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Soursop (*Annona muricata*) Attenuates Diabetes-Induced Cerebellar Neurodegeneration and Motor Dysfunction in Wistar Rats

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Abstract

Diabetes mellitus has important neurological consequences, yet cerebellar involvement in diabetes-associated motor dysfunction remains underexplored. Soursop (*Annona muricata*) possesses reported antidiabetic and antioxidant properties; however, its protective effects against diabetes-induced cerebellar injury and motor impairment remain insufficiently investigated. Following two weeks of acclimatization, 24 Wistar rats were randomly assigned to four groups (n=6): control, streptozotocin (STZ; 60 mg/kg, i.p.), STZ + soursop (5,000 mg/kg), and soursop alone. Body weight and fasting blood glucose were monitored throughout the experiment. Motor coordination was assessed using the beam-walking test before euthanasia. The cerebellum was harvested for biochemical and histological analyses. Data were expressed as mean $\pm$ SD and analysed statistically, with significance set at $p < 0.05$. STZ-induced diabetes caused progressive weight loss and hyperglycaemia, impaired motor performance, evidenced by increased crossing latency, falls, and slips, elevated malondialdehyde and nitric oxide levels, and reduced glutathione, catalase, superoxide dismutase, and haeme oxygenase-1 activities. Diabetes also reduced cerebellar serotonin and dopamine levels, increased caspase-3, and produced reduced Nissl staining intensity with Purkinje cell shrinkage and chromatolysis. Soursop treatment substantially ameliorated these alterations, improving glycaemic control, body weight, motor performance, antioxidant status, neurotransmitter levels, and cerebellar histoarchitecture while suppressing oxidative stress and caspase-3 activity. Collectively, these findings demonstrate that soursop attenuates diabetes-induced cerebellar neurodegeneration and motor dysfunction, potentially through coordinated antidiabetic, antioxidant, anti-apoptotic, and neuroprotective mechanisms.

Keywords: Diabetes mellitus, Soursop, cerebellar, glycemic

Diabetes mellitus is a major metabolic disorder associated with significant morbidity and mortality, with complications that extend to the central nervous system (Peeyush et al., 2009; Mirzadeh et al., 2022). The mechanisms in diabetes-induced neural dysfunction is therefore essential. Fundamentally, this disease is a long-term metabolic condition characterized by sustained elevation of blood glucose levels resulting from impaired insulin secretion, diminished insulin sensitivity, or a combination of both (Ajibola et al., 2025; Genitsaridi et al., 2026). This dysregulation of glucose metabolism in hyperglycemic state results in disturbances in carbohydrate, lipid, and protein metabolism, leading to progressive and often irreversible damage to multiple organ systems including the eyes (Luty, 2013), kidneys (Ajibola et al., 2025), cardiovascular system (Herrera et al., 2023), and the nervous system (Zhang et al., 2023). Globally, Diabetes has reached epidemic proportions; according to the International Diabetes Federation (IDF, 2024), approximately 537 million adults were living with diabetes in 2021, with the global prevalence projected to increase substantially by 2050 (Ogle et al., 2025). The prevalence of diabetes is increasing rapidly across sub-Saharan Africa, with Nigeria bearing a substantial national burden (Manne-Goehler et al., 2024).

Oxidative stress plays a significant role in the development and progression of diabetes-associated complications (Singh et al., 2024). Persistent hyperglycaemia increases mitochondrial production of reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, and Genotoxic damage (Dash et al., 2025). These oxidative insults are particularly harmful to the nervous tissue, which is highly metabolically active and vulnerable to oxidative injury (Lemche et al., 2025). Within the CNS, the functional anatomy of cerebellar cortex is not only essential for motor coordination, balance, and fine-tuned voluntary movements but also for cognitive processes such as attention and language (Niyomchan et al., 2019). The high lipid content and comparatively limited antioxidant defense capacity of the cerebellum render it particularly vulnerable to oxidative stress, which can result in Purkinje cell loss, dendritic degeneration, and disrupted neural circuitry (Akanbi et al., 2022). Beyond mitochondrial oxidative damage and disruption of cellular architecture, excessive oxidative stress may trigger the caspase cascade and promote apoptotic cell death through signalling pathways involving apoptosis signal-regulating kinase 1 (ASK1), Src tyrosine kinase, and other downstream mediators (Prause et al., 2016; Zhang et al., 2020). Neurotransmitters are key chemical mediators within the central and peripheral nervous systems and play important roles in the regulation of energy homeostasis,

glucose metabolism, appetite, and insulin sensitivity. Increasing evidence indicates a complex interplay between neurotransmitter signalling and metabolic regulatory pathways. In particular, brain insulin resistance (BIR) may disrupt dopaminergic and serotonergic neurotransmission involved in reward processing and appetite regulation, thereby contributing to hyperphagia and exacerbating metabolic dysregulation.(Gruber et al., 2023; Alagiakrishnan and Halverson, 2024;Ahrićulesei et al., 2025)

Ethnomedicine remains an important component of the healthcare system in Nigeria. Among the plethora of botanicals, *Annona muricata* (soursop) is notable for its diverse composition of biologically active phytochemicals including acetogenins, flavonoids, and alkaloids-with demonstrated antioxidant, anti-inflammatory, and hypoglycaemic properties (Ezekiel and Emeka, 2022; Jadhav & Selvakumarsamy, 2024).

