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Ameliorative Effects of *Vernonia amygdalina* and *Moringa oleifera* Extracts on Cognitive Impairment in Diabetic Wistar Rats

Koofreh G. Davies[1], Emmanuel O. Olorunsola[2], Nathaniel P. Udokang[1], Kubiat T. Umana[1], Mfonobong F. Alozie[3], Innocent A. Edagha[4]

[1]Department of Medical Physiology, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria;
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria;
[3]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria;
[4]Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria

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ABSTRACT

This study investigated the ameliorative effects of *Vernonia amygdalina* and *Moringa oleifera* on cognitive impairment in alloxan-induced diabetes mellitus in male Wistar rats. The animals were allotted into eight groups of five. Group 1 were not induced nor treated. Diabetes was induced with alloxan (135 mg/kg body weight) in groups 2-8. Group 2 which served as diabetic control received distilled water (10 mL/kg). Groups 3-8 were administered ethanol extracts of *V. amygdalina* (200 mg), *M. oleifera* (500 mg), *V. amygdalina* (400 mg), *M. oleifera* (1,500 mg), *V. amygdalina* (300 mg) + *M. oleifera* (1,000) mg, and Metformin (14.29 mg) per kg body weights respectively, for 28 days starting 72 h post induction of diabetes. Novel object recognition, T-maze simple alternation, transfer latency and neurohistology were assessed. Rats in diabetic control had negative discrimination ratio and scored less than 50% in simple alternation. These memory deficits were reversed in the treated groups. The nootropic effect was higher in *M. oleifera* 1,500 mg/kg than any other group. Severe neuronal degeneration, shrinkage and clumping observed in the diabetic group were ameliorated with administration of *V. amygdalina* and *M. oleifera* extracts individually and in combination. Histological findings showed decreased glial fibrillary acidic protein expression. *V. amygdalina* (400 mg/kg) and *M. oleifera* (500 mg/kg) were the most effective in ameliorating neuronal damage. The neuroprotective effects of both plants are attributed to their constituent antioxidants, and appear not to be synergistic.

Keywords: *Diabetes mellitus; Cognitive impairment; Neurodegeneration; Vernonia amygdalina; Moringa oleifera*

INTRODUCTION

Diabetes mellitus is a common metabolic disorder with chronic complications including a state of mild to moderate cognitive impairment, in particular psychomotor slowing and reduced mental flexibility, and shares many symptoms that are best described as accelerated brain ageing (Muriach et al. 2014). Complications of diabetes can dramatically impair the quality of life and cause long-lasting impairment. They may be influenced by non-modifiable factors such as age at onset of disease, type of diabetes, sex and genetic makeup. The most common cognitive deficits identified in patients with type 1 diabetes mellitus (T1DM) are slowing of information processing speed (Wessels et al. 2007) and worsening psychomotor efficiency (Weinger et al. 2008). Other reported impairments with diabetes mellitus include deficits in somatosensory examination and motor strength (Skenazy and Bigler 1984), motor speed, vocabulary, general intelligence, psychomotor speed, attention, memory and executive

Correspondence: Koofreh G. Davies, PhD; Department of Medical Physiology, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria. Email: koofrehdavies@uniuyo.edu.ng; Phone: +2348063408977; ORCID: 0000-0002-8223-4155

function (Wessels et al. 2007; Weinger et al. 2008). Functions such as psychomotor efficiency, motor speed, attention, verbal IQ scores, memory and academic achievement improve with better glycaemic control in patients with T1DM (Jacobson et al. 2007).

In Nigeria, most communities broadly use several herbal preparations from different plant parts (leaves, roots, barks and twigs) for the treatment of varieties of diseases including diabetes mellitus, breast cancer, hypertension, asthma, tuberculosis, ulcers, diarrhoea and dysentery (Li and Schellhorn 2007; Nwaehujor et al. 2013). Two of such plants reported with medicinal values are the bitter leaf (*Vernonia amygdalina*) and Moringa (*Moringa oleifera*). These two plants have been widely used in preventive and treatment in most West African countries.

Moringa oleifera is used in folk medicine to treat diabetes (Dieye et al. 2008; Nku-Ekpang et al. 2015; Frederick et al. 2020), as well as in the treatment of rheumatism, venom and microbial infections (Rajnish et al. 2012). It is a rich source of antioxidant phytochemicals such as phenolic acids and flavonoids which play important roles in the defence against free radicals (Xu et al. 2019; Olaoye et al. 2021). It is believed that the antidiabetic properties of *M. oleifera* may be due its terpenoid constituents. The terpenoids cause insulin release by inhibition of ATP-sensitive potassium channels just like the sulphonylureas (Idakwoji et al. 2015). According to the researchers, *M. oleifera* also mop up free radicals generated by alloxan leading to the regeneration of beta-cells, as well as showing a synergistic effect between the anti-diabetic activity of Metformin and ethanol extract of *M. oleifera*. The LD_{50} of ethanol extract of *M. oleifera* roots in mice was reported as 15.0-17.8 g/kg (Kasolo et al. 2011), while the leaves was 6,500 mg/kg (Awodele et al. 2012).

