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ORIGINAL PAPER

Neuroprotective effects of *Vernonia amygdalina* and *Moringa oleifera* in alloxan-induced diabetic Wistar rats

Chidimma Amarachi Acharaike [1,2], Uzozie Chikere Ofoego [1], Damian Nnabuihe Ezejindu [1], Paul Matthew Demshimeno [3], Makuachukwu Francisca Obinwa [4], Benedict Nzube Obinwa [1]

[1] Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria
[2] Department of Physical and Health Education, School of Sciences, Federal College of Education Pankshin, Plateau State, Nigeria
[3] Department of Nursing Science, School of Basic Medical Sciences, American University of Nigeria, Yola, Adamawa State, Nigeria
[4] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria

ABSTRACT

Introduction and aim. Diabetes mellitus is a metabolic disorder that affects multiple organs, including the hippocampus, a key region involved in memory. This study aimed to investigate the neuroprotective and antidiabetic effects of *Vernonia amygdalina* and *Moringa oleifera* in an alloxan-induced diabetic rat model.

Material and methods. Thirty-five adult Wistar rats were randomized into seven groups and treated with aqueous extracts of *V. amygdalina*, *M. oleifera*, their combination, or glibenclamide for 30 days following alloxan-induced diabetes. Fasting blood glucose (FBG), hippocampal acetylcholinesterase (AChE) activity, cognitive performance (Morris Water Maze test) and histopathological changes in the hippocampus were evaluated.

Results. Alloxan significantly increased FBG (20.68±1.04 mmol/L), AChE activity (40.40±0.40 nmol/mL), and escape latency (51.75±4.39 sec), and reduced hippocampal cell density. Treatment with *V. amygdalina* and *M. oleifera* reduced FBG (8.29±0.93 mmol/L), AChE activity (34.50±0.30 nmol/mL), and escape latency (3.39±0.45 sec), and improved hippocampal histoarchitecture.

Conclusion. *V. amygdalina* and *M. oleifera* demonstrated neuroprotective and antidiabetic effects in diabetic rats. These results support their potential as adjunct agents to prevent diabetes-induced cognitive dysfunction.

Keywords. acetylcholinesterase, diabetes mellitus, hippocampus, *Moringa oleifera, Vernonia amygdalina*

Introduction

Diabetes mellitus is a significant global health concern, with its complications significantly impairing the health of affected individuals, making it a worldwide concern.[1,2] It is a metabolic disorder caused by both aberrant insulin secretion and disrupted insulin action.[3,4] The hormone insulin, which is released by the islets of Langerhans' pancreatic β-cells, enables cells to absorb

Corresponding author: Benedict Nzube Obinwa, e-mail: bn.obinwa@unizik.edu.ng

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glucose and utilize it as fuel; however, excess glucose in the blood stream leads to hyperglycemia which in turn impairs health.[5] Although diabetes mellitus exists in several forms, type 2 diabetes mellitus (T2DM) is the most common among middle-aged and older adults. Early-onset T2DM is linked with extended periods of hyperglycemia, rapid disease progression, significant insulin resistance, and declining beta-cell function, all of which impair glycemic control and heighten the likelihood of complications.[6]

Interestingly, T2DM is increasing exponentially worldwide, largely due to physical inactivity, sedentary lifestyles, and poor dietary habits.[7] The International Diabetes Federation estimates indicate the number of people aged 20-79 living with diabetes will surge from over 530 million in 2021 to more than 780 million by 2045.[8] It has also been estimated that a person with diabetes mellitus has higher chances (1.5 times more prone) to experience cognitive dysfunction than a normal healthy person.[9] In Nigeria, a study carried out in Kano and other states estimated that T2DM caused impaired cognitive function.[10-13] In another study, it was estimated that 40% of the T2DM patients had cognitive impairment.[14]

