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Neuroprotective Effect of Ethanolic Leaf Extracts of Vernonia Amygdalina and Cymbopogon Citratus on Diabetes Induced Cognitive Impairments in Mice

Monday AB[1*], Anibeze CIP[2], Ijioma SN[3], Vidona WB[4] and Baaba TC[5]

[1]Department of Nursing Science, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria

[2]Department of Anatomy, Enugu State University of Science and Technology, Nigeria

[3]Department of Forestry and Environmental Science, Michael Opara University of Agriculture, Umudike, Nigeria

[4]Department of Anatomy, Edo University, Iyamho, Nigeria

[5]University of Port Harcourt Teaching Hospital, Nigeria

ABSTRACT

Evaluation of learning and memory enhancing efficacy of ethanolic leaf extracts of vernonia amygdalina and cymbopogon citratus was studied in alloxan-induced diabetic mice to scientifically validate its usefulness traditionally. Upon induction and confirmation of diabetes using alloxan monohydrate, forty mice were randomly divided into 8 groups of 5 mice each and treated with low and high doses of the extracts (400 and 800mg/kg). Metformin, a standard antidiabetic drug was used as a reference agent and given to group 8. Results revealed that diabetes induced memory impairment in neurobehavioural assessment and histomorphological examination of the hippocampus. However, administration of ethanolic leaf extracts of vernonia amygdalina and cymbopogon citratus reveals its neuroprotective potential by ameliorating the histological alterations in the hippocampus (CA1 region) relative to the control and attenuating the learning and memory impairments when compared with the control. Findings from this study suggests the ethanolic leaf extracts of vernonia amygdalina and cymbopogon citratus are potentially efficacious in attenuating diabetes induced memory deficit and alterations in the hippocampus of mice and could be utilized for the treatment and management of diabetes and dementia.

***Corresponding author**

Monday AB, Department of Nursing Science, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria.

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Introduction

The ability to acquire and retain information is memory. All cognitive functions and all characteristics domiciled in humans, are centered in memory. It is fundamental to our sense of self and our ability to maneuver the day to day task. Hence, impairment associated with it has serious consequences to our wellbeing. Recently, discoveries have linked some form of cognitive impairment to diabetes mellitus. Studies have shown that diabetes increases the risk of dementia in adults [1]. These cognitive deficits with diabetes often results in decrease in attention, executive function, psychomotor speed and memory [2]. Both types of diabetes mellitus has the capacity to induce abnormalities in cortical and subcortical structures in the brain. Evidence from epidemiological and biological assessment, suggest a pathophysiological link between type 2 diabetes mellitus and cognitive deficits [3-5]. However, there is paucity of information regarding the actual mechanism involved in the genesis of memory impairments associated with diabetes. The conventional treatment protocols for the management of diabetes mellitus have really help to mitigate against this disease, but none offer complete relieve. Also, they are as well associated with many side effects and not readily available in some part of the African continent (especially, Nigeria), hence, the quest for an alternative therapeutics. The attention given to herbal medication worldwide is at all-time high, given the lesser side effect, cost effectiveness and availability to locals in poor communities across Africa. In Nigeria, herbal medications are frequently used for treating diabetes, malaria, high blood pressure, memory loss, ulcer etc. Vernonia amygdalina and cymbopogon citratus are two plants commonly used for its therapeutical benefits. These plants possess some bioactive phytocomponents which are known to be beneficial to health. Since the standard medications for the treatment of diabetes are expensive, laced with side effects and not readily available to patients in rural communities in Africa, the need to develop an alternative agent for those who can't access the conventional medications became a necessity.

Objective

In this study, we aim to evaluate the cognitive enhancing potentials of vernonia amygdalina and cymbopogon citratus in alloxan-induced memory and learning impairments in mice.

Materials and Methods

ANIMALS – A total of forty (40) mice with body weight of 30-35kg were procured from the animal house of the department of Forestry, College of Natural and Environmental Resources, Michael Opara University of Agriculture, Umudike (MOUAU). They were housed in a non-stressful, well ventilated cages and provided with standard rat pellets (Vital Feed, Nigeria) and water ad libitum. Prior to the commencement of the experiment, the mice were allowed to acclimatized for two weeks. During this period, thorough observations were made to ensure that the mice were disease free. Agility and appetite for food were closely monitored during these two weeks of acclimatization. Animal ethical committee of MOUAU approval was acknowledged and all procedures were carried out in strict compliance with NIH guidelines for care and use of laboratory animals.

Preparation of Plant Material

Fresh leaves of cymbopogon citratus and vernonia amygdalina were washed with clean water to remove sand, dirt and debris. The leaves were air dried at room temperature for 12 days and thereafter gound into powder using electric blender. One hundred (100) grams of each powdered plant material was introduced into the extraction chamber of the soxhlet extractor and extraction was done with ethanol as solvent. Extraction temperature was maintained at 600C for 48 hours after which the solvent was evaporated at low temperature in a hot air oven to obtain crude extract which weighed 7.98g and represented a percentage yield of 7.98% for vernonia amygdalina and 4.24g which represents percentage yield of 4.24% for cymbopogon citratus respectively. The extracts were preserved in a refrigerator until needed.

Induction of Diabetes Mellitus

The rats were fasted for 12 hours (overnight) and diabetes was induced by simple intraperitoneal administration of freshly prepared Alloxan. The prepared alloxan at a dose of 160mg/kg body weight was used for the induction. Diabetes was confirmed after fourth day of administration by measuring fasting blood glucose concentration, using commercial glucose strip (Accu-chek glucometer). Only animals with fasting blood glucose level of 200mg/dl and above were considered diabetic and used for the study.