Therefore, this study evaluated the effects of soursop on blood glucose levels, behavioural phenotypes, monoamine neurotransmitters, caspase-3 expression, and histostructural changes in the cerebellar tissue of diabetic Wistar rats.

2.0 Methods

Ethical Consideration

All procedures involving the experimental animals were performed in accordance with the ethical standards and guidelines of the Faculty of Basic Medical Sciences with approval code (FUOYE/BMS/0076) and in line with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1986).

Animal Procurement, Housing and Husbandry

Twenty-four healthy adult male Wistar rats weighing 90–120 g were obtained from an accredited laboratory animal breeding facility. Upon arrival, the animals were housed in clean, well-ventilated cages and maintained under standard laboratory conditions, including controlled ambient temperature and humidity and a 12-hour light/dark cycle. The rats were allowed to acclimatize to the experimental environment for two weeks before the commencement of the study. During this period and throughout the experiment, they were fed a standard pelletized laboratory diet and provided unrestricted access to clean drinking water.

Experimental Grouping and Treatment

Following two weeks of acclimatization, the animals were randomly assigned to four groups and all treatments were administered for a period of 4 weeks, and the soursop extract was administered daily by oral gavage

Group	Experimental grouping	STZ (60 mg/kg, I.P.)	Normal saline (0.5 mL, oral)	Soursop fruit extract (5000 mg/kg, oral)
A	Control	No	Yes	No
B	Diabetic only	Yes	Yes	No
C	Diabetic + soursop fruit extract	Yes	No	Yes
D	Soursop fruit extract only	No	No	Yes

3.4.1 Induction of Diabetes

A single intraperitoneal dose of streptozotocin (STZ) was administered at 60 mg/kg body weight to animals that had been fasted for 12 hours. Three days after STZ administration, fasting blood glucose levels were assessed to confirm diabetes induction. Rats with fasting blood glucose levels ≥ 200 mg/dL were classified as diabetic. This method was described by Akwu et al. (2022)

Preparation of Soursop (*Annona muricata*) fruit Extract

Fresh fruits of soursop were obtained from farms at Ado-Ekiti and subsequently authenticated. Following collection, the fruits were allowed to dry under ambient conditions at room temperature before being pulverized. The powdered material was immersed in 1 L of distilled water and subjected to maceration for 24 h at room temperature with continuous agitation using a magnetic stirrer. The suspension obtained after extraction was passed sequentially through Whatman No. 1 filter paper and hydrophilic cotton to remove particulate matter. The aqueous filtrate was concentrated under reduced pressure using a rotary evaporator maintained at 60 °C. The resulting concentrate was further dried in an oven at 45 °C for 48 h to obtain the crude extract. The dried extract was cooled and stored in a desiccator before its mass was determined. The extract was subsequently preserved under refrigeration until required for experimental use. The percentage extraction yield was calculated as the ratio of the weight of the dried extract (W_2) to the initial weight of the extracted plant material (W_1), multiplied by 100, following the method described by Ezekiel et al. (2022).

Lethal Dose of the Soursop

Acute toxicity of the soursop extract was evaluated using the Lorke procedure, as previously described by Ajibola et al. (2025). Following administration of the test extract, the animals were closely monitored for behavioural and clinical signs of toxicity during the initial 4 h, at 24 h, and subsequently once daily for 14 days. Based on the observed responses and mortality pattern, the median lethal dose (LD_{50}) of the soursop extract was estimated to be 5000 mg/kg body weight. The estimated LD_{50} of 5000 mg/kg body weight is consistent with the findings of Ekweaga et al. (2025), who reported a comparable LD_{50} for soursop extract.

Body weight and Blood Glucose Assessment

Body weight was measured weekly using a precision weighing balance. Prior to the commencement of the study, baseline blood glucose levels were determined, followed by weekly monitoring throughout the experimental period. Blood samples were collected from the tail vein, and blood glucose concentrations were measured using a ACCUCHECK glucometer and its test strip

Beam walk test

Motor coordination and balance were assessed using the beam-walking test, adapted from a method described by Hausser et al. (2018). A wooden beam measuring 100 cm in length, 2.5 cm in width, and 4.0 cm in height was elevated approximately 1 m above the floor and connected at one end to a black goal box (28 × 18 × 18 cm). Bright light and white noise were used as aversive stimuli at the starting end of the beam to encourage movement toward the goal box. Before behavioural assessment, the rats underwent beam-walking training to familiarize them with the apparatus and were considered adequately trained when they could traverse the beam within ≤ 5 s. During testing, each rat was placed at the starting end of the beam, and the stopwatch was simultaneously initiated. Timing was terminated when the rat's forepaws crossed the threshold of the goal box. Three trials were conducted for each animal, with a 30-s rest interval between trials. The latency (in seconds) to traverse the beam, number of foot slips, number of falls, and percentage of successful crossings were recorded.

3.6 Euthanasia and Tissue Collection

At the end of the experimental period around 7:00 A.M to 10: 00 A. M , Animals designated for histological analysis were deeply anaesthetized and transcardially perfused with phosphate-buffered saline (PBS; pH 7.4), followed by 10% neutral buffered formalin (NBF) for fixation. The skull was opened, and the whole brain was carefully removed. The cerebellum was dissected from the cerebrum and brainstem and fixed in 10% NBF for histological processing.