Cognition is closely linked to the brain, which comprises various structures, including the hippocampus, which is widely recognized for its critical role in memory and learning. It functions as a memory control center, directing the storage and retrieval of memories in appropriate brain regions for long-term use.[15,16] However, the hippocampus is particularly vulnerable to metabolic diseases like diabetes mellitus because of its complex anatomical makeup.[17,18] Hyperglycemia is the metabolic syndrome component most strongly linked to cognitive impairment, a growing comorbidity of diabetes mellitus.[19,20] This impairment is caused by injury to the hippocampus as well as shrinkage and disruption of cholinergic transmission brought about by the key factors of diabetes mellitus: chronic hyperglycemia, and oxidative stress.[21] Extreme hyperglycemia and oxidative stress modify the hippocampus's neuronal membrane and structure, as well as its capacity to receive neural impulses, which alters the signal pathways needed for memory.[22,23]

The search for remedies for hippocampal degeneration and memory impairment has gained prominence in recent years. In order to manage diabetes mellitus and prevent these associated complications, tight glycemic control must be achieved and maintained.[24] For this reason, World Health Organization (WHO) suggested the creation of oral hypoglycemic medicines from medicinal plants as a form of herbal treatment for diabetes mellitus.[25] *Moringa oleifera* (Lam) and *Vernonia amygdalina* are two examples of medicinal plants that have been used for centuries because of their therapeutic and dietary value in the management of diabetes mellitus.[26] *M. oleifera* belongs to the Moringaceae family, found abundantly in the tropics and widely used in folklore and traditional medicine. Because of the numerous health benefits of the plant's various parts, hence its reputation as the miracle tree.[27] According to reports, the leaves of this plant contain high levels of micro-nutrients such as potassium, iron, vitamins, and vital amino acids as well as numerous phytochemicals including flavonoids, phenols, alkaloids, nitrile glycoside, and tannins.[28-31]

Likewise, *V. amygdalina* commonly called bitter leaf is native to tropical regions. The Asteraceae family including *V. amygdalina* are best identified due to their bitter leaves caused by the availability of anti-nutritional elements such as alkaloids, saponins, glycosides, and tannins.[32] They are also packed with essential micronutrients, including vitamins and minerals, and a good source of dietary fiber, rendering them vital for human nutrition.[33] The plant is consumed locally as food and is also used for medical purposes such as suppression of cancer, neuroprotective effects, hepato-protective effects, and other several purposes.[34-36]

Aim

This study focuses on investigating the protective effect of *V. amygdalina* and *M. oleifera* against diabetes-induced neurodegeneration and acetylcholinesterase (AChE) activity in the hippocampus and cognitive decline in adult Wistar Rats.

Material and methods

Study location

This study was conducted in the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus.

Collection, identification and preparation of plant materials

M. oleifera and *V. amygdalina* were procured from Nkwo Market, a well-known market in Nnewi town, Anambra State. Identification and authentication of the specimens were conducted at the Department of Botany, Nnamdi Azikiwe University, with the herbarium numbers NAUH-17B and NAUH-20B respectively. The leaves were washed thoroughly to remove dust particles. They were air-dried and pulverized. The aqueous extracts of both *M. oleifera* and *V. amygdalina* were separately prepared according to the method described by Thilza et al.[37] The extracts were freeze-stored at 4°C in a refrigerator (Model: HTF-319, China) until use. The extracts were made into varying doses on each administration day and administered using orogastric tube according to the treatment group doses and animal's body weight.

Procurement and handling of experimental animals

Thirty-five adult Wistar rats, with weights ranging from 130g to 170g were sourced and housed at the College of

Health Sciences Animal House, Nnamdi Azikiwe University, for two weeks to acclimatize according to standard ethical procedure (NAU/CHS/NC/FBMS/472). The animals had access to rat chow and water ad libitum throughout the duration of the experiment. The animal welfare and health status were monitored throughout the experiment, following the guidelines set by the Federation of European Laboratory Animal Science Associations.[38]