Experimental Design

The mice in this study were randomly divided into eight groups of five mice each [6]. Group one and two served as the normoglycemic and diabetic control. Groups 3 and 4 received 400 and 800mg/kg body weight of vernonia amygdalina, groups 5 and 6 received 400 and 800mg/kg body weight of cymbopogon citratus. Group 7 were given 400+400mg/kg body weight of vernonia amygdalina and cymbopogon citratus. The last group (group 8) received 150mg/kg of metformin. All animals in the groups were administered with the extracts through the oral route with the aid of oral gavage. The extracts administration lasted for twenty one (21) days.

Behavioural Test

Moris Water Maze

The Morris water maze is made of a circular plastic pool (100cm diameter) divided into four quadrants. Water was filled to a height of about 30cm and milk was added to make the water opaque. The water was left to sit overnight in order to achieve room temperature (25 ± 1°C). The test consisted of 3 days of acquisition training and 3 days of reversal training (with the platform in the opposite quadrant) where the mice were trained to use extra-maze visual cues to locate an escape platform hidden just below the surface of the opaque water, in one of the quadrants (Morris, 1984). On the 7th day, the platform was removed and the duration in each quadrant noted, to test for visuo-spatial learning and memory, which is hippocampus dependent [7].

Procedure

The Moris water maze test took 8 days in our study;
Day 1: Acquisition 1
Day 2: Acquisition 2
Day 3: Acquisition 3
Day 4: Reversal 1
Day 5: Reversal 2
Day 6: Reversal 3
Day 7: Probe trial
Day 8: Visible-platform day

Acquisition and reversal training used hidden platform submerged in water (water is 0.5-cm above platform). During reversal, the platform is moved to the opposite side of the maze, while in the probe trial, there is no escape platform so that visuo-spatial memory can be assessed. On the visible-platform day the platform was moved to another quadrant of the pool and the visible top is added to the platform. This assesses basic visual ability and motivation to locate the platform. Each day of the test, the mouse is removed from the cage and is placed in a clean holding cage without woodchip bedding. Paper towel is shredded into strips and placed in the bottom of the holding cages to allow the mice to dry quickly.

During acquisition training, the platform is placed at the centre of the Northeast quadrant. Each mouse receives 4 trials per day. Each mouse is given 60-sec to locate the escape platform per trial. The starting positions of the mice are predetermined using a Latin square design, this is to prevent the repetition of starting location sequences on back-to-back test days. Possible start positions were at the boundaries of the quadrants (e.g. West, North, East or South). For each trial, a mouse is removed from its holding cage using a small, clean 500-mL plastic container to minimize handling stress. The animal is then placed into the water at the appropriate start position and then allow to explore the pool and to search for the hidden escape platform for 60-sec. As soon as the animal locates the platform, the timer is stopped and the mouse is allowed to stay on it. Once on the platform, the mice are permitted to view the extra-maze environment for 10-sec, at which point the mouse is picked in the plastic container and returns it to the appropriate holding cage. If the mouse does not find the platform during the allotted time, the animal is guided onto the platform using the plastic container. The next mouse is then placed in the pool and the same procedure followed.

Reversal training starts on day 4. Here, the invisible platform is moved to the opposite quadrant (Southwest quadrant), and mice are again assigned to appropriate start positions. The same procedures as in acquisition training are carried out during reversal training. Each of the animals completes 4 trials per day for 3 days. A probe trial is conducted on day 7 to assess visuo-spatial memory. In this trial, there is no escape platform in the maze. Each mouse is placed in the pool from one of the four possible start positions and allowed to navigate through the pool for 60-sec, after which the time spent in each quadrant of the maze is recorded. When the 60-sec is complete the mouse is scooped up using the container

and placed in a holding cage to dry. The visible platform task is conducted on day 8. The visible platform is placed in a new location within the Northwest quadrant of the pool. The same procedures as in acquisition and reversal training are carried out and mice complete 4 trials.

Data Collection for Morris Water Maze

In acquisition, reversal and visual training, the following behaviors were measured: (1) swim latency (time to find and mount the escape platform) During the probe trial, the data measured were (1) duration of time spent in each quadrant, (2) the number of times the mouse crossed the location of the platform during reversal training (annulus reversal crossing), (3) the number of times the mouse crossed the location of the platform during acquisition training (annulus acquisition crossing).

Statistical Analysis

Graph pad prism version 7 was used for the statistical analysis and the results were express as mean±SEM. Hypothesis testing method was by one-away analysis of variance (ANOVA) and least significance difference (LSD) dunnett post hoc for multiple comparism. P-value of less than 0.05 was considered statistically significant, 0.01 as highly statistically significant.

Results

Results showing the effect of the extracts on spatial memory and learning using Morris Water Maze.

Comparison of Swim Latency of Day One of Acquisition Training In Morris Water Maze Tests

Figure 1 shows the swim latency of the acquisition training (Day 1) between the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p< 0.001$) dose dependent decrease in the swim latency of the 400mg/kg of V. amygdalina, 400mg/kg of C. citratus groups and 400+400mg/kg combination of the two extracts when compared to the normal Control (Ctrl) and Diabetic Control (DM Ctrl) groups respectively. In contrast, there was significant increase in the swim latency of the 800mg/ kg of V. amygdalina, 800mg/kg of C. citratus groups and metformin groups when compared to the controls.