For biochemical analyses, cerebellar tissues were rapidly excised, homogenized in PBS, and stored frozen until analysis.

Cerebellar tissue homogenates were subjected to various biochemical evaluations relevant to the central nervous system, focusing on oxidative stress, neurotransmitter levels, and apoptosis.

Determination of antioxidant activities, oxidative markers and Heme oxygenase-1 (HO-1) protein levels

Cerebellar tissue homogenates were subjected to various biochemical evaluations.

Reduced glutathione (GSH) concentration was determined using the colorimetric method described by Ellman (1959). Superoxide dismutase (SOD) activity was assessed according to the method of Misra and Fridovich (1972). Briefly, 0.2 mL of the brain homogenate supernatant was combined with 0.8 mL of 50 mM glycine buffer (pH 10.4), after which 0.02 mL of epinephrine was added to initiate the reaction. Following a 5-min incubation period, absorbance was measured spectrophotometrically at 480 nm. Catalase (CAT) activity was determined following the method of Aebi et al. (1974). For the assay, 0.1 mL of the supernatant was added to 1.9 mL of 50 mM phosphate buffer (pH 7.0), followed by the addition of 1.0 mL of freshly prepared 30 mM hydrogen peroxide (H_2O_2) to initiate the reaction. Catalase activity was expressed as $\mu\text{M H}_2\text{O}_2$ decomposed per minute. Malondialdehyde (MDA), an index of lipid peroxidation, was quantified using the method described by Wills (1966), and the results were expressed as nmol/mg tissue. Nitrite concentration was determined using the Griess reaction as described by Green et al. (1982) with the findings expressed as $\mu\text{g/mg}$ tissue. Heme oxygenase-1 (HO-1) protein levels in cerebellar tissue lysates were quantified using a rat-specific HO-1 enzyme-linked immunosorbent assay (ELISA) kit (Assay Designs, SIGMA ALDRICH, USA). Tissue lysates were prepared using the lysis buffer supplied with the assay kit, following the manufacturer's instructions. The optical density (OD) of the samples was measured at 450 nm using a Bio-Rad Benchmark Plus microplate spectrophotometer. HO-1 assays were done by previous method described by Wang et al. (2010).

Determination of Neurotransmitters and caspase-3

The concentrations of the cerebellar monoamine neurotransmitters, serotonin and dopamine, were quantified in tissue homogenates using specific enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Neuro-apoptosis was assessed by quantifying the level of the pro-apoptotic protein caspase-3 in the cerebellar tissue homogenates using a highly sensitive sandwich ELISA kit, following the manufacturer's instructions.

Histological Analyses

The excised cerebellum tissues were fixed in 10% NBF and subsequently dehydrated through ascending concentrations of alcohol (70% to 100%). The tissues were then cleared in xylene, embedded in paraffin wax, and sectioned at a thickness of 5 μ m using a microtome. The sections were subjected to the Haematoxylin and Eosin (H&E) and Cresyl Fast Violet (Niss staining)

Histological examination

Following staining, the cerebellar tissue sections were examined microscopically using an OPTO-EDU binocular microscope equipped with a 5.1-megapixel MV550 digital camera. The microscope-camera system was connected to a computer equipped with image acquisition and analysis software. The imaging system was appropriately calibrated to ensure optimal image quality and resolution. Representative photomicrographs of the cerebellar sections were captured at $\times 400$ magnification. Quantitative cell counting was performed on the captured photomicrographs using ImageJ software.

Statistical analysis

Statistical analyses were conducted using appropriate statistical analysis software. Differences among experimental groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison post hoc test. Statistical significance was established at $p < 0.05$. Treatment- and time-dependent changes in the measured outcomes were evaluated using a two-way repeated-measures analysis of variance (ANOVA), followed by Sidak's post hoc test for multiple comparisons.

3.0 Result

Figure I: Assessment of body weight following soursop treatment on diabetic- induced Wistar rats.