Induction of diabetes

Alloxan diabetes induction was done as described by Ibrahim et al.[39] All rats, except those in the control group (Group A), received intraperitoneal injections of alloxan monohydrate at a dose of 150 mg/kg body weight. Alloxan was prepared by dissolving alloxan monohydrate in sterile normal saline solutions. To prevent alloxan-induced hypoglycemia, rats were provided with a 10% glucose solution for 24 hours after alloxan injection.[40,41] Diabetes was confirmed 72 hours post-alloxan administration by assessing fasting blood glucose levels in overnight-fasted rats using a glucometer (Accu-Chek Active Performa, Roche, Germany). Rats were classified diabetic if their blood glucose levels, measured from tail punctures, were >200 mg/dL (11 mmol/L); levels were checked daily for 5 consecutive days. Treatment began 5 days after alloxan injection and lasted for 30 days. Alloxan was used at a moderate dose to induce partial β-cell dysfunction, replicating the impaired insulin production characteristic of late-stage T2DM. This model reflects the key features of T2DM, including insulin resistance and β-cell exhaustion.

Experimental design

A total of 35 adult Wistar rats were randomly allocated into 7 groups (A-G) of 5 rats each.

Group A: each control rat received only water and rat chow

Group B: each diabetic control rat received only water and rat chow

Group C: alloxan-induced diabetic rats were administered 500 mg/kg body weight aqueous leaf extract of *V. amygdalina* and *M. oleifera* once daily.

Group D: alloxan-induced diabetic rats were administered 700 mg/kg body weight aqueous leaf extract of *V. amygdalina* and *M. oleifera* once daily.

Group E: alloxan-induced diabetic rats were treated once daily with the standard antidiabetic drug glibenclamide (5 mg/kg body weight).[42]

Group F: alloxan-induced diabetic rats were administered 350 mg/kg body weight aqueous leaf extract of *V. amygdalina* once daily.

Group G: alloxan-induced diabetic rats were administered 350 mg/kg body weight aqueous leaf extract of *M. oleifera* once daily.

Determination of fasting blood sugar (FBS)

FBS was determined four times during the course of the study. The blood glucose level was determined after induction and also on the 7th day, 15th day and 30th day. This was measured by pricking the tail with a lancet after cleaning with 10% alcohol, applying the blood to a test strip in a glucometer (Accu-Chek Active Performa), and obtaining a direct reading in mmol/L.[43]

Determination of spatial memory with Morris Water Maze test

Spatial memory was assessed via the Morris Water Maze test according to Stewart and Morris.[44] The Morris water maze setup included a 1m diameter pool with 30 cm deep water, divided into four quadrants (NE, SE, SW, NW). A submerged platform was placed in one quadrant, with external visual cues guiding the rats. Rats underwent 24-hour training, where they were placed in different quadrants to find the hidden platform within 60 seconds, with 15-minute intervals. Training continued until they could find the platform in under 15 seconds. During the test, the water was made opaque, the platform taken out, and the rats placed in different quadrants. The duration for rats to locate the platform's previous position was measured as escape latency.

Termination of experiment

After the experiment, the Wistar rats were fasted for 24 hours, then euthanized by decapitation following chloroform anesthesia, and their brains harvested. One cerebral hemisphere was preserved in 10% formal saline for histopathological analysis while the other hemispheres were kept in the refrigerator until ready for biochemical analysis.

Brain homogenate preparation

The cerebral hemispheres for biochemical analysis were homogenized with 10 mL of ice-cold 0.01 M phosphate buffer at pH 7.4. The homogenate was centrifuged at 7,000 rpm for 15 minutes in the cold medium and the supernatant obtained was stored at 4°C for biochemical evaluation.

Determination of brain AChE activity

The AChE assay was performed using a modified protocol, with a reaction mixture containing brain homogenate, phosphate buffer (0.01M, pH 7.4), and 100 µl of 5,5-dithiobis-2-nitrobenzoic acid.[45] A baseline reading was taken before adding acetylthiocholine to start the reaction, and absorbance changes were monitored at 412 nm at 2-minute intervals for 10 minutes.

Histopathological analysis

The cerebral hemispheres were immersed in 10% formal saline for fixation at room temperature for 24 hours.