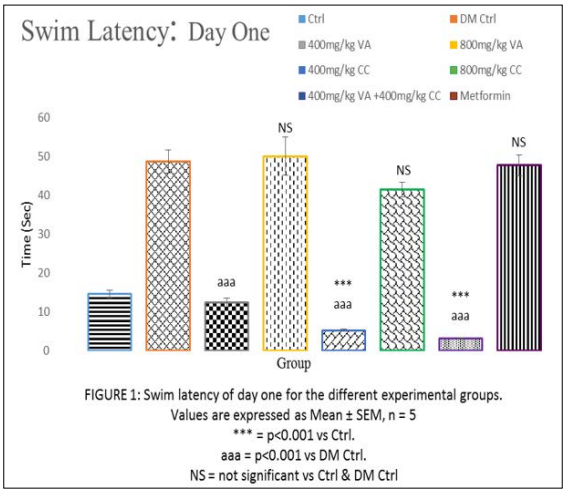

Figure 1

Comparison of Swim Latency of Day Two of Acquisition Training In Morris Water Maze Tests

Figure 2 shows the swim latency of the acquisition training (Day 2) for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p< 0.001$) dose dependent decrease in the swim latency of the 400mg/kg V. amygdalina, 400mg/kg of C. citratus groups and 400+400mg/kg combination of the two extracts when compared with the normal Control (Ctrl) and Diabetic Control (DM Ctrl) groups respectively. There was also a significant ($p<0.01$ and $p<0.05$) decrease in the swim latency of the 800mg/kg of V. amygdalina and 800mg/kg of C. citratus groups respectively when compared to the Diabetic control group. There was no significant decrease (rather increase) for the metformin group when compared to the controls.

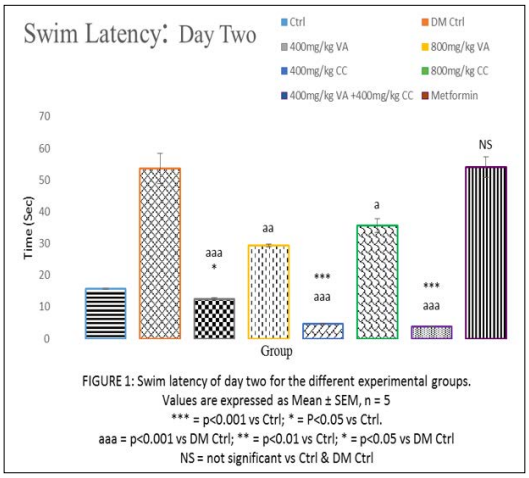

Figure 2

Comparison of Swim Latency of Day Three of Acquisition Training In Morris Water Maze Tests

Figure 3 shows the swim latency of the acquisition training (Day 3) for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p< 0.05$ and $p< 0.001$) dose dependent decrease in the swim latency of the 400mg/kg of V. amygdalina, 400mg/kg of C. citratus groups and 400+400mg/kg combination of the two extracts when compared to the normal Control (Ctrl) and Diabetic Control (DM Ctrl) groups respectively. In contrast, there was no significant decrease (rather increase) in the swim latency of the 800mg/kg of V. amygdalina, 800mg/kg of C. citratus groups and metformin groups when compared to the controls

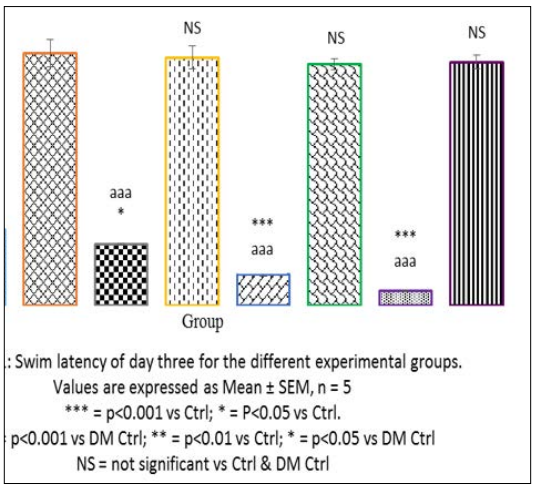

Figure 3

Comparison of Swim Latency of Day Four of Reversal Training In Morris Water Maze Tests

Figure 4 shows the swim latency of the reversal training (Day 4) for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p< 0.05$ and $p< 0.001$) dose dependent decrease in the swim latency of the 400mg/kg and 800mg/kg of V. amygdalina, C. citratus groups and 400+400mg/kg combination of the two extracts and Metformin when compared to the normal Control (Ctrl) and Diabetic Control (DM Ctrl) groups respectively

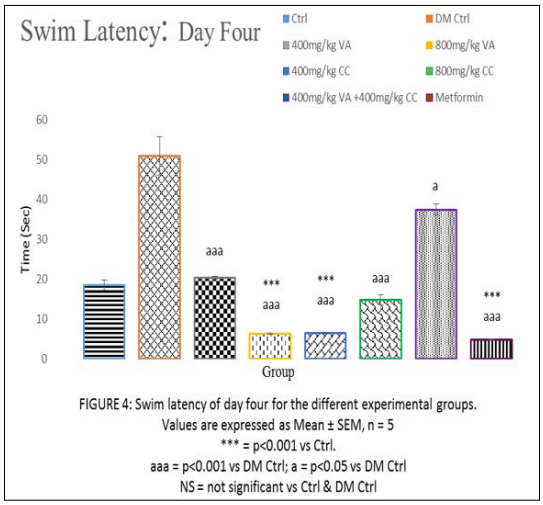

FIGURE 4: Swim latency of day four for the different experimental groups.
Values are expressed as Mean ± SEM, n = 5
*** = p<0.001 vs Ctrl.
aaa = p<0.001 vs DM Ctrl; a = p<0.05 vs DM Ctrl
NS = not significant vs Ctrl & DM Ctrl