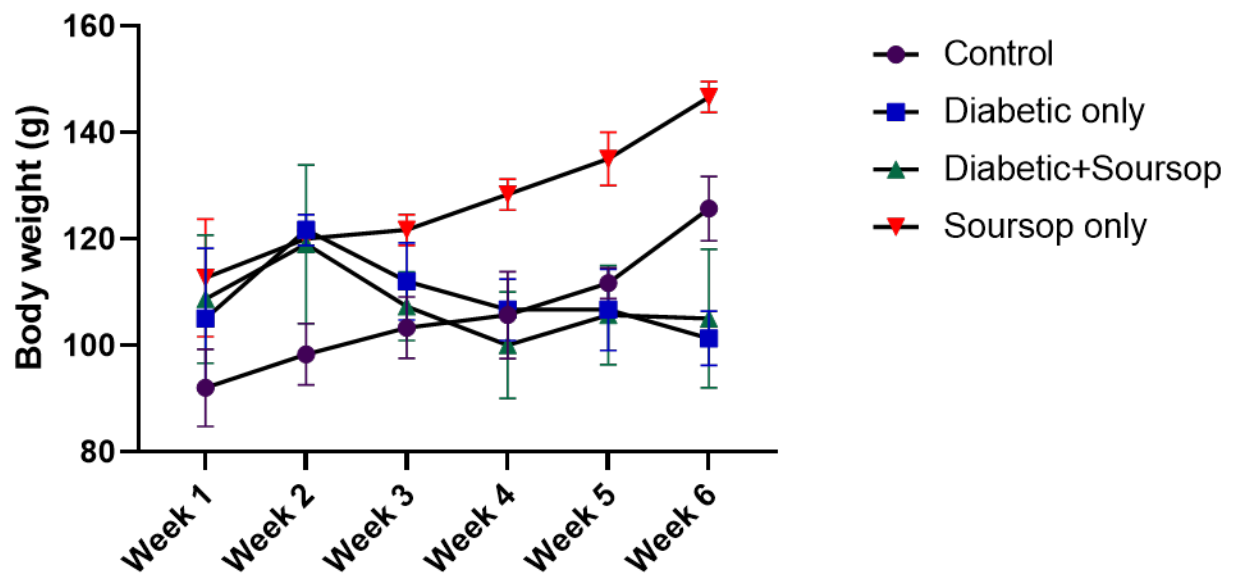


Figure I: Assessment of body weight following soursop administration on diabetic Wistar rats. Data are expressed as mean $\pm$ SD and analysed by one-way ANOVA followed by Tukey post hoc test. ; n=6

Figure II: Assessment of blood glucose level following soursop administration on Diabetic-Induced Wistar rats

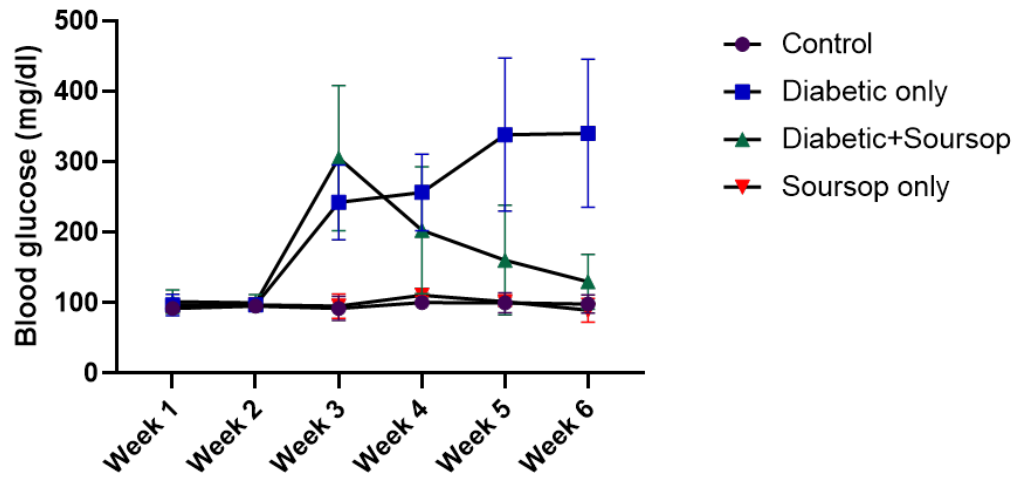


Figure II: Assessment of blood glucose level following soursop administration on diabetic Wistar rats. Data are expressed as mean $\pm$ SD and analysed by one-way ANOVA followed by Tukey post hoc test. ; n=6

Figure III: Effect of Soursop on Beam-Walking Performance in Diabetic Wistar Rats

Comparison among groups using one-way ANOVA followed by Tukey's multiple-comparisons test revealed a statistically significant increase in the time taken to traverse the beam, number of foot slips, and number of falls in the diabetic group compared with the control and soursop-only groups (**** $p < 0.0001$). Conversely, treatment with soursop significantly reduced the time taken to traverse the beam (**** $p < 0.0001$), number of foot slips (*** $p < 0.0001$), and number of falls ($p < 0.1$) compared with the untreated diabetic group. There was also a statistically significant reduction in the percentage of successful crossings in the untreated diabetic group compared with the control (**** $p < 0.0001$), soursop-only (**** $p < 0.0001$), and soursop-treated diabetic groups (** $p < 0.01$).