Vernonia amygdalina is commonly used in traditional medicine to treat fever, malaria, diarrhoea, dysentery, and hepatitis, as a laxative and as a fertility inducer (Ucheck Fomum 2004; Challand and Willcox, 2009). It has antidiabetic properties (Akah et al. 2004; Ong et al. 2011; Oshotse and Ifeanacho 2021), which normalized associated biochemical and haematological abnormalities (Zakaria et al. 2018). It acts by increasing peripheral glucose utilization just like Metformin (Zakaria et al. 2018). Many studies have reported the antioxidant effects of *V. amygdalina* (Iwalewa et al. 2005; Eyong et al. 2018), which is attributed to its flavonoids, luteolin, luteolin 7-0-glucosides and 7-0-glucuronide (Alara and Abdurahman, 2021). The LD_{50} of the leaves extract in rats was reported as over 6 g/kg (Eyong et al. 2018).

Previous works have established the antidiabetic, as well as the antioxidant properties of *V. amygdalina* and *M. oleifera* (Dieye et al. 2008; Oguwike et al. 2013). Some studies have also demonstrated the neuroprotective properties of these two plants in animal models of neurodegenerative diseases (Sutalangka et al. 2013; Ekong et al. 2017; Oladele et al. 2020). This study was to evaluate the effects of these two plants on cognitive impairments associated with alloxan-induced diabetes in Wistar rats. The study was limited to behavioural activities, and histological and immunohistochemical evaluation of the brain tissues.

MATERIALS AND METHODS

Collection and Identification of Plants

Vernonia amygdalina leaves were collected in a farm within the Uyo metropolis, while fresh leaves of *M. oleifera* were obtained from the botanical garden of the Faculty of Pharmacy, University of Uyo. The plants were identified and authenticated in the Department of Botany, University of Uyo. Samples were preserved and allocated voucher numbers UUPH/10(J) for *V. amygdalina* and UUPH/A/50(I) for *M. oleifera*.

The leaves of both plans were each air-dried, pulverized and then macerated in absolute ethanol for 72 h. Each was filtered with Whatman filter paper no. 1, dried in the water bath at 40℃ and the resultant extracts were preserved at 4℃.

Experimental Animals

Adult Wistar rats weighing 100-130 g were obtained from the Animal House of the Department of Pharmacology, University of Uyo, Nigeria. They were maintained under standard conditions (12-h light/dark cycle), and allowed to acclimatize for two weeks before the commencement of the experiment. All the study procedures were carried out following the Guide for the Care and Use of Laboratory Animals (National Research Council 2011). This research was approved by the Health Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Uyo, with protocol number FBMS/HREC/07/18/012.

Preparation of Alloxan and Induction of Diabetes

Diabetes was induced in the experimental animals using alloxan (Sigma-Aldrich, Germany). The alloxan (5 g) was dissolved in 100 mL of water, giving a stock concentration of 50 mg/mL. Initially, fifty-six male Wistar rats were divided into eight groups. Group 1 was maintained as the normal control, while groups 2-8 were administered intraperitoneally with alloxan at the dose of 135 mg/kg based on previous studies (Ighodaro et al. 2017; Ighodaro et al. 2018), after determining the basal glucose level of the animals.

Seventy-two hours after the administration of alloxan, blood glucose levels were measured again using a glucometer. Animals with glucose level above 150 mg/dL were selected as diabetic (Martín-Mateos et al. 2016). Forty Wistar rats divided into eight groups of five animals were then used for the study.

Experimental Design

The eight groups were handled as follows: Group 1 (negative control) no treatment given; Group 2 (diabetic control) induced, and administered distilled water subsequently; Group 3 (diabetic rats) treated with *V. amygdalina* (200 mg/kg); Group 4 (diabetic rats) treated with *M. oleifera* (500 mg/kg); Group 5 (diabetic rats) treated with *V. amygdalina* (400 mg/kg); Group 6 (diabetic rats) treated with *M. oleifera* (1,500 mg/kg); Group 7 (diabetic rats) treated with *M. oleifera* (1,000 mg/kg + *V. amygdalina* 300 mg/kg); Group 8 (diabetic rats) treated with Metformin 14.29 mg/kg (standard drug).

The extracts' doses were fixed such that the low and high doses were respectively, not below 3.3% or above 10% of the LD_{50} (Kasolo et al. 2011; Oguwike et al. 2012). The treatment was for 28 days by oral administration, and neurobehavioural assessments were carried out.

