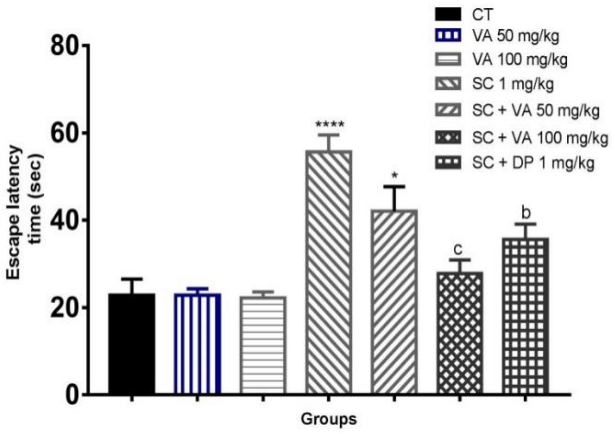


**Figure 1.**
PEP during NORT. Values are expressed as mean ± SEM, n = 5. $^{****}p<0.0001$ *versus* Control (CT); $^{d}p<0.0001$ *versus* scopolamine (SC) treated.

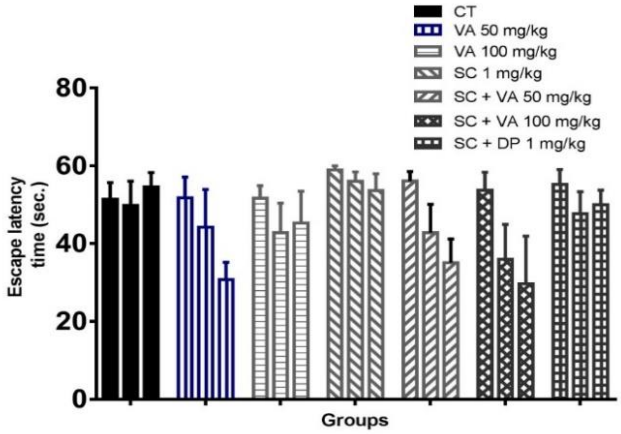


**Figure 2.**
Escape latency during acquisition trial in MWM. Values are expressed as Mean ± SEM, n = 5.

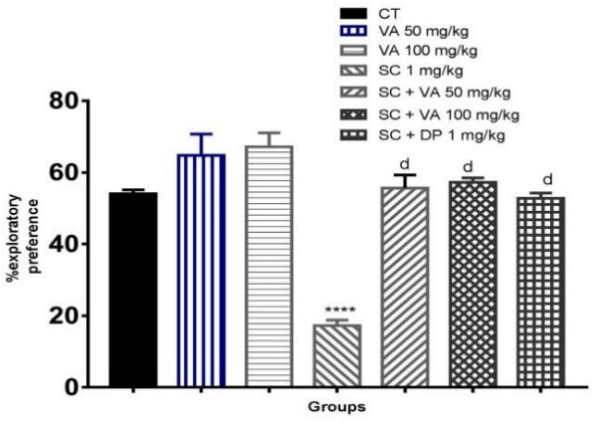


**Figure 3**
Escape latency during probe trial in MWM. Values are expressed as mean ± SEM, n = 5. $^{*}p<0.05$ and $^{****}p<0.0001$ *vs* CT; $^{b}p<0.01$ and $^{c}p<0.001$ *vs* SC treated.

**Comparison of protein concentration in in experimental rats:** As shown in Fig. 4, one-way ANOVA showed that scopolamine injection produced significant changes in protein concentration. Post hoc analysis showed that Protein concentration decreased significantly ($p<0.001$) in the SC group compared with the control. However, the SC+*VA* (50 and 100 mg/kg) and SC+DP pretreatments groups showed significant decreases ($p<0.0001$ and $p<0.00$) and ($p<0.01$) respectively compared with the SC group (fig. 4).