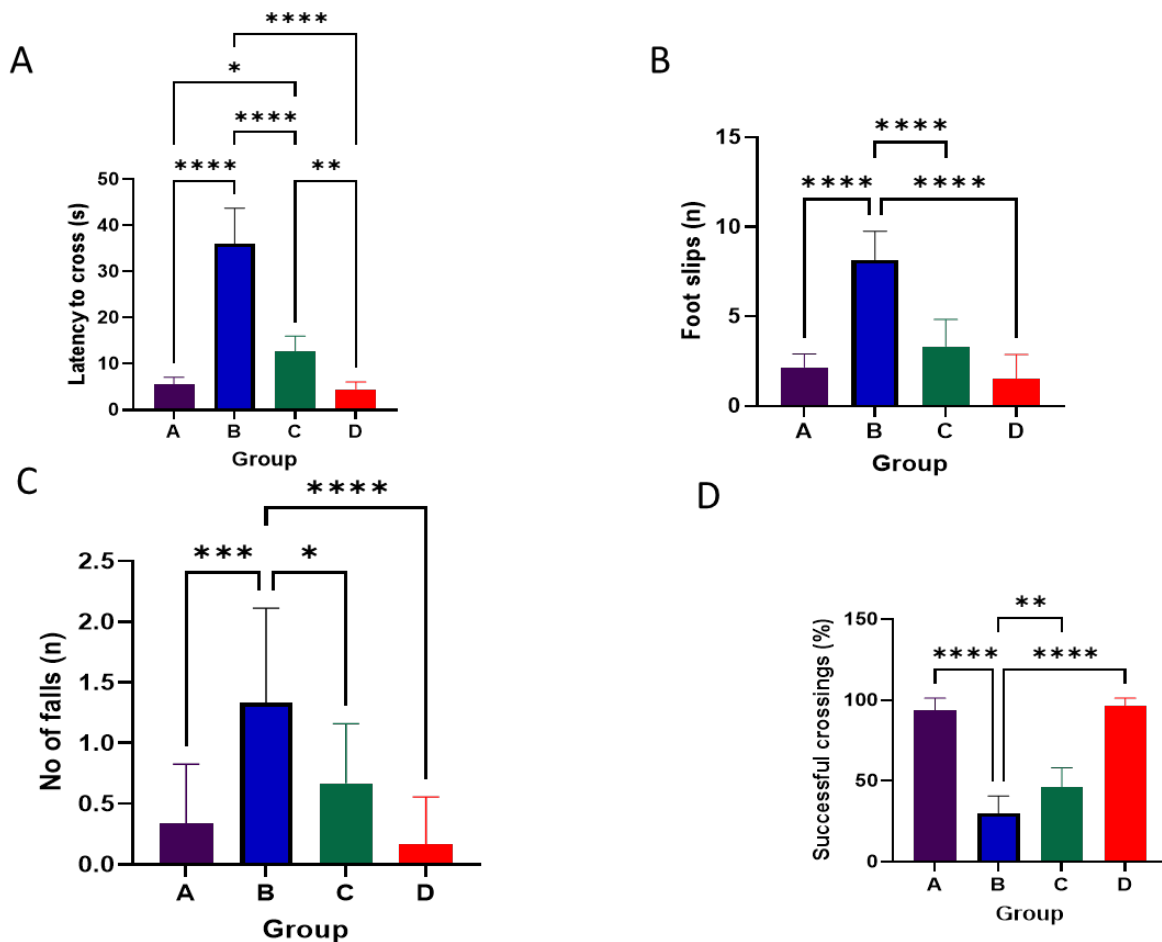


Figure III: Effects of Soursop on Beam-Walking Performance in STZ-Induced Diabetic Wistar Rats. (A) Latency to traverse the beam, (B) number of foot slips, (C) number of falls, and (D) percentage of successful crossings. Data are expressed as mean $\pm$ SD and analysed by one-way ANOVA followed by Tukey post hoc test. (n=6) A= Control group; B=Diabetic group; C= Diabetic+soursop; D= soursop group only

Figure IV: Effect of Soursop on Oxidative Stress Markers Levels in Diabetic Wistar Rats

Comparison among groups using one-way ANOVA followed by Tukey's multiple-comparisons test revealed a significant increase in cerebellar MDA and NO levels in the diabetic group compared with the control group (**** $p < 0.0001$), alongside significant reductions in SOD (**** $p < 0.0001$), GSH (** $p < 0.01$), CAT (*** $p < 0.001$), and HO-1 (**** $p < 0.0001$) levels. Treatment with soursop significantly attenuated these diabetes-associated alterations, as evidenced by reduced MDA (*** $p < 0.001$) and NO (*** $p < 0.001$) levels and increased SOD (* $p < 0.05$), GSH (* $p < 0.05$), CAT (** $p < 0.01$), and HO-1 (*** $p < 0.001$) levels compared with the untreated diabetic group. Furthermore, the diabetic group differed significantly from the control and soursop-only group in MDA, SOD, NO, GSH, CAT, and HO-1 levels.