Neurobehavioural Assessments

Novel-Object Recognition (NOR) Test

The NOR test was performed in a wooden chamber (35.0 x 41.5 x 50.0 cm). Three sides of the chambers were fully protected, while one side was covered with a Plexiglas. Four days prior to the test, mice were placed in the box for 5 min every day to habituate. Test consisted of training and retention sessions. During a training session two objects (various objects differing in their shape and colour but similar in size) were placed in the box 35.5 cm apart (symmetrically), and each animal was allowed to explore the box for 5 min. The animals were considered to have explored the object when the head or any part of the body, except for the tail faced it within a distance of 2 cm. The time that mice spent exploring each object was recorded. After training, mice were immediately returned to their home cages, and the box and objects were cleaned with 75 % ethanol to eliminate odorant cues. Retention tests were carried out at one day intervals following the respective training. During the retention test, each mouse was placed back into the same box, in which one of the objects used during training was replaced by a novel one. The mice were then allowed to freely explore for 3 min, and the time spent exploring each object was recorded.

Throughout the tests, objects were used in a counter-balanced manner in terms of their physical complexity. A preference index, a ratio of the amount of time spent exploring any one of the two objects (training session) or the novel one (retention session) over the total time spent exploring both objects, was used to measure memory performance (Taglialatela et al. 2009; Cohen and Stackman 2014).

T-Maze Simple Alternation Test

The T-maze was shaped as 'T', with a base, the left arm and the right arm (length of 50 cm and 10 cm width). Animals were introduced from the base of the T-maze and allowed to choose one of the goal arms abutting the other end of the stem. The trial was carried out twice in quick succession. At the second trial, the rodent tended to choose the arm not visited before, reflecting a memory of the first choice, and known as 'spontaneous alternation'. Each trial was completed in less than 2 min (Dember and Richman 1989). The percentage of alternation (number of turns in each goal arm) and total trial duration was recorded.

Elevated Plus Maze (EPM) Test

This test was in an elevated-plus shaped apparatus with two open (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) arms extended from a central platform (5 × 5 cm), and the maze was elevated to a height of 25 cm from the floor. The behavioural model is based on the general aversion of rodents to open spaces. This aversion leads to the behaviour termed thigmotaxis, a preference for remaining in enclosed spaces or close to the edges of a bounded space. This assay essentially determines a preference between a comparatively safe and comfortable environment (the closed arms) and a risky environment (elevated open spaces). Memory was assessed by the transfer latency, the time it took the rats to move from the open to the enclosed arm (Lister 1990). On completion of the behavioural test, the rats were anaesthetized with ketamine (100 mg/kg, i.p), and euthanized as described by Hewett et al. (1993).

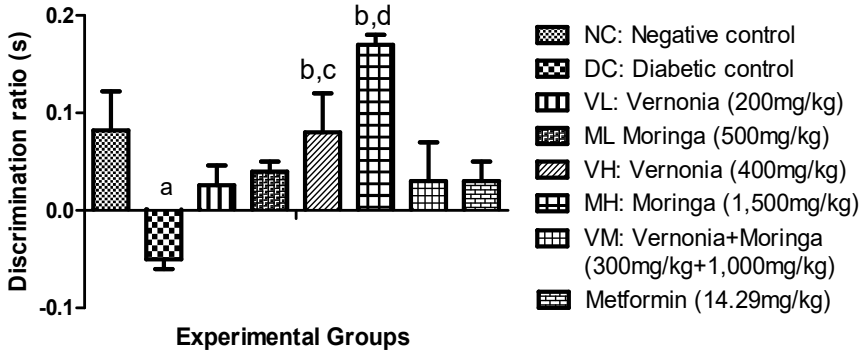

Fig. 1: Discrimination ratio of alloxan-induced diabetic rats treated with different doses of *V. amygdalina, M. oleifera* and Metformin. a= p<0.05 when compared with normal control; b=p<0.05 when compared with the diabetic control; c=p<0.05 when with the VL treated group; d=p<0.05 when compared with *M. oleifera* low dose treated group.

Tissue Processing for Microscopic Study
Whole brain perfusions were carried out using phosphate buffered saline for 2 min and thereafter with 4% paraformaldehyde until tail stiffness. The brains were then dissected out, and then processed for light microscopy within 72 h. The paraffin wax blocked tissues were sectioned at 5 µm, sampling 5 ribbons sections per tissue blocks using the rotary microtome (Thermo Scientific–Microm HM 325, England). Sections on slides were dewaxed in xylene and hydrated in decreasing alcohols, stained with haematoxylin and eosin (H&E) and mounted with (dibutylphthalate polystyrene xylene (DPX). Upon the examination of at least five fields, photomicrographs were made from sections using a light microscope (Olympus CX31) coupled to an AmScope® digital camera (MU 1000, China), and interpreted by at least three independent histopathologists.

Immunohistochemical (IHC) Analysis
Dewaxed tissue slides were placed in a Coplin jar containing a solution of sodium citrate (pH 6.0) before being transferred into the water bath at 95°C. Tissue sections were washed in wash buffer, blocked with peroxidase and incubated with diluted GFAP primary antibody (Dako, Lot 00083681 used at 1:100 dilution) and incubated for 1 h at room temperature. The secondary antibody; anti-GFAP immunoglobulin incubated the sections for 25 min at room temperature, while 3, 3'-diaminobenzidine (DAB) was applied, then counterstained with haematoxylin, washed in wash buffer, dehydrated, cleared and mounted on DPX. The immunoscoring system was performed as described by Adams et al. (1999) and was blindly assessed by three independent histopathologists. All photomicrographs were obtained as described previously.