Figure 4

Comparison of Swim Latency of Day Five of Reversal Training In Morris Water Maze Tests

Figure 5 shows the swim latency of the reversal training (Day 5) between the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p< 0.001$) dose dependent decrease in the swim latency of the 400mg/kg V. amygdalina, C. citratus groups and 400+400mg/kg combination of the two extracts when compared with the normal Control (Ctrl) and Diabetic Control (DM Ctrl) groups respectively. In contrast, there was no significant decrease (rather increase) in the swim latency of the 800mg/kg of V. amygdalina, C. citratus groups and metformin groups when compared to the controls.

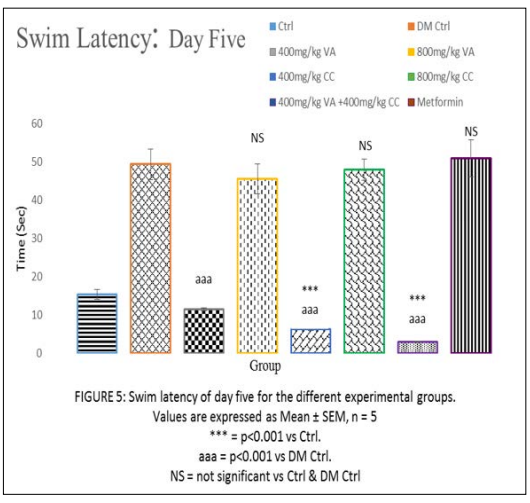

FIGURE 5: Swim latency of day five for the different experimental groups.
Values are expressed as Mean ± SEM, n = 5
*** = p<0.001 vs Ctrl.
aaa = p<0.001 vs DM Ctrl.
NS = not significant vs Ctrl & DM Ctrl

Figure 5

Comparison of Swim Latency of Day Six of Reversal Training In Morris Water Maze Tests

Figure 6 shows the swim latency of the reversal training (Day 6) for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p< 0.001$) dose dependent decrease in the swim latency of the 400mg/kg V. amygdalina, C. citratus groups and 400+400mg/kg combination of the two when compared with the normal Control (Ctrl) and Diabetic Control (DM Ctrl) groups respectively. There was also a significant ($p<0.01$ and $p<0.05$) decrease in the swim latency of the 800mg/kg of V. amygdalina and C. citratus groups respectively when compared to the Diabetic control group. There was no significant decrease (rather increase) for the metformin group when compared to the controls.

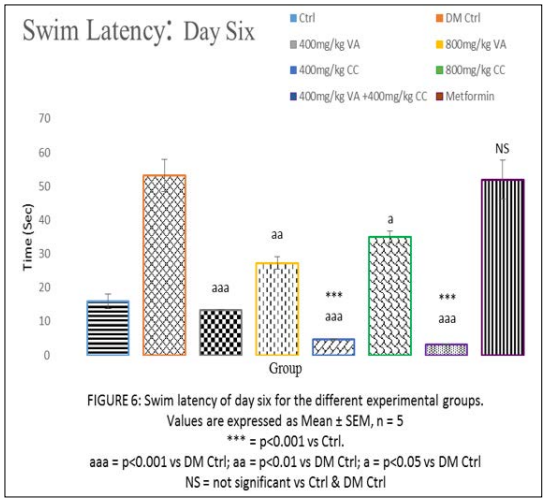

FIGURE 6: Swim latency of day six for the different experimental groups.
Values are expressed as Mean ± SEM, n = 5
*** = p<0.001 vs Ctrl.
aaa = p<0.001 vs DM Ctrl; aa = p<0.01 vs DM Ctrl; a = p<0.05 vs DM Ctrl
NS = not significant vs Ctrl & DM Ctrl

Figure 6

Comparison of Preferencial Quadrant (North-East) Duration during the Morris Water Maze Test

Figure 7 shows the comparison of quadrant (North-East) duration in the probe trial of the Morris water maze test for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p<0.05$) increase in the preferential North-East quadrant duration across all the test groups (V.amygdalina, C.citratus and Metformin) when compare with the control groups.

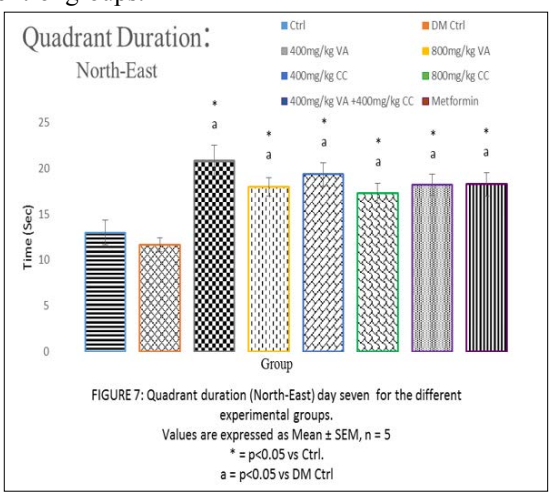

FIGURE 7: Quadrant duration (North-East) day seven for the different experimental groups.
Values are expressed as Mean ± SEM, n = 5
* = p<0.05 vs Ctrl.
a = p<0.05 vs DM Ctrl

Figure 7

Comparison of Preferencial Quadrant (South-West) Duration during the Morris Water Maze Tests

Figure 8 shows the comparison of quadrant (South-West) duration in the probe trial of the Morris water maze test for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p<0.05$) increase in the preferential South-West quadrant duration across all the test groups (V. amygdalina, C. citratus and Metformin) when compare to the control groups.