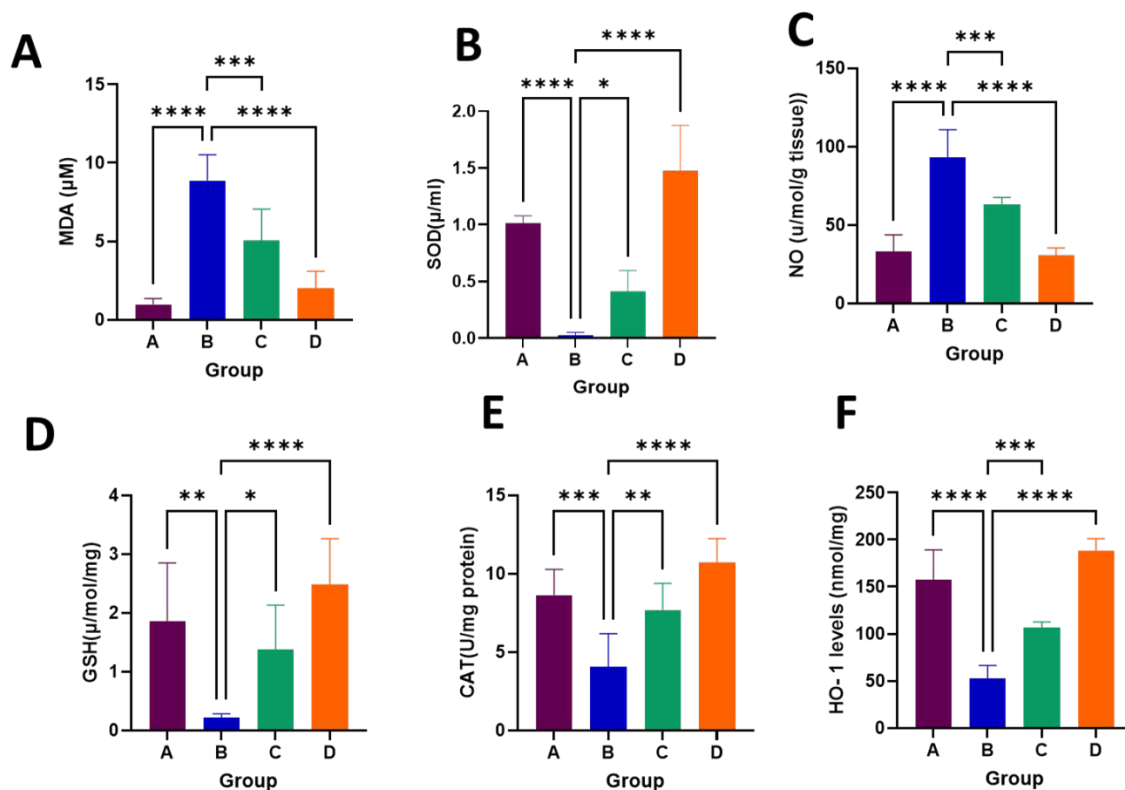


Figure IV: Effects of Soursop on oxidative stress markers in STZ-Induced Diabetic Wistar Rats. (A) Lipid peroxidation (MDA); (B) Superoxide dismutase (SOD); (C) Nitric Oxide (NO); (D) Gluthathione (GSH); Catalase (CAT); and Heme Oxygenase-1 (HO-1). Data are expressed as mean $\pm$ SD and analysed by one-way ANOVA

followed by Tukey post hoc test. (n=6) A= Control group; B=Diabetic group; C= Diabetic+ soursop; D= soursop group only

Figure V: Effect of Soursop on Neurotransmitter Levels in Diabetic Wistar Rats

Comparison between groups by one-way ANOVA followed by Tukey's multiple tests shows a statistically significant decrease in serotonin in diabetic-induced (B) group compared to control (A) group (**** $p < 0.0001$) and normal + Soursop (D) group (** $p < 0.01$). There is also significant decrease in serotonin level in diabetic group (B) compared to Diabetic treated with Soursop group (C) (* $p < 0.05$). Dopamine level showed statistical reduction in diabetic group (B) only compared to diabetic treated with soursop (C) (* $p < 0.05$), normal + Soursop (D) (*** $p < 0.001$), and control (A) group (*** $p < 0.001$). Diabetic group treated with soursop shows a statistically significant difference with that of control group (* $p < 0.05$)

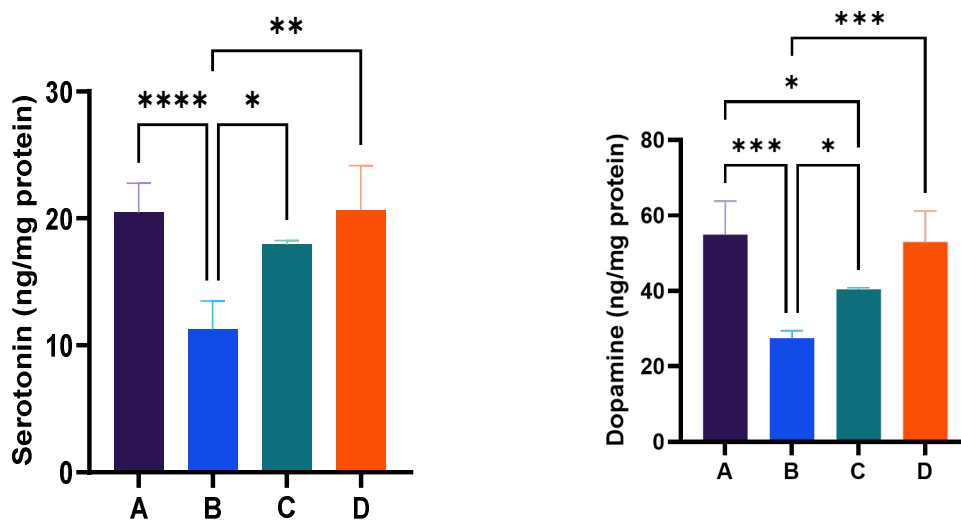


Figure V: Effects of soursop on neurotransmitters (serotonin and dopamine) of STZ induced diabetic Wistar rats. Data are expressed as mean $\pm$ SD and analysed by one-way ANOVA followed by Tukey post hoc test. A= Control group; B=Diabetic group; C= Diabetic+soursop; D= Normal + soursop group

Figure VI: Effect of Soursop on Apoptotic Marker (Caspase-3) of Diabetic-induced Wistar Rat