GFAP Immunostaining Score
A semi-quantitative score was utilized as described by Adams et al. (1999). Each immunostain expressed in the photomicrograph was scored: (a) by percentage of labelled reactive astrocytes (0 = absence of labelling, 1 = less than 30% of astrocytes labelled, 2 = 30 to 60%, and 3 = more than 60%); and (b) and for the intensity of the immunostaining (0 = no staining; 1 = weak; 2 = mild; and 3 = strong staining). Both scores were summed to obtain the final, ranging from 0 - 6. To avoid bias, the evaluation was performed by at least two independent pathologists.

Statistical Analysis
Data were expressed as group mean ± standard error of mean (SEM). All data were analysed by one-way analysis of variance, followed by post hoc Dunnett's tests. $P < 0.05$ was considered significant. GraphPad Prism 5.04 software (GraphPad Software, Inc) was used for the data analysis and preparation of graphs.

RESULTS

Discrimination Ratio between Treatment Groups of Alloxan-Induced Diabetic Rats
The discrimination ratio between different treatment groups of alloxan-induced diabetic rats is shown in Figure 1. The discrimination ratio was significantly reduced in the diabetic control (-0.05 ± 0.01 s) when compared with the negative control (0.08 ± 0.04 s). The discrimination ratio was significantly increased ($p<0.05$) in the *V. amygdalina* + *M. oleifera* medium doses (0.03 ± 0.09 s), and *M. oleifera* high dose (0.17 ± 0.10 s) treated groups when compared with the diabetic control, while the discrimination ratio was significantly increased ($p<0.05$) in the *M. oleifera* high dose when compared with the *V. amygdalina* low dose (0.03 ± 0.02 s) treated group. The discrimination ratio was significantly increased ($p<0.05$) in the *M. oleifera* high dose treated groups when compared with the *M. oleifera* low dose (0.04 ± 0.02 s) treated group, while there was no significant difference in discriminatory ratio in the *V. amygdalina* high dose (0.08 ± 0.04 s) and Metformin (0.03 ± 0.02 s).

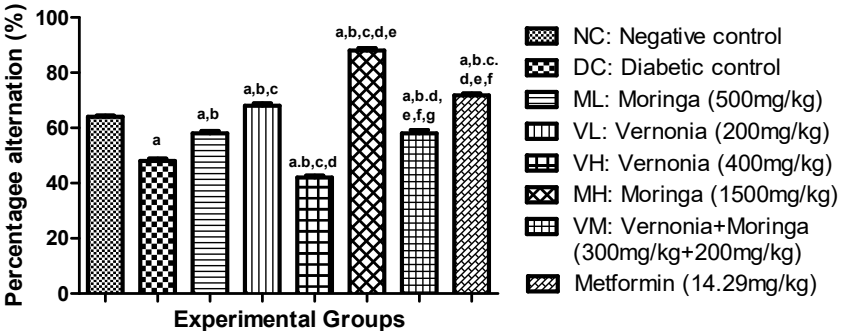

Fig. 2: Percentage alternation of alloxan-induced diabetic rats treated with different doses of *V. amygdalina, M. oleifera* and Metformin. a= $p<0.05$ when compared with normal control; b=$p<0.05$ when compared with the diabetic control; c=$p<0.05$ when with the VL treated group; d=$p<0.05$ when compared with ML treated group. e=$p<0.05$ when compared with MH treated group; f=$p<0.05$ when compared with MH treated group; g=$p<0.05$ when compared with VM treated group.

Percentage Alternation between Treatment Groups of Alloxan-Induced Diabetic Rats
The percentage alternation of different treatment groups of alloxan-induced diabetic rats is shown in Figure 2. The percentage alternation were significantly decreased ($p<0.05$) in the diabetic control (48 ± 0.83%), *M. oleifera* low dose (58 ± 0.84%), *V. amygdalina* high dose (42 ± 0.71%), and *V. amygdalina* + *M. oleifera* medium dose (58 ±

1.23%) treated groups when compared with the negative control group (64 ± 0.44%). The percentage alternation were significantly increased ($p<0.05$) in the *V. amygdalina* low dose (68 ± 0.84%), *M. oleifera* high dose (88 ± 0.84%) and Metformin (72 ± 0.66%) treated groups when also compared to the negative control group. The percentage alternation were significantly increased ($p<0.05$) in the *M. oleifera* low dose, *V. amygdalina* low dose, *M. oleifera* high dose and Metformin treated group but significantly decreased ($p<0.05$) in the *V. amygdalina* high dose treated groups when compared with diabetic control group.