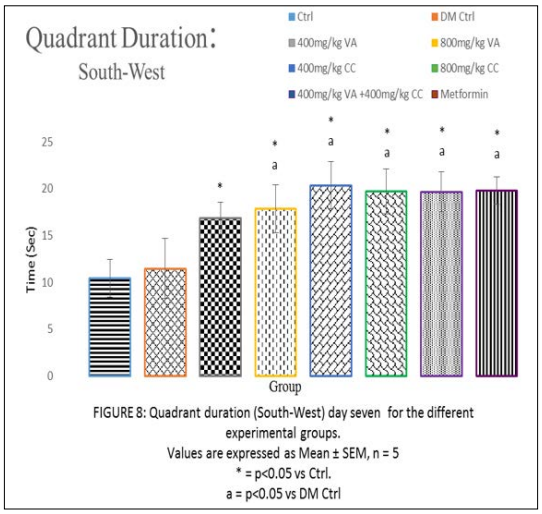

FIGURE 8: Quadrant duration (South-West) day seven for the different experimental groups.
Values are expressed as Mean ± SEM, n = 5
* = p<0.05 vs Ctrl.
a = p<0.05 vs DM Ctrl

Figure 8

Comparison of Annulus Crossing (Acquisition) In the Moris Water Maze Tests

Figure 9 shows the comparison of annulus crossing (Acquisition) in the probe trial of the Morris water maze test for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p<0.05$) increase in the frequency at which the V. amygdalina, 400+400mg/kg combination of V.amygdalina and C. citratus and Metformin treated groups crossed the position of the platform during the acquisition training in North-East quadrant when compare to the normal control group, but the C. citratus treated groups had a significant ($p<0.05$) increase when compared to the diabetic control group.

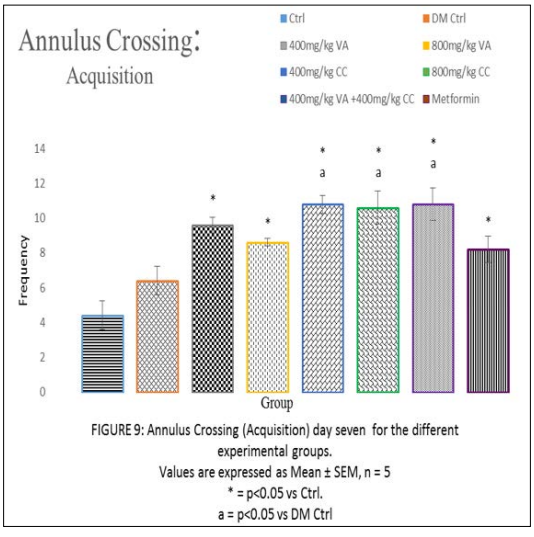

FIGURE 9: Annulus Crossing (Acquisition) day seven for the different experimental groups.
Values are expressed as Mean ± SEM, n = 5
* = p<0.05 vs Ctrl.
a = p<0.05 vs DM Ctrl

Figure 9

Comparison of Annulus Crossing (Reversal) In the Moris Water Maze Tests

Figure 10 shows the comparison of annulus crossing (Reversal) in the probe trial of the Morris water maze test for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p<0.05$) increase in the frequency at which the V. amygdalina, 400+400mg/kg combination of V. amygdalina and C. citratus and Metformin treated groups and also ($p<0.05$) for the C. citratus crossed the position of the platform during the reversal training in South-West quadrant when compare to the Normal and Diabetic control groups respectively.

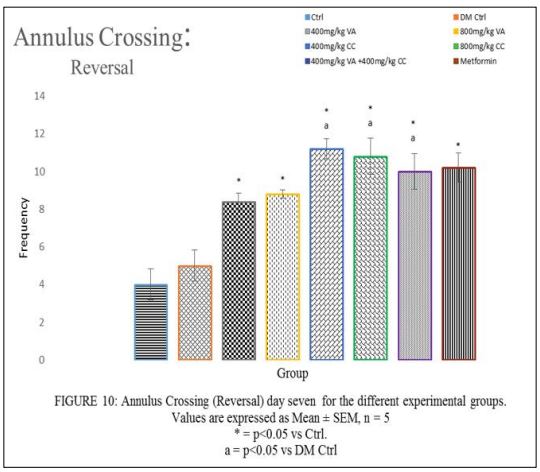

FIGURE 10: Annulus Crossing (Reversal) day seven for the different experimental groups.
Values are expressed as Mean ± SEM, n = 5
* = p<0.05 vs Ctrl.
a = p<0.05 vs DM Ctrl

Figure 10

Comparison of Swim Latency Day Eight (Visible Platform) In the Moris Water Maze Tests

Figure 11 showed comparison of swim latency during the visible platform of the Morris water maze test for the control and graded doses of Vernonia amygdalina and Cymbopogon citratus fed mice groups.