Comparison between groups by one-way ANOVA followed by Tukey's multiple tests shows a statistically significant increase in serum caspase-3 in diabetic-induced (B) group compared to control (A) group (**** $p < 0.0001$), normal + Soursop (D) (** $p < 0.01$) and diabetic treated with soursop (** $p < 0.01$). Also, diabetic group treated with soursop shows a statistically significant difference with control group (** $p < 0.01$) and normal + soursop (*** $p < 0.001$)

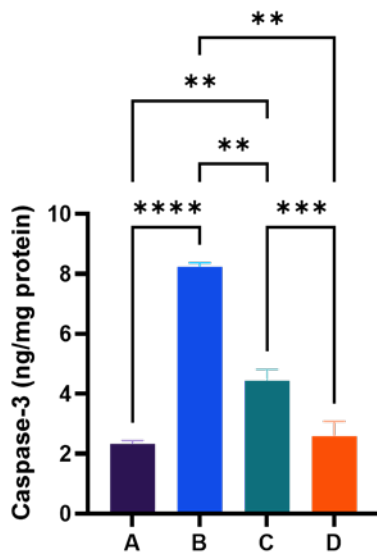


Figure VI: Effects of soursop on caspase-3 level of STZ- induced diabetic Wistar rats. Data are expressed as mean $\pm$ SD and analysed by one-way ANOVA followed by Tukey post hoc test. A= Control group; B=Diabetic group; C= Diabetic+soursop; D= Soursop only

Figure VII: Histological Observation of Cerebellum Using Cresyl Fast Violet (CFV)

Cerebellar sections from Group A showed normal distribution and well-stained Nissl substance compared with Group B. An improvement in Nissl staining intensity was observed in Group C, following treatment with soursop, while Group D exhibited a Nissl staining pattern comparable to that of the control group. Quantitative analysis revealed a statistically significant reduction in the number of Purkinje cells in the diabetic group (Group B) compared with the control group ($p < 0.01$), soursop-only group ($***p < 0.001$), and diabetic group treated with soursop ($p < 0.01$).

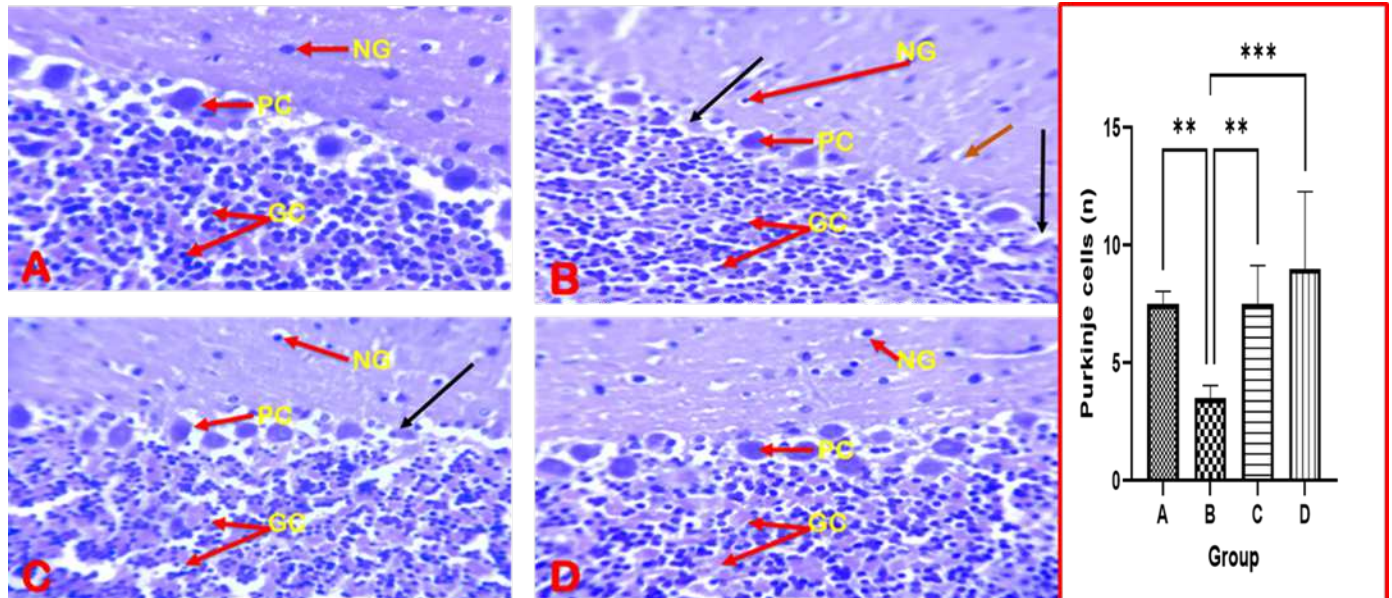


Figure VII: Photomicrograph of cerebellum of diabetic-induced Wistar rats treated with soursop using CFV

(Mag×400). A= Control group; B=Diabetic group; C= Diabetic+soursop; D= Soursop only. NG (neuroglial cell); PC (Purkinje cells); and GC (Granular cells); Black arrow (Purkinje cell with reduced Nissl substance); Brown arrow (Pyknotic glial cell)

Figure VIII: Histological Observation of Cerebellum Using Haematoxylin and Eosin (H&E)

Cerebellar sections from Group A showed normal neuronal, Purkinje, and granular cell architecture compared with Group B, which exhibited notable pyknotic neurons and a few flattened Purkinje cells. Group C, the diabetic group treated with soursop, showed an increased number of viable neurons and preservation of the normal pear-shaped morphology of Purkinje cells compared with the untreated diabetic group, which exhibited flattened and shrunk Purkinje cell architecture. Histomorphometric of purkinje cells showed reduction in number in diabetic group compared to control ($***p<0.001$); soursop only group ($***p<0.001$) and diabetic group treated with soursop ($***p<0.001$).