The hippocampus was excised from each for histological assessment using hematoxylin and eosin (H&E) and done by a blind observer. Tissues were dehydrated in alcohol (70%, 80%, 90%, and absolute) for one hour each and cleared in xylene (2 changes; 30 minutes each). The cleared tissues were impregnated in molten paraffin wax, allowed to cool and a paraffin block formed and ready for sectioning using rotary microtome. Tissues were mounted on glass slides aided with albumin of egg and the section (5 µm) immersed in water bath at 50–55°C temperature to remove wrinkles. Water was drained off the slides and placed inside the incubator fix sections on the slide and dry. Tissues were then stained with hematoxylin and eosin technique, viewed under a compound light microscope (Zeedo microscope) and photomicrographs taken from each group and labelled using Photoscape v3.7.

Measurement of the neuron density in the CA1 area

The neuron numbers of the hippocampus in the CA1 area were counted under a compound microscope (Zeedo microscope) at 400× magnification. An accompanying software of the microscope, USB 2.0 camera viewer was used in the counting process to determine the thickness in the CA1 area.

Statistical analysis

IBM Statistical Package for Social Sciences version 23 for Windows (IBM Incorporation, Armonk, New York, USA) was used for data analysis, and the results expressed as mean±SEM. One-way analysis of variance (ANOVA) followed by post Hoc LSD was applied for determining the significance. Level of significance was established at p≤0.05.

Results

Effect of aqueous leaf extracts of M. oleifera and V. amygdalina on FBG

The FBG level was evaluated in the treatment groups after the administration of *M. oleifera* and *V. amygdalina*. The results in table 1 showed a significant elevation (p<0.05) in blood glucose level at day 1 in groups B, C, D, E, F, and G compared to A, which is indicative of the successful induction of diabetes mellitus by the alloxan. This is consistent across days 7, 15, and 30, which is indicative of sustained diabetes mellitus in the Wistar rats. However, treatment of the Wistar rats with *M. oleifera* and *V. amygdalina* resulted in a significant reduction (p<0.05) in FBG level across the groups when compared to the diabetic control (group B). This reduction in the FBG level of *M. oleifera* and *V. amygdalina* treated groups are comparable to the glibenclamide treated group (group E), suggesting hypoglycemic effects of the extracts.

Table 1. Effect of aqueous leaf extracts of *M. oleifera* and *V. amygdalina* on FBG level at day 1, 7, 15 and 30 in diabetic adult Wistar rats*

	FBG day 1 (mmol/L)	FBG day 7 (mmol/L)	FBG day 15 (mmol)	FBG day 30 (mmol/L)
	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
Group A (control)	4.60±0.29 [b]	4.50±0.31 [b]	4.42±0.22 [b]	4.82±0.322 [b]
Group B (diabetic control)	20.68±1.04 [a]	21.07±0.85 [a]	23.40±2.06 [a]	25.50±1.16 [a]
Group C (DM+500 mg/kg of *M. oleifera*+*V. amygdalina*)	22.45±2.28 [a,b]	23.18±2.08 [a,b]	19.15±0.43 [a,b]	12.60±3.98
Group D (DM+700 mg/kg of *M. oleifera*+*V. amygdalina*)	20.30±0.67 [a,b]	21.02±0.85 [a,b]	17.68±1.82 [a,b]	8.29±0.93 [a,b]
Group E (5 mg/kg of glibenclamide)	20.47±1.20 [a,b]	20.30±1.28 [a,b]	12.73±2.59 [a,b]	7.57±1.22 [a,b]
Group F (350 mg/kg of *V. amygdalina*)	18.97±0.65 [a,b]	38.15±18.88	19.40±0.45 [a,b]	9.23±1.08 [a,b]
Group G (350 mg/kg of *M. oleifera*)	20.17±1.43 [a,b]	21.18±1.16 [a,b]	19.95±0.60 [a,b]	10.44±0.08 [a,b]

* data was analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at considered significant at p≤0.05, SEM – standard error of mean. [a] – p≤0.05 compared with control (group A), [b] – p≤0.05 compared with diabetic control (group B)

Effect of aqueous leaf extracts of M. oleifera and V. amygdalina on cognitive function

An evaluation of cognitive status in the diabetic Wistar rats after treatment with *M. oleifera* and *V. amygdalina* using Morris Water Maze test is shown in Table 2. Induction of diabetes mellitus significantly increased the time spent before finding the escape platform in MWMT across all the treatment groups suggesting cognitive decline. Treatment with *M. oleifera* and *V. amygdalina* significantly decreased (p<0.05) the time spent in finding the platform in all the treatment groups suggesting the anti-diabetic and nootropic effects of the extracts.