Transfer Latency (EPM) for the Initial and Final Tests

The transfer latency for the initial and final tests is shown in Table 1. The final transfer latencies were significantly decreased ($p<0.05$) when compared with the initial latencies in the diabetic control, *V. amygdalina* high dose, *M. oleifera* high dose, *M. oleifera* + *V. amygdalina* medium doses and Metformin treated groups.

Table 1: Transfer latency of alloxan-induced diabetic rats treated with *V. amygdalina*, *M. oleifera* and Metformin

Groups	Initial (s)	Final (s)
NC	1.30 ± 0.06	1.27 ± 0.06^{NS}
DC	1.32 ± 0.08	1.10 ± 0.05*
VL	0.37 ± 0.01	0.33 ± 0.06^{NS}
ML	0.22 ± 0.02	0.19 ± 0.06^{NS}
VH	0.69 ± 0.03	0.40 ± 0.02***
MH	0.42 ± 0.01	0.10 ± 0.02***
VM	0.47± 0.01	0.38 ± 0.01***
MET	1.15 ± 0.05	0.66 ± 0.02***

***=p<0.001; *=p<0.05; NS= Not significant compared to initial transfer latency

Cytoarchitecture of the Hippocampus

The section of the CA 1 region of the hippocampus of the negative control (Fig. 3A) showed intact molecular, pyramidal and granular layer respectively. In contrast, similar section in the diabetic control (Fig. 3B) showed severe neuronal shrinkage and degeneration. The section of the hippocampus of diabetic rats administered with low dose (200 mg/kg) of *V. amygdalina* (Fig. 3C) also demonstrated severe neuronal shrinkage in comparison to the control group. In comparison, the section of the hippocampus of diabetic rats administered with low dose (500 mg/kg) of *M. oleifera* (Fig. 3D) presented better hippocampal cytoarchitecture compared to the diabetic control (Fig. 3B and the negative control).

The hippocampus of the groups administered high dose extracts (400 mg/kg of *V. amygdalina* and 1,500 mg/kg of *M. oleifera*), showed moderate neuronal shrinkage (Fig 3E and 3F) when compared to the diabetic control group. The section of the hippocampus of diabetic rats co-administered with *M. oleifera* (1,000 mg/kg) and *V. amygdalina* (300 mg/kg) showed mildly affected hippocampus (Fig. 3G) compared to the diabetic control group. In contrast, the diabetic group administered with Metformin 14.29 mg/kg demonstrated severely affected cytoarchitecture characterized by neuronal shrinkage (Fig. 3H).

Immunohistochemistry of Glial Fibrillary Acidic Protein (GFAP)

Immunohistochemically, GFAP anti-body demonstrated strong astrocyte reactivity in the hippocampal sections from extracts-administered groups 4-7 (Fig. 4D-G) with lesser intensity when compared with diabetic control (Fig. 4B), indicative of moderate astrogliosis which was different from the normal control (Fig 4A), low dose *V. amygdalina* at 200 mg/kg (Fig. 4C) and Metformin-treated group (Fig. 4H).

DISCUSSION

The role of diabetes in memory impairment has been highlighted in some studies (Ryan et al. 2003; Brands et al. 2006; Wessels et al. 2007; Muriach et al. 2014). Diabetes is a common metabolic disorder associated with chronic complications, including a state of cognitive impairment. Alloxan-induced diabetes is a common animal model for evaluating the anti-diabetic potential of many compounds and plant extracts (Ighodaro et al. 2018). Alloxan acts by selective inhibition of glucose-stimulated insulin release and by formation of reactive oxygen species which lead to necrosis of beta cells of the islet of Langerhans, thus producing hypo-insulinaemia and hyperglycaemia and other metabolic abnormalities associated with type 1 diabetes mellitus (Szkudelski 2001; Ighodaro et al. 2018).

The novel objects recognition test was to evaluate the ability of the animal to recognize a novel object. This test evaluates short-term, intermediate and long-term memories through the manipulation of the retention intervals. That is, the amount of time the animal must retain memory of the objects presented during the familiarization phase, before the test phase, when one of the familiar objects was replaced by a novel one (Baxter 2010). The main advantage of this test over others that evaluate memory is the reliance on rodents' natural tendency for exploring novelty, and therefore, may not require many training sessions or reinforcement to motivate behaviour. The NOR test is less stressful, requires less time to run, and the conditions closely resembles those used in studying human cognition. In this test, memory is usually assessed by the discrimination ratio. A

positive discrimination ratio indicates good recognition memory, while negative suggests memory impairment (Taglialatela et al. 2009; Antunes and Biala 2012; Cohen and Stackman 2014). In the present study, the discrimination ratio was negative in the diabetic control but was positive in all other groups. One of the most well-established tests of spatial working memory is the spontaneous alternation. Spontaneous alternation is based on the natural tendency of rodents to explore a novel arm over a familiar one, making them alternate their choice of the goal arm. This test has a high ecological validity since it relies on natural tendency of the animals to explore and not on experimenter-imposed sources of motivation. The index of memory evaluation is percentage alternation (Dember and Richman 1989). In this study, percentage alternation was higher than 50% in all the groups except diabetic control and *V. amygdalina* (400 mg/kg). Score of 50% or less is indicative of memory impairment.