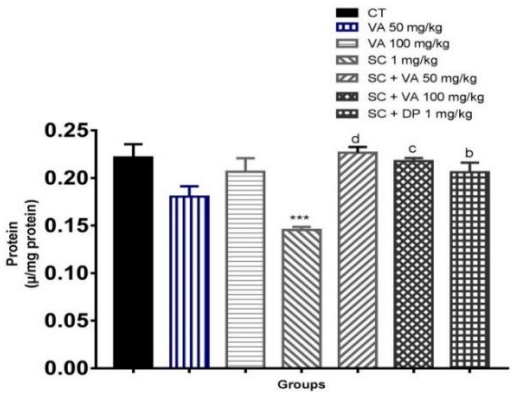


**Figure 4.**
Effects of *VA* on protein concentration. Values are expressed as mean ± SEM, n = 5. $^{***}p<0.001$ *vs* CT; $^{b}p<0.01$, $^{c}p<0.001$ and $^{d}p<0.0001$ *vs* SC treated.

**Comparison of Malonaldehyde (MDA) levels in experimental rats:** Fig. 5 shows the effects of scopolamine injection caused significant changes in the MDA levels in rats' brains as revealed by one-way ANOVA. Post hoc analysis showed that MDA levels significantly increased in the SC ($p<0.0001$), SC+*VA* 100 mg/kg ($p<0.05$), and SC+DP ($p<0.001$) groups compared with the CT group. While MDA significantly decreased in the SC+*VA* (50 mg/kg) compared with the SC group, it also decreased significantly in SC+ *VA* 100 mg/kg ($p<0.0001$), and SC+DP ($p<0.001$) groups compared with the SC group (fig. 5).

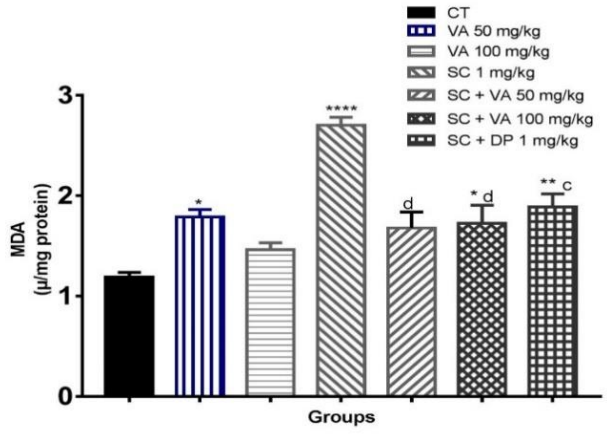


**Figure 5.**
Effects of *VA* on MDA level. $^{*}p<0.05$ $^{**}p<0.01$ and $^{****}p<0.0001$ *vs* CT; $^{c}p<0.001$ and $^{d}p<0.0001$ *vs* SC treated.

**Comparison of Nitrite levels in experimental rats**
One-way ANOVA showed significant differences in nitrite levels across the groups. Post hoc analysis showed that nitrite level was significantly increased in SC+ *VA* (50 and 100 mg/kg) groups ($p<0.001$, $p<0.05$, respectively), and SC group ($p<0.0001$) groups compared with the CT group. Nitrite levels also increased significantly in the SC+ *VA* (50 and 100 mg/kg) or SC+DP groups ($p<0.0001$, $p<0.001$, $p<0.01$, respectively) compared with the control. However, pretreatment of rats with *VA* inhibited the severity of scopolamine-induced effects by significant decreases ($p<0.0001$) in the Nitrite levels across the combination groups (Fig. 6).

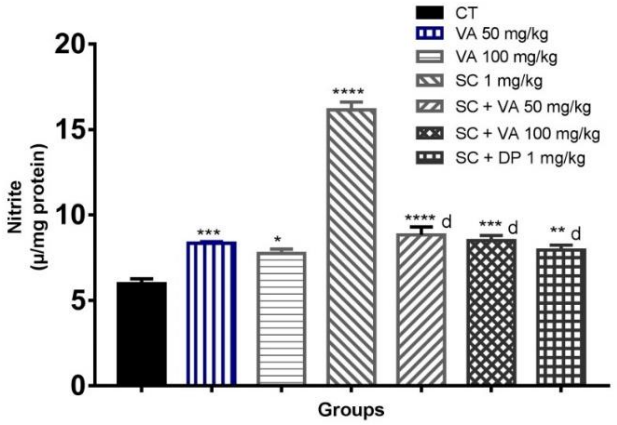


**Figure 6**
Effects of *VA* on Nitrite level. Values are expressed as mean ± SEM, n = 5. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ *vs* CT; $^{d}p<0.0001$ *vs* SC treated.