Table 2. Effect of aqueous leaf extracts of *M. oleifera* and *V. amygdalina* on Morris Water Maze test in diabetic adult Wistar rats[a]

	Escape latency (secs) initial	Escape latency (secs) day 1	Escape latency (secs) day 30
	Mean±SEM	Mean±SEM	Mean±SEM
Group A (control)	1.91±0.40	4.81±0.32*	2.65±0.67*
Group B (diabetic control)	1.77±0.52	51.75±4.39*	17.33±4.39*
Group C (DM+500 mg/kg of *M. oleifera*+*V. amygdalina*)	1.84±0.33	24.40±9.23	7.13±2.49
Group D (DM+700 mg/kg of *M. oleifera*+*V. amygdalina*)	1.59±0.32	9.23±1.08*	3.39±0.45*
Group E (5 mg/kg of glibenclamide)	1.76±0.15	7.58±1.23*	2.93±0.47*
Group F (350 mg/kg of *V. amygdalina*)	1.58±0.24	8.32±0.91*	4.43±0.59*
Group G (350 mg/kg of *M. oleifera*)	1.53±0.15	9.92±01.01*	5.37±0.38*

[a] data were analyzed using t-test, and values considered significant at p≤0.05, SEM – standard error of mean, * – p≤0.05 indicates statistical significance

Effect of aqueous leaf extracts of M. oleifera and V. amygdalina on AChE activity

The Ache activity in the hippocampus was evaluated after treatment with *M. oleifera* and *V. amygdalina*. As shown in Table 3, the Ache activity was significantly lower in group A compared to B (p=0.03). Groups C, D, E, F, and, G had significantly lower AChE activity compared to group B. Also, AChE showed a significantly higher activity levels in groups B, C, D, E, F, and, G compared to A.

Table 3. Effect of aqueous leaf extracts of *M. oleifera* and *V. amygdalina* on AChE (Ache) activity in diabetic adult Wistar rats

	AChE (nmol/mL) Mean±SEM
Group A (control)	21.25±0.85 [b]
Group B (diabetic control)	40.40±0.40 [a]
Group C (DM+500 mg/kg of *M. oleifera*+*V. amygdalina*)	37.35±0.95 [a, b]
Group D (DM+700 mg/kg of *M. oleifera*+*V. amygdalina*)	34.50±0.30 [a, b]
Group E (5 mg/kg of glibenclamide)	31.20±1.20 [a, b]
Group F (350 mg/kg of *V. amygdalina*)	35.00±0.80 [a, b]
Group G (350 mg/kg of *M. oleifera*)	36.10±0.30 [a, b]

* data was analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at p≤0.05, SEM: standard error of mean, [a] – p≤0.05 compared with control (group A), [b] – p≤0.05 compared with diabetic control (group B)

Histopathological evaluation

The histoarchitecture of the hippocampus of each group was evaluated and the photomicrographs presented in Figure 1. Histopathological section of the hippocampus of the diabetic control (group B) revealed change in the cell density with average thickness in the CA1 area reaching 68.73 µm suggesting loss of neurons (neurodegeneration) whereas *M. oleifera* and *V. amygdalina* treated Wistar rats maintained higher cell densities in the CA1 areas suggesting neuroprotective activity.

Discussion

This study evaluated the combined effects of *M. oleifera* and *V. amygdalina* on the hippocampus of alloxan-induced diabetic adult Wistar rats. Both plants significantly lowered fasting blood glucose levels, demonstrating anti-hyperglycemic effects comparable to glibenclamide. The results indicate that both plant extracts, whether administered alone or in combination, are effective in controlling hyperglycemia. The hypoglycemic effects of the extracts could be a result of their bioactive phytochemicals with antioxidant activity.[46-48] These antioxidant activities may promote more insulin production by the remaining functional pancreatic beta cells, facilitate the repair of beta cells damaged by alloxan, or enhance glucose uptake and metabolism across various organ systems. Kempferol, quercetin and tannins have been identified as very potent antioxidants and they are all found in the plant materials used.[49-51] Findings by Momoh and his colleagues indicate that both leaf extract of *M. oleifera* and *V. amygdalina* have immense anti-hyperglycemic effect when administered to diabetic rats.[52]