The diabetic control group had a negative discrimination ratio and decreased percentage alternation. This poor performance in the memory tasks is indicative of impairment in recognition and spatial memory. This memory impairment could be as a result of neuronal insult triggered by alloxan-induced hyperglycaemia and associated metabolic changes. Our finding is in line with some earlier studies which reported impaired short-term memory in alloxan-induced diabetes (Akinola et al. 2012; Balogun et al. 2015). Our finding, on the other hand, contradicted an earlier report by Ceretta et al. (2012), who showed that alloxan-induced diabetes did not cause alteration in animal's recognition memory. The reason for this may be due to differences in the methodology. This is also true of Balogun et al. (2015), which also reported memory impairment. Concerning the duration of the test session, some researchers are of the view that 5 min is rather long as the animals may familiarize with the novel object and stops exploring. Furthermore, it has been shown that animals explore intensely within the first 3 min (Taglialatela et al. 2009; Cohen and Stackman 2014). These factors may have contributed to our unravelling a borderline deficit that otherwise would have gone undetected.

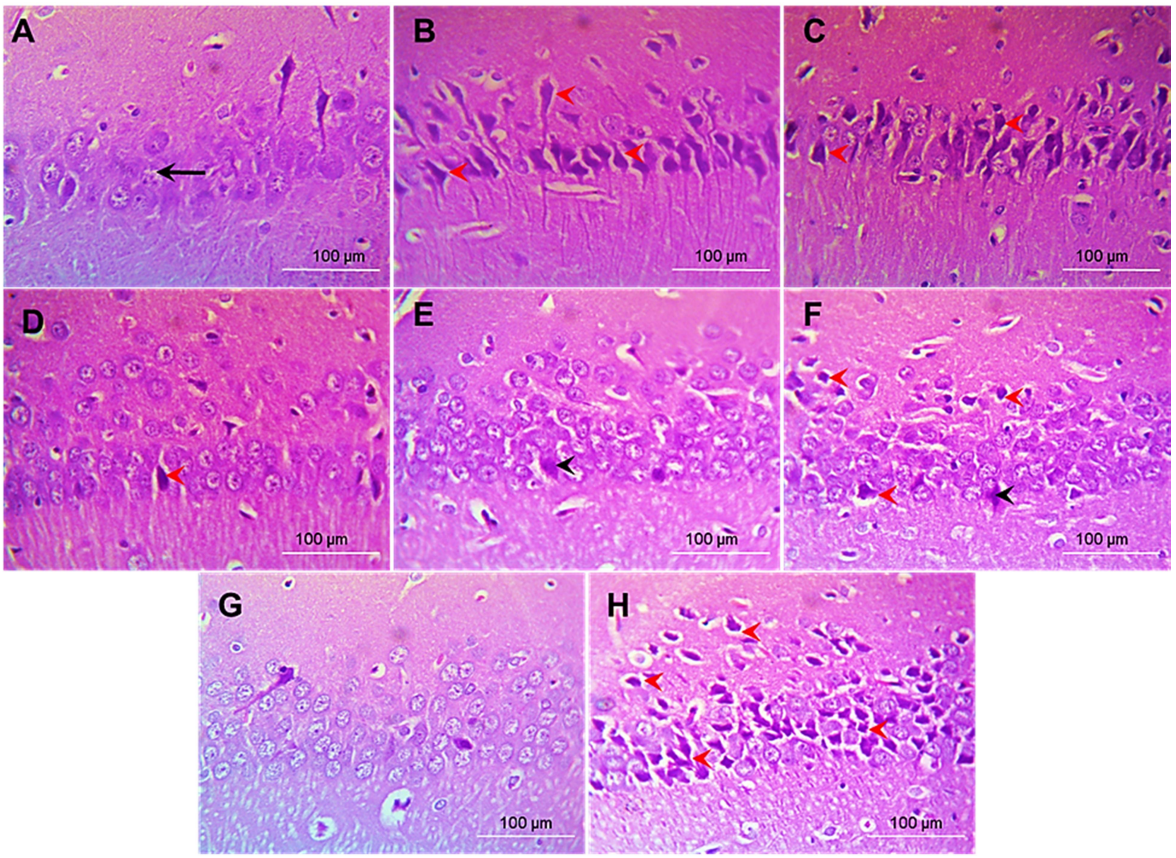

Fig. 3: Representative sections of the CA1 region of hippocampus. H&E stain; ×400 magnification; 100 µm scale bar; normal pyramidal neurons (black arrow), with shrunken and atrophic pyramidal neurons (red arrowhead). A - negative control, B - diabetic control, C - diabetic rats treated with *V. amygdalina* (200 mg/kg), D - diabetic rats treated with *M. oleifera* (500 mg/kg), E - diabetic rats treated with *V. amygdalina* (400 mg/kg), F - diabetic rats treated with *M. oleifera* (1,500 mg/kg), G - diabetic rats treated with *M. oleifera* (1,000 mg/kg + *V. amygdalina* 300 mg/kg), H - diabetic rats treated with Metformin (14.29 mg/kg)