**Comparison of SOD level in experimental rats:** One-way ANOVA showed significant differences in the SOD level across the groups. Post hoc analysis showed significant decreases in the SOD levels in the SC group ($p<0.0001$) compared with the CT group. SOD also showed significant decreases in the SC+*VA* (50 and 100 mg/kg) and SC+DP groups ($p<0.05$ and $p<0.05$; and $p<0.01$), respectively compared to the CT group. SOD was however significantly increased SC+*VA* (50 and 100 mg/kg) and SC+DP groups ($p<0.0001$); and $p<0.001$), respectively compared to SC group (Fig. 7).

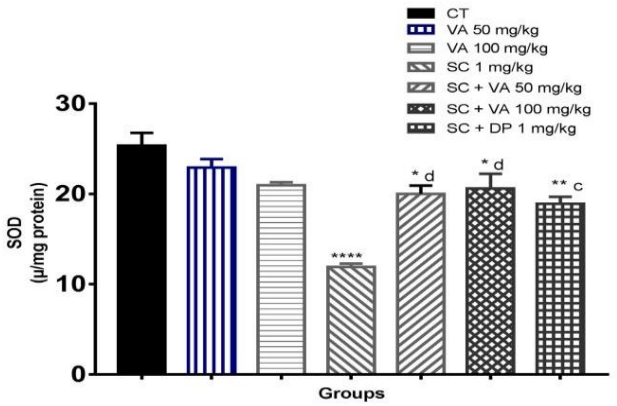


**Figure 7**
Effects of *VA* on SOD level. $^{*}p<0.05$, $^{**}p<0.01$, and $^{****}p<0.0001$ *vs* CT; $^{c}p<0.001$, and $^{d}p<0.0001$ *vs* SC treated.

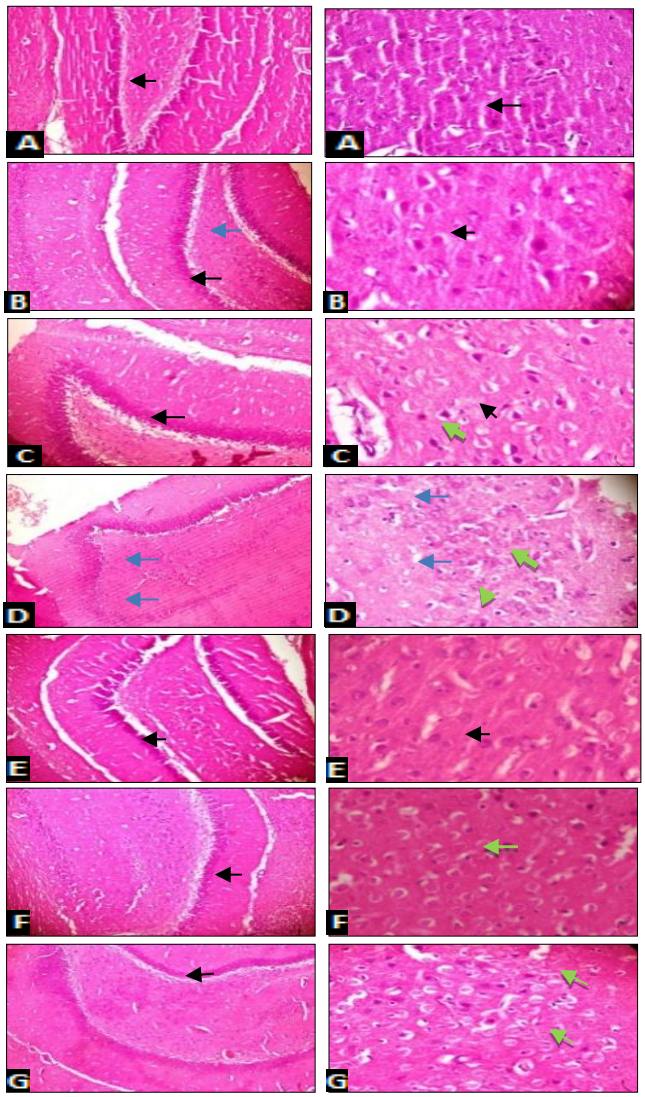

**Plate 1a:**
Photomicrograph of brain sections of the **hippocampus, *hp* (***left)*, and prefrontal cortex, *pc* (*right)* **stained with H&E**.