Aqueous leaf extracts of *M. oleifera* and *V. amygdalina* attenuate AChE activity, thereby enhancing acetylcholine levels in the hippocampus and inadvertently enhancing cognitive performance, marked by decreased escape la-

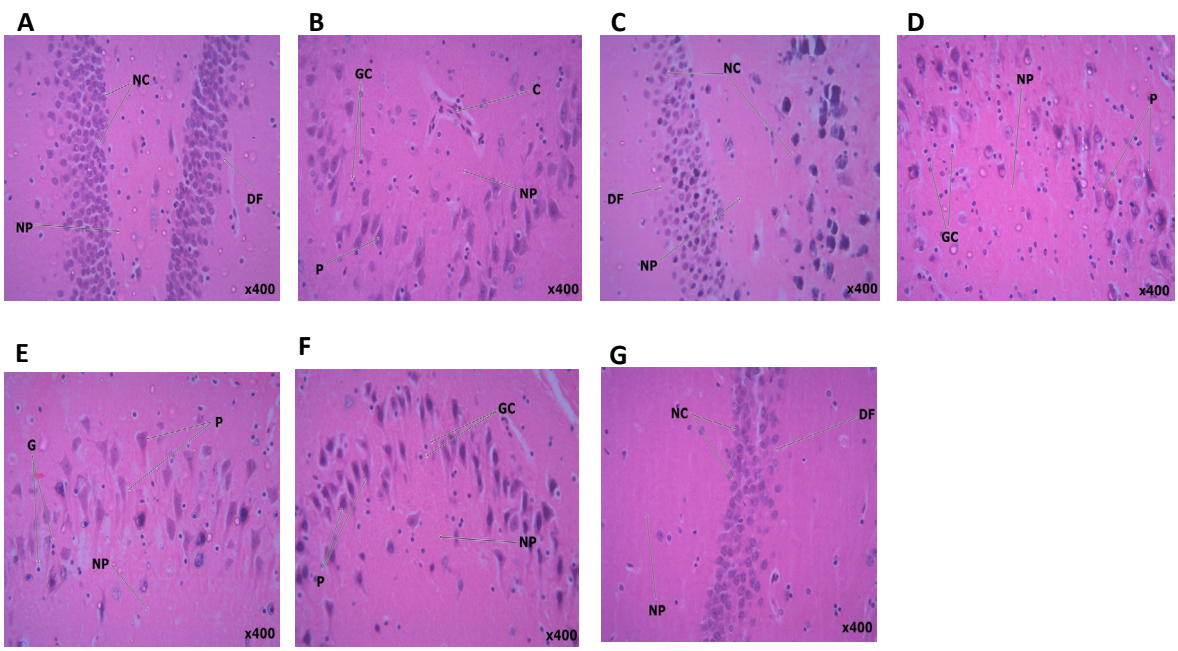

Fig. 1. Effects of *M. oleifera* and *V. amygdalina* on histopathological changes induced by diabetes on the hippocampus of adult Wistar rats. A: control group, B: diabetic control, C: DM+500 mg/kg of *M. oleifera*+*V. amygdalina*, D: DM+700 mg/kg of *M. oleifera*+*V. amygdalina,* E: 5 mg/kg of Glibenclamide, F: 350 mg/kg of *V. amygdalina,* and G: 350 mg/kg of *M. oleifera*

tency in the Morris water test compared to the diabetic control (group B). The significant decrease in AChE activity leads to a corresponding increase in hippocampal acetylcholine, as less is metabolized by the enzyme. This confirms the extracts' ability to enhance synaptic acetylcholine availability, modulate cholinergic neurotransmission, and function as potent AChE inhibitors comparable to established drugs. Csernansky et al. and Muthuraju et al., revealed that down-regulation of AChE activities using AChE inhibitors such as physostigmine and galantamine restored learning and memory deficits and increased acetylcholine level.[53,54] Oladele and colleagues, in their study also found that administration of methanolic leaf extracts of *V. amygdalina* significantly decreased the activity of AChE in different regions of the brain.[35]