There are many reports that diabetes mellitus patients have impaired memory and cognitive deficit (Ryan et al. 2003; Brands et al. 2006; Wessels et al. 2007). Apart from hyperglycaemia-induced neuronal damage, cognitive impairment may also be due to hypoinsulinaemia. Recently, studies have demonstrated the role of insulin and insulin receptors in synaptic transmission, cognition and memory. Even insulin spray has been shown to enhance memory in humans and animals (Duarte et al. 2012). The discrimination ratio was positive in all the treatment groups, and percentage alternation was higher in all the treated groups than the diabetic group except for the group treated with 400 mg/kg of *V. amygdalina*. *M. oleifera* (1,500 mg/kg) extract produced the best improvement in cognitive impairment based on the discrimination ratio and percentage alternation. The effect was much higher than that of the combined extracts. Normal or improved performance in memory tasks is an indication that an administered treatment was able to prevent or ameliorate cognitive deficit, caused by alloxan-induced diabetes.

The histology results showed several structural changes which are indicative of neuronal damage of varying degrees. Neuronal damage is most likely resulting from hyperglycaemia induced by alloxan. It has been reported that high glucose concentration in patients with diabetes affect neurons through osmotic insults, oxidative stress and continued chronic hyperglycaemia, which leads to the formation of advanced glycation end products (AGE). The AGE coupled with free radicals can cause oxidative damage, which can, in turn, lead to neuronal injury (Umegaki 2013; Frederick et al. 2020). From the histology results, it is evident that interventions with *M. oleifera* (500 mg/kg), *V. amygdalina* (400 mg/kg) and combination of *M. oleifera* and *V. amygdalina* could prevent this damage. The *V. amygdalina* (200 mg/kg), *M. oleifera* (1,500 mg/kg) and Metformin treatment showed little protective effects.

Since the integrity of the neuronal tissue was better preserved by *V. amygdalina* (400 mg/kg), the inability of the *V. amygdalina* (200 mg/kg) to confer an equal protection may be due to particular plant constituents not being adequate enough, to elicit the effect.