The Plate 1a shows (A) Control rats exhibit a normal histological structure with intact neuronal cells in both the hippocampus (hp) and prefrontal cortex (pc)**;** (**B**) Rats treated with *Vernonia amygdalina (VA*, 50 mg/kg) display a few degenerated neurons (hp), and normal neuronal cells (pc); (**C**Rats treated with *VA* (100 mg/kg) show normal neuronal cells (hp), and chromatolysis in a few neuronal cells (pc); (**D**) scopolamine-induced AD rats showing several degenerated neurons (blue arrows) in both **hp and pc. D**egenerated neurons in the pc appear transparent and exhibit chromatolysis; (**E**) scopolamine-induced AD rats pretreated with *VA* treatment (50 mg/kg) showing normal neuronal cells (in hp and pc); (**F**) scopolamine-induced AD rats pretreated with *VA* treatment (100 mg/kg) display normal neuronal cells (hp), **and** moderate chromatolysis (pc); (**G**) scopolamine-induced AD rats pretreated with Donepezil (1 mg/kg) exhibit moderately normal neuronal cells (hp), with some degenerated neuronal cells showing degree of hyalinization and chromatolysis (pc).

Plate 1b **shows (A)** show a normal distribution of Nissl bodies in neurons within the hippocampus (hp) and prefrontal cortex (pc)**;** (**B**) *VA*-treated rats (50 mg/kg) show a moderately normal distribution of Nissl bodies (hp), with a few neuronal cells displaying loss of Nissl bodies (pc); (**C**) *VA*-treated rats (100 mg/kg) display moderately normal distribution Nissl bodies (hp), and some neuronal cells with a loss of Nissl bodies in the pc; (**D**) Scopolamine-induced AD rats exhibit a poor distribution of Nissl bodies (hp), and significant loss of Nissl bodies (red arrows) with a reduced neuronal population (pc). (**E**) scopolamine-induced AD rats pretreated with *VA* (50 mg/kg) show moderately restored distribution of Nissl bodies in both hp and pc. (**F)** Scopolamine-induced AD rats pretreated with *VA* (100 mg/kg) show a moderate distribution of Nissl bodies, although a few neurons exhibit a loss of Nissl bodies (hp), some neuronal cells in the prefrontal cortex, particularly within the hypocellular layer, display a loss of Nissl bodies (pc); (**G**) Scopolamine-induced AD rats pretreated with Donepezil (1 mg/kg) exhibit a moderate distribution of Nissl bodies followed by a low neuronal population (hp), there is a reduction in neuronal cells and a significant loss of Nissl bodies (pc).