The result showed a significant ($p< 0.001$) dose dependent decrease in the swim latency of the 400mg/kg V. amygdalina, C. citratus groups and 400+400mg/kg combination of the two extracts when compared to the normal Control (Ctrl) and Diabetic Control (DM Ctrl) groups respectively. There was also a significant ($p<0.01$ and $p<0.05$) decrease in the swim latency of the 400mg/kg, 800mg/kg of V. amygdalina and 800mg/kg of C. citratus groups respectively when compared to the Diabetic control group. There was no significant decrease for the metformin group when compared to the controls.

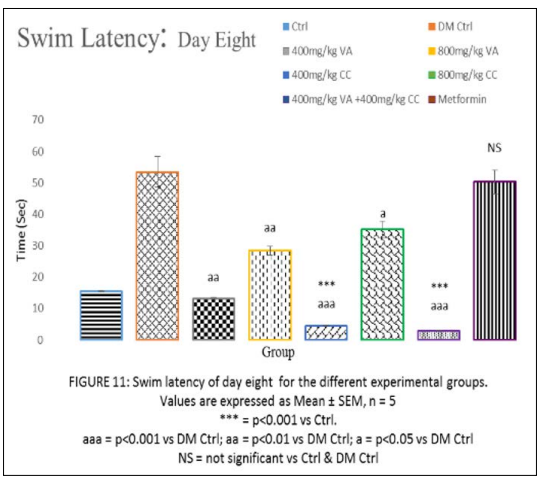

FIGURE 11: Swim latency of day eight for the different experimental groups.
Values are expressed as Mean ± SEM, n = 5
*** = p<0.001 vs Ctrl.
aaa = p<0.001 vs DM Ctrl; aa = p<0.01 vs DM Ctrl; a = p<0.05 vs DM Ctrl
NS = not significant vs Ctrl & DM Ctrl

Figure 11

Result on the Effect of the Extracts on the Cytomorphology of the Hippocampus of Mice

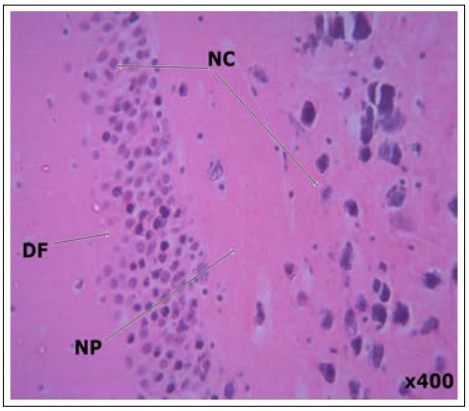

Plate 1: Photomicrograph of Group 1 hippocampal formation show the Cornu Ammnonis (CA) with variable cellularity in the constituent layers (CA1-4). The average thickness in the CA1 is 79.69µm. The CA1 is shown in the image and shows rich neuronal cell density within the dentate fascia and adjacent areas. Other regions show variable cellularity with evenly distributed glial cells and pyramidal cells, all set in a meshwork of neuropils. H&E X400

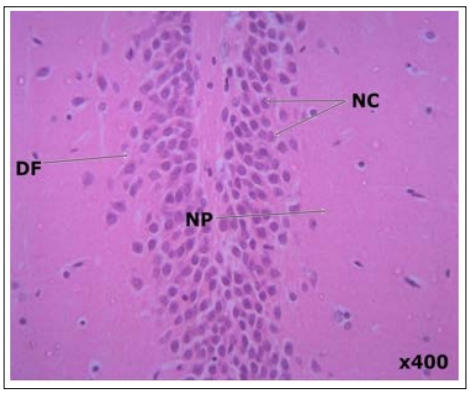

Plate 2: Photomicrograph of Group 2 (diabetic untreated group) shows atrophy with loss of up to 75% of thickness in the CA1. The average thickness in the CA1 is 22.31µm. Other areas show no significant thinning or loss of cellular density. H&E X400

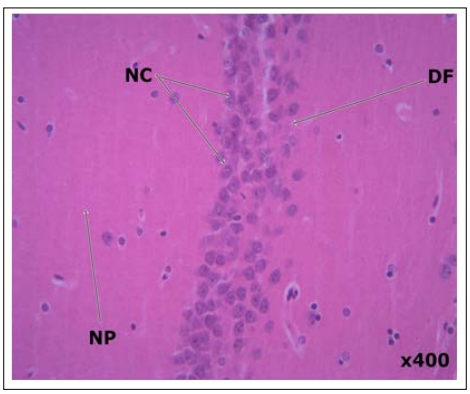

Plate 3: Photomicrograph section of Group 3 showing significant restoration of thickness and density of cells in the dentate fascia of the CA1 area with the average thickness reaching 40.63µm. Other areas show no significant thinning or loss of cellular density after treatment with 400mg/kg of V.A. H&E X400

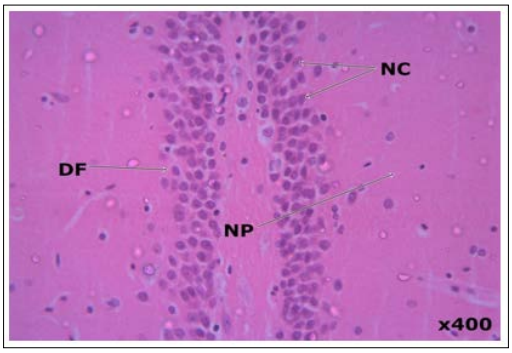

Plate 4: Photomicrograph of Group 4 shows no significant change in cell density with average thickness in the CA1 area reaching 22.31µm. Other areas show no significant thinning or loss of cellular density after treatment with 800mg/kg of V.A. H&E X400