Additionally, patients with diabetes mellitus often exhibit impaired attention, memory, general intelligence, and slower information processing.[6,18,21] These findings support the present study, which observed increased time in the Morris water test and significantly elevated AChE activity in the diabetic control group compared to Group A. However, administration of *M. oleifera* and *V. amygdalina* extracts significantly reduced both the time spent in the Morris Water Maze test and AChE activity in the treatment groups, except at the lower dose (group C), which showed no significant time reduction from baseline but a significant reduced time compared to the diabetic control. These findings align with those of Rahmath et al. as well as Kirisattayakul et al., supporting the nootropic effects of the extracts.[55,56]

Neurodegeneration in the hippocampus is closely linked to memory impairment, and diabetes mellitus has been shown to cause neuronal death in this region, likely due to oxidative stress and lipid peroxidation.[18,57] In this study, average thickness in the CA1 region was used to estimate neuronal density. Histopathological analysis revealed reduced CA1 thickness in the diabetic control group (68.73 μm) compared to group A (79.61 μm). However, treatment with *M. oleifera* and *V. amygdalina* extracts conferred neuroprotection, with CA1 thickness values of 71.46 μm, 74.39 μm, 76.31 μm, 73.79 μm, and 73.44 μm in groups C through G, respectively, indicating improved neuronal density relative to the diabetic control. These findings are supported by Sutalangka et al. and Aguwa et al., who reported that *M. oleifera* and *V. amygdalina* extracts enhance spatial memory and reduce hippocampal neurodegeneration.[58-60] The neuroprotective effects observed may be attributed to the antioxidant phytochemicals, such as flavonoids and tannins, which scavenge free radicals and inhibit lipid peroxidation and neuronal cell death.

Overall, co-administration of *M. oleifera* and *V. amygdalina* leaf extracts significantly improved fasting blood glucose levels, reduced AChE activity, enhanced cognitive function, and mitigated neurodegeneration, comparable to the effects of the standard antidiabetic drug glibenclamide. Between the two, *V. amygdalina* demonstrated greater efficacy, likely due to its higher flavonoid and tannin content, compounds known to support cognitive function and lower blood glucose levels.[61,62]

Study limitations and recommendations

This study evaluated only one neurotransmitter enzyme, AChE; therefore, the findings should be validated by assessing additional neurotransmitter enzymes. Furthermore, the use of more specific histological stains would provide greater insight into the underlying neuronal changes.

Conclusion

Our result confirms the age long traditional and therapeutic value of *V. amygdalina* and *M. oleifera* in the treatment of diabetes mellitus and its associated complications. It presents empirical and compelling evidence that diabetes mellitus adversely affects the hippocampus and cognition, and that administration of aqueous extracts of *V. amygdalina* and *M. oleifera* protected against neurodegeneration, cognitive decline, and attenuated AChE activity. This proves the neuroprotective effect of these plant extracts and makes them relevant for use in preventing and managing neurodegeneration and other neurological disorders associated with diabetes mellitus.

Declarations

Funding

The authors received no external funding.

Author contributions

Conceptualization, C.A.A. and D.N.E.; Methodology, U.C.O. and D.N.E.; Software, B.N.O.; Validation, U.C.O. and P.M.D.; Formal Analysis, B.N.O.; Investigation, C.A.A.; Resources, P.M.D. and M.F.O.; Data Curation, P.M.D.; Writing – Original Draft Preparation, C.A.A. and U.C.O.; Writing – Review & Editing, B.N.O. and M.F.O.; Visualization, D.N.E.; Supervision, U.C.O. and D.N.E.; Project Administration, B.N.O.; Funding Acquisition, C.A.A.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

The protocol for the study was approved by the Ethics Committee, Faculty of Basic Medical Sciences, Nnamdi Azikiwe Universitty, Nnewi Campus and was assigned the reference number NAU/CHS/NC/FBMS/472.

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