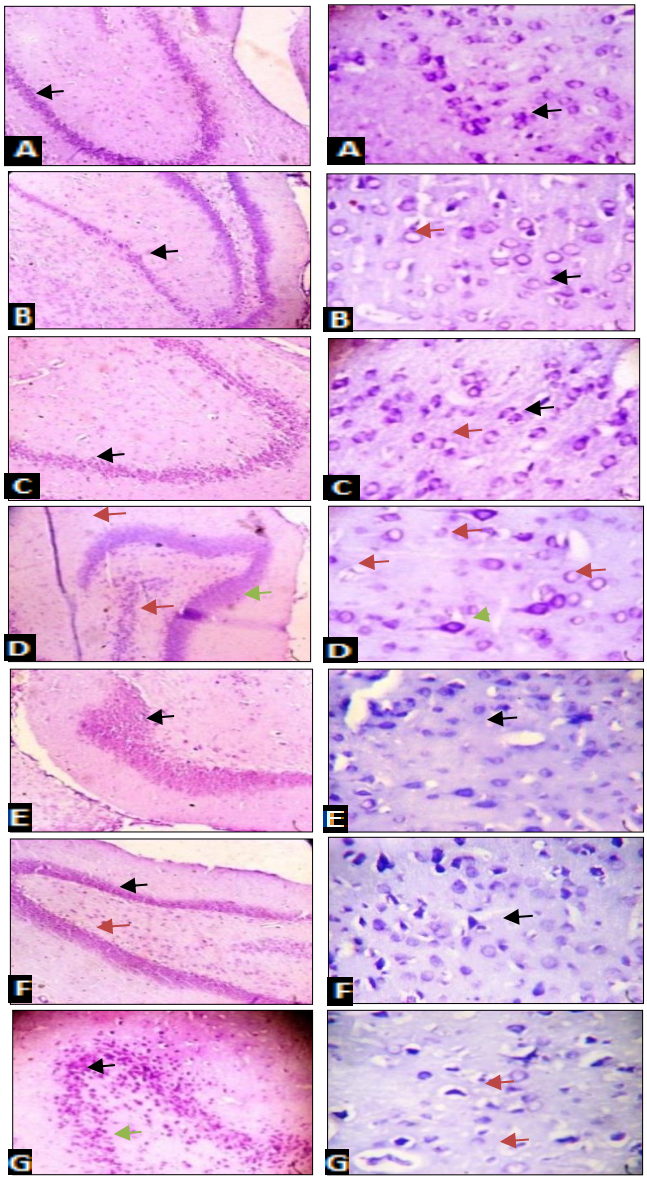

**Plate 1b:**
Photomicrograph of brain sections of the **hippocampus**, ***hp*** **(***left)*, and prefrontal cortex, *pc* (*right)* **stained with cresyl fast violet.**

## DISCUSSION

Our study showed that scopolamine, a non-selective muscarinic receptor antagonist caused significant damages in neuronal architecture in both the prefrontal cortex and the hippocampus with associated cognitive impairment that relates to AD. Scopolamine alters neuronal processes and facilitates neurodegeneration of cortical and hippocampal neurons. Previous studies have reported that scopolamine interferes with cognitive processes involved in memory consolidation and retrievals thus causing short- and long-term memory impairment (Ishola *et al.,* 2013; Souza *et al.,* 2013). Behavioural, neurochemical, and histological changes induced by scopolamine in the current study were attenuated by pretreatment with *Vernonia amygdalina*.

Scopolamine significantly impaired rodents' recognition memory in the novel object recognition task (NORT), a task widely used to assess rodent's long-term, non-spatial recognition memory (Ennaceur, 2010; Pezze *et al.,* 2017). The NORT takes advantage of rodent's unprompted nature to explore its natural surroundings especially novel objects (Ennaceur, 2010). While a higher time spent with the novel object compared to a familiar one within the rodent's environment indicates good residual memory for the familiar object, the opposite indicates memory deficits. Consequently, pre-treatment with *Vernonia amygdalina* (*VA*) efficiently prevented memory impairment in the NORT when recognition memory was assessed.

Morris water maze (MWM), a widely used neurobehavioral task, was used to assess spatial memory which is a hippocampal-dependent task. Animals were trained to acquire information about a spatial location and reach the hidden platform in the circular pool. Escape latency time (ELT) which is the time taken to reach the hidden platform was significantly increased following scopolamine treatment indicating impairment in memory. Pretreatment with *Vernonia amygdalina* however inhibited and reversed the severe memory deficits recorded in groups that received only scopolamine.

It has also been reported that the central nervous system is very susceptible to oxidative stress (Walton *et al*., 2012). Biochemical assay**s** in numerous studies relating to AD shows low levels of neuronal antioxidant defense enzymes and elevated levels of oxidative stress biomarkers**,** which contributes greatly to neuronal damage and cognitive dysfunction (Walton *et al*., 2012). In **a** similar context, scopolamine has been reported in several studies to cause memory impairment via oxidative stress pathology in rodents (Khalifa, 2004; Fan *et al.*, 2005).