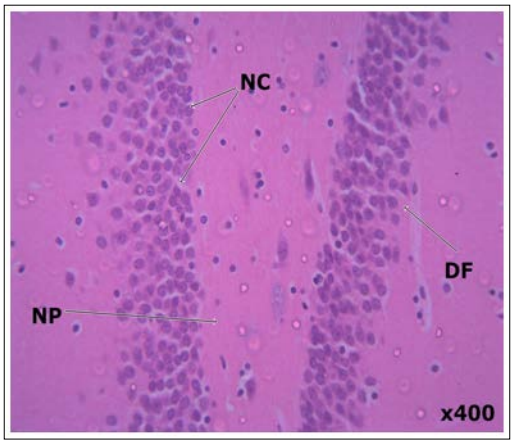

Plate 5: Photomicrograph of Group 5 shows no significant change in the cell density with average thickness in the CA1 area reaching 24.93µm. Other areas show no significant thinning or loss of cellular density after treatment with 400mg/kg of C.C for 21 days. H&E X400

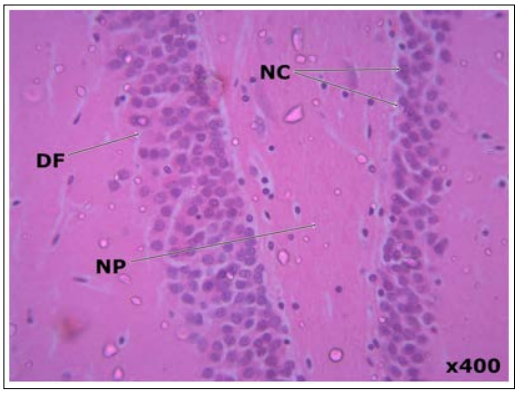

Plate 6: Photomicrograph of Group 6 shows a mild change in the cell density with average thickness in the CA1 area reaching 31.44µm. Other areas show no significant thinning or loss of cellular density after treatment with 800mg/kg of C.C for 21 days. H&E X400

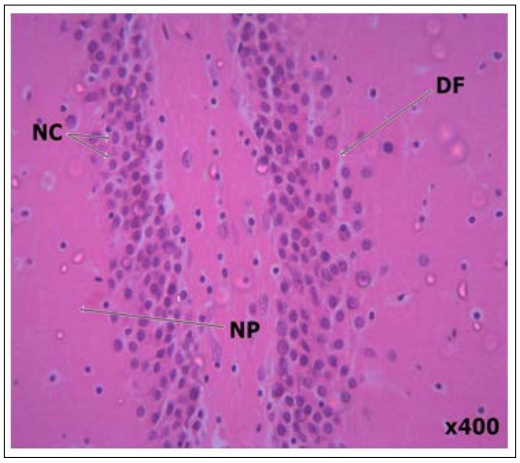

Plate 7: Photomicrograph of Group 7 showing moderate change in the cell density with average thickness in the CA1 area reaching 44.56µm. Other areas show no significant thinning or loss of cellular density after treatment with the combined extracts of 400mg/kg of V.A and 400mg/kg 0f C.C. H&E X400

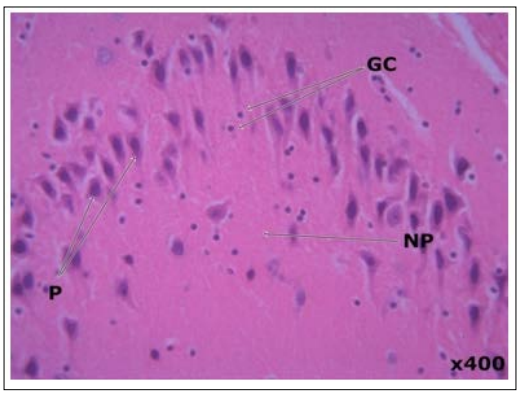

Plate 8: Photomicrograph of Group 8 showing moderate change in the cell density with average thickness in the CA1 area reaching 44.50µm. Other areas show no significant thinning or loss of cellular density after treatment with 160mg/kg of metformin. H&E X400

Discussion

Memory is an organism's mental ability to store, retain and recall information [8]. In this study, the Morris water maze which is one of the most widely used task in behavioural neuroscience for studying psychological processes, served as an exteroceptive behavioural model to evaluate spatial learning and memory in mice [9]. Memory forms can be classified as declarative or explicit (the ability to recall past event deliberately) and are hippocampus-dependent; and non-declarative or procedural (also called implicit), defined by unconsciously perform skills (cognitive) that are mainly dependent on the striatum and cerebellum [6]. Morris water maze is based on the ability of the mice to remember what it was thought (how to locate a platform) [9]. The task exploits the ability of the mice to locate an escape platform after training under the influence of the extracts of vernonia amygdalina and cymbopongon citratus. The swim or escape latency refers to the time taken for the mice to locate the escape platform. The measure is accessed by the swimming speed [10]. In the Morris water experiment, during the acquisition period which lasted for three days, it was observed that the low doses of the extracts of vernonia amygdalina and cymbopogon citratus administered to the diabetic groups showed a significant decrease in swim latency when compared with the normoglycemic and diabetic control groups, while high doses of the extracts showed increase in swim latency (Figures 1 and 3). During the reversal training, the swim latencies for the groups treated with low doses of the extracts showed significant decrease when compared with the controls (Figure. 5). High doses of the extracts did not exert significant decrease in swim latency when also compared with the controls (Figures 4, 5 and 6). This suggest that the low dose of the extracts of VA and CC enhances better learning and memory in mice than the high doses of the same extracts when compared with the controls. During the probe trial, there was significant increase in preference to North-East quadrant which bore the escape platform during acquisition training when compared with the controls. There was increase preference to South-West which bored the platform in reversal training when compared with the controls (Figure. 8). This shows that the extracts treated groups had better learning and memory than the controls.