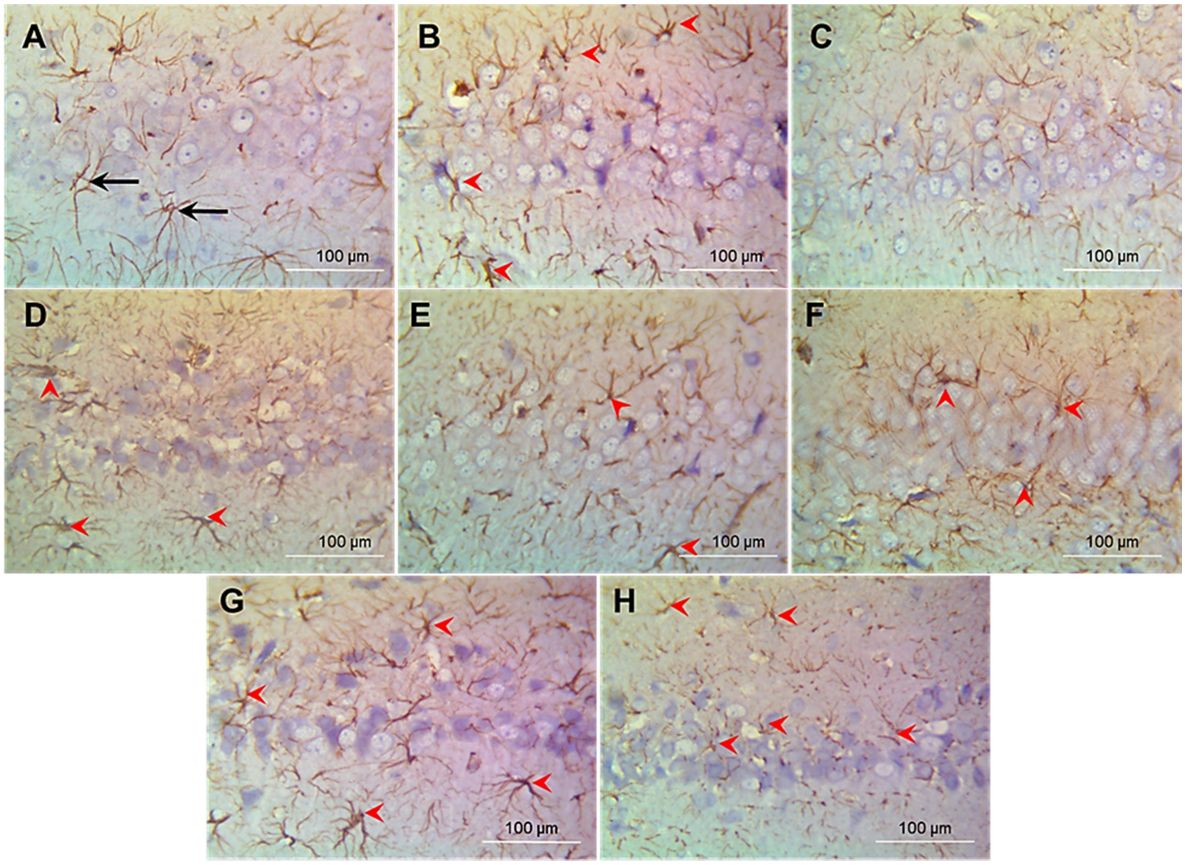

Fig. 4: Representative sections of GFAP expressed CA1 region of hippocampus. ×400 magnification; 100 µm scale bar; normal astrocyte expression of GFAP (black arrow), and reactive astrogliosis (red arrow head). A - negative control, B - diabetic control, C - diabetic rats treated with *V. amygdalina* (200 mg/kg), D - diabetic rats treated with *M. oleifera* (500 mg/kg), E - diabetic rats treated with *V. amygdalina* (400 mg/kg), F - diabetic rats treated with *M. oleifera* (1,500 mg/kg), G - diabetic rats treated with *M. oleifera* (1,000 mg/kg + *V. amygdalina* 300 mg/kg), H - diabetic rats treated with Metformin (14.29 mg/kg)

Unlike the lower dose levels (500 mg/kg), *M. oleifera* at 1,500 mg/kg did not offer protection against tissue damage. It is difficult to understand why rats treated with *M. oleifera* at 1,500 mg/kg showed little or no tissue protection even though the animals in this group exhibited the highest performance in the memory task discussed above. This finding needs to be further investigated. Curiously, in one of our previous studies, *M. oleifera* at the dose of 1,500 mg/kg caused a negative discrimination index in the novel object recognition test (Davies et al. 2020). The combination of *M. oleifera* and *V. amygdalina* did not show any difference from that of *M. oleifera* administered alone. It, therefore, suggests that the actions of both plant extracts may not be synergistic.

Since Metformin, a standard anti-diabetic drug employed in this study could not to protect against neuronal damage sufficiently similar to a precious report (Ekong et al. 2022), it could be adduced that *M. oleifera* and *V. amygdalina* confer tissue protection by mechanism other than normalizing blood sugar. *M. oleifera* and *V. amygdalina* are rich sources of phenols, vitamins C and E which are well known antioxidants (Ganguly et al. 2005; Olaoye et al. 2021). This neuroprotective effect may be attributed to these antioxidants (Ekong et al. 2017; Oladele et al. 2020; Alara and Abdurahman 2021).

Table 2: Effect of *V. amygdalina*, *M. oleifera* and Metformin on Hippocampal GFAP Expression of Alloxan-Induced Diabetic Rats

Group	% of IHC (A)	Intensity of IHC (B)	Final score (A+B)	GFAP Antibody Expression
NC	< 30% (1)	Mild (2)	3	Low
DC	> 60% (3)	Strong (3)	6	High
VL	< 30% (1)	Mild (2)	3	Low
ML	> 60% (3)	Strong (3)	6	High
VH	> 60% (3)	Strong (3)	6	High
MH	> 60% (3)	Strong (3)	6	High
VM	> 60% (3)	Strong (3)	6	High
M	> 60% (3)	Strong (3)	6	High

% IHC: 0 = 0 %; 1 = < 30 %; 3 = > 60 %; Intensity of IHC: 0 = No reaction; 1 = Weak; 2 = Mild; 3 = Strong; Final Score: Range = 0 to 6; 0/6 = Negative Reaction; 1/6, to 3/6 = Low expression; 4/6, to 6/6 = High expression

Astrocytes are the major glial cell population within the central nervous system that play significant physiological role in brain function. They react to diverse neurodegenerative insults rapidly, leading to strong astrogliosis (Reier 1986; Eng et al. 1992). The reaction produces more GFAP, leading to intense astrogliosis (Sriram et al. 2004). The results of immunoreactivity showed moderate astrogliosis in all the groups except those treated with *V. amygdalina* (200 mg/kg) and Metformin. Astrogliosis may indicate an ongoing neuronal damage or recovery process. Almost all the groups with moderate astrogliosis had near normal histological appearance.

Conclusion

Ethanol extract of *V. amygdalina* and *M. oleifera* could prevent or reverse the tissue damage and memory impairment caused by alloxan-induced diabetes in male Wistar rats. The ameliorative effect of *M. oleifera* was more pronounced than that of *V. amygdalina*. The standard drug Metformin did confer much tissue protection. The neuroprotective effects of these plant extracts may be attributed to their constituent antioxidant phytochemicals.

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Conflict of Interest

None declared.

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Authors Contribution

KGD and IAE - Conception and design of the research; NPU - Experimentation and data collection; NPU, EOO and MFA - Data analysis and interpretation; NPU, KTU and IAE - Drafting of the manuscript; KGD and EOO - Critical revision of the manuscript.

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