The current study also shows that scopolamine-induced oxidative stress significantly causes increases in the brain lipid peroxidation with parallel decreases in total protein concentrations (TPC) and superoxide dismutase activity (SOD). However, pretreatment with *VA* protected the brain from this oxidative stress impact of scopolamine by causing a decrease in Malonaldehyde (MDA) level, an index of lipid peroxidation MDA level, and parallel increases in TPC and SOD. SOD is an anti-oxidant that detoxifies superoxide anions by converting it to hydrogen peroxide and water (Abhinav, 2010). Although previous studies have shown that the central nervous system is very susceptible to oxidative stress (Walton *et al.,* 2012), the *VA* was able to exert neuroprotective effects. The rich antioxidant properties of *VA* have been reported in previous studies (Owolabi *et al.,* 2008). Ogunlade *et al.* (2012) also reported that SOD and MDA levels significantly improved following *Vernonia amygdalina* treatment in liver oxidative stress caused by alcohol ingestion.

Scopolamine also caused significant elevation in brain Nitric oxide (NO) levels, but this was ameliorated by *VA* pretreatment. NO is an important oxidative biological signalling molecule with diverse physiological functions including neurotransmission and learning and memory processes (Susswein *et al.,* 2004), however, when generated in excess during oxidative outburst and inflammation, In excess, NO combines with superoxide anion ($O_2^-$) forming the highly reactive and neurotoxic product peroxynitrite ($ONOO^-$), which leads to further oxidative and nitrosative stress in part via mitochondrial injury (Rao and Balachandran, 2002; Lipton, 2004). Very high level of NO have been implicated in oxidative stress and neuroinflammation in brain parenchyma, neurodegeneration, and neuronal cell death through NO-mediated neurotoxicity (Law *et al.,* 2004; Togo, 2004).

Histological findings in this study support earlier reports that scopolamine extensively destroys neuronal architecture and hippocampal circuits that may play vital roles in learning and memory (Haroutunian *et al.,* 1997; Choi *et al.,* 2012; Chen *et al.,* 2014), as well as depopulates neuronal cells via neurotoxicity (Ramirez-Rodriguez *et al.,* 2011). It has also been reported that scopolamine attenuates memory task-induced increases of regional cerebral blood flow in the prefrontal cortex and the right anterior cingulate region (Grasby *et al.,* 1995). The histological report of the current study showed that scopolamine caused neurodegeneration, chromatolysis, hyalination several losses of nissil bodies in the hippocampus and prefrontal cortex.

Pretreatment with *VA* however protected the brain from the severity of scopolamine-induced damages. While *VA* was able to reduce the level of damage induced by scopolamine exposure, there were however other negative side effects across the groups with *VA* treatment. This may be due to the presence of bioactive compounds such as oxalate previously reported to be insoluble in the methanol form of *VA* extractions (Nerurkar *et al.,* 2004; Eleyinmi *et al.,* 2006). The presence of oxalate in food has been associated with acidity and toxicity (Eleyinmi *et al.,* 2006). However, studies that involved an aqueous form of *Vernonia amygdalina* extraction recorded no histopathological conditions (Ogunlade *et al.,* 2012; Mebratu *et al.,* 2013). On the other hand, the donepezil treatment group was associated with adverse negative side effects characterized by undifferentiated layers, degeneration, chromatolysis, reduction in neuronal cells, and loss of Nissl bodies, thus making it less effective to protect the brain compared with *VA*. Moreover, a study by Zaki *et al.* (2013) reported that donepezil was not strong enough in protecting the brain against scopolamine insults and was associated with some levels of degeneration (Zaki *et al.,* 2013). Donepezil works via a cholinergic pathway to inhibit scopolamine activity and enhance acetylcholine levels which improves cognition. While the scope of this study didn't consider this pathway, *VA* may be working via the same route to execute its beneficial function.

In conclusion, *VA* demonstrated neuroprotective effects and improved cognitive deficits induced by scopolamine in

rats thus suggesting a beneficial role in conditions associated with cognitive dysfunctions. Although the cognitive enhancing effect of *VA* was parallel to that of donepezil, the underlying pathological adverse effects reported in the present and previous studies limit its wide usage. Further research into the mechanism via which *VA* executes its function may position it as a better alternative to donepezil.

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