The annulus acquisition and reversal crossing refers to the number of times that the mouse crossed the location of the platform during acquisition and reversal training. There was significant increase at the frequency at which the extracts treated groups and metformin group crossed the position of the platform during acquisition training in North-East when compared with the normal and diabetic controls. Significant increase of crossing frequency was observed in the control in the treatment groups when compared with the controls. There was a significant decrease in swim latency in the visible platform training compared to the control (Figure. 11). Shorter swim latency indicates poor cued learning. This present study has shown that ethanolic extracts of vernonia amygdalina and cymbopogon citratus enhance memory and learning in mice and it consistent with the work done by Ademosun et al., 2017), Akram and Nawaz (2017) [11, 12]. Ekong et al. (2017) Reported that plants extracts may offer neuroprotection effects through the inhibition of acetylcholinesterase (AChE) [13].

Administration of plant extracts has been reported to successfully reverse chemical-induced memory deficits in rodents and correlated memory enhancing properties is by virtue of their bioactive phytochemical constituents [14, 15]. Phytoconstituents includes: flavonoids, steroids, terpenoids and organic acids (ascorbic, benzoic, shikinic and vanillic acid). Memory enhancing action shown by medicinal plants is by increased supply of oxygen, and free radical elimination from the body, thereby improving memory. Recently, there has been intense interest in the potential of flavonoids to modulate neuronal function and prevent age-related neurodegeneration. Flavonoids exert a multiplicity of neuroprotective actions within the brain, including a potential to protect neurons against injury induced by neurotoxins and ability to suppress neuroinflammation, and also the potential to promote memory, learning, and cognitive function. These effects appear to be underpinned by their interaction with critical protein and lipid kinase signaling cascades in the brain leading to an inhibition of apoptosis triggered by neurotoxic species and to a promotion of neuronal survival and synaptic plasticity. Though these mechanisms, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration, decrease neuroinflammation, and prevent or reverse age-dependent loses in cognitive performance [16]. Result suggest that the extracts of Vernonia amygdalina and Cymbopogon citratus may serve as a potential neuroprotective agent against diabetes-induced cognitive dysfunction.

Effect on the Histology of the Hippocampus

In this study, light microscopic examination of (Haematoxylin and Eosin, H&E) stained histological sections of mice hippocampus was conducted. Neurodegeneration is a process involved in both neuropathological conditions and brain ageing [15]. Histoarchitectural distortion of neural tissue manifesting as neuronal degenerative

changes are indicative of neurotoxicity in the central nervous system [17, 18]. Diabetes mellitus has repeatedly been reported to be associated with cognitive deficits and an increased risk of dementia. And these deficits are parallel by neurophysiological and structural changes in the hippocampus, the major area associated with memory and learning. The process of learning and memory involves distinct, and interlinked, groups of efferent system for episodic memory, affective and social learning, and sensory processing and integration, all of which could be affected by chronic diseases like diabetes [19]. In this study, examination of section of the hippocampus of normoglycemic control rats revealed normal Cornu Ammonis (CA) with variable cellularity in the constituent layers (CA1-4). The average thickness in the CA1 is 79.69µm. The CA1 shown in Plate1 shows rich neuronal cell density. There were evenly distributed glial cells and the pyramidal cells were all normal. Conversely, histoarchitectural distortion of the hippocampus (Region CA1), such atrophy, loss of thickness (75%) in the CA1 and irregular arrangement of CA1 hippocampal neurons was evident in the diabetic untreated control group (Plate 2). This is an indication of diabetes-induced neurodegenerative change. Findings is in accordance with previous report on hyperglycemic effect on the hippocampus [20, 21]. Other regions of the hippocampus showed no significant thinning or loss of cellular density, except the CA1 region and its neurons in the pyramidal layer which is the most vulnerable to toxic events [22]. These changes observed in the diabetic untreated group were attenuated moderately by the administration of the extracts to the diabetic test groups. It was observed that diabetic group three which received 400mg/kg body weight of vernonia amygdalina had significant change in the regeneration of neurons in the CA1 region of the hippocampus with thickness of 40.63µm when compared with the diabetic control (Plate 3). It further evaluates the neuroprotective properties of vernonia amygdalina and cymbopogon citratus.

Conclusion

The result of the present study suggests that, ethanol extracts of Vernonia amygdalina and Cymbopogon citratus may prove efficacious in ameliorating diabetes-induced cognitive impairments in the mice. The neuroprotective properties of the extracts, relative to the standard drug (metformin), is rather similar, and is attributed to antioxidants properties of constituent phytochemicals, such as flavonoids. Thus, ethanolic extracts of vernonia amygdalina and cymbopogon citratus are potential candidates for application in the management and treatment of diabetes-induced neurodegenerative diseases.

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Conflict of Interest

The authors declared no conflict of interest

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