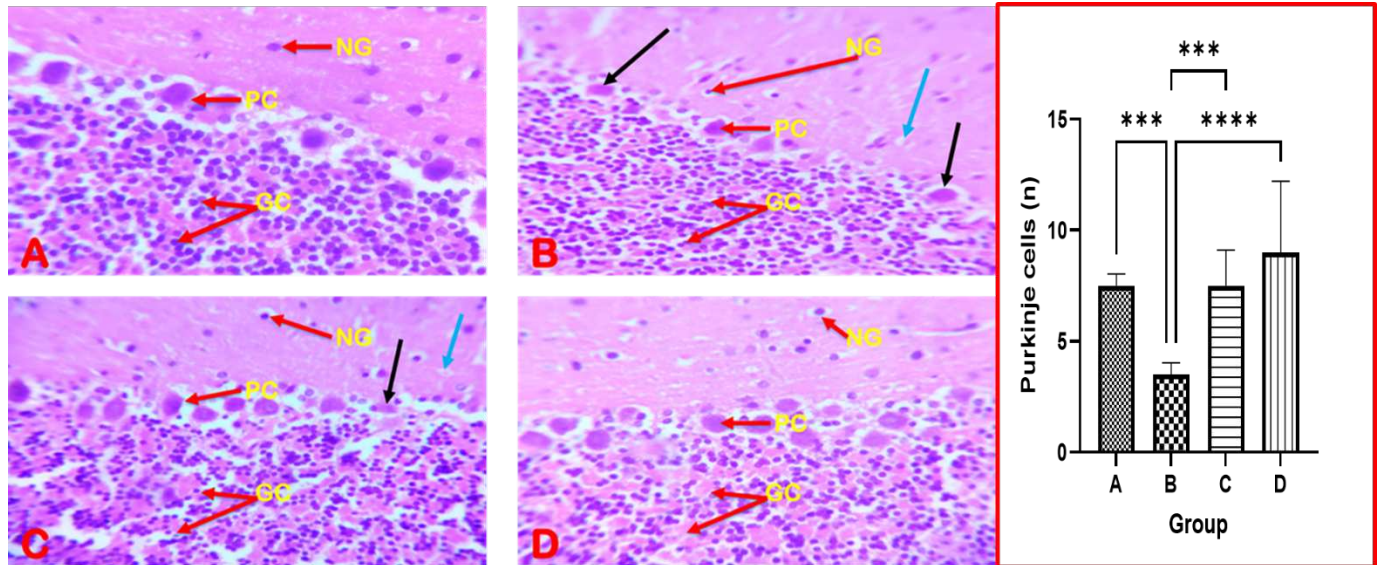


Figure VIII: Photomicrograph of cerebellum of diabetic-induced Wistar rats treated with soursop using H&E (Mag×400). A= Control group; B=Diabetic group; C= Diabetic+ soursop; D= Soursop only. NG (Neuroglial cell); PC (Purkinje cells); and GC (Granular cells); Black arrow (flattening and distortion of Purkinje cell bodies); Blue arrow (pyknotic neuronal cells).

Discussion:

Chronic hyperglycaemia in diabetes can induce neuronal injury through intracellular glucose accumulation, resulting in disrupted neurotransmitter homeostasis (Sukhov et al., 2016), impaired motor function (Nagayach et al., 2014), mitochondrial oxidative stress (singh et al., 2004) and apoptotic signalling (singh et al., 2004).

The reduction in body weight observed in the diabetic animals, together with the marked elevation in fasting blood glucose, is consistent with the characteristic metabolic disturbances associated with streptozotocin-induced diabetes. Similar findings were reported by Belkacemi et al. (2012) and Adeeyo et al. (2023), when they observed a progressive decline in body weight in STZ-induced diabetic rats accompanied by significantly increased glycaemia compared with non-diabetic controls. The improvement in fasting blood glucose and body weight following soursop treatment corroborates previous reports that soursop exerts antihyperglycaemic effects and promotes recovery of body weight in STZ-induced diabetic rats (Adeyemi et al., 2008; Florence et al., 2014).

Balance and motor coordination are fundamental cerebellar functions that rely on its functional connectivity with cortical motor regions, particularly the motor cortex, to facilitate the precise control and execution of voluntary movements (Koziol et al., 2014). Our findings showed that hyperglycemia impaired performance on the beam-walking test, as evidenced by increased slips and falls and increased latency to traverse the beam. However, soursop treatment improved motor coordination. The impaired beam-walking performance observed in the diabetic group is consistent with previous evidence showing that diabetes can compromise sensorimotor function and motor learning in experimental animals (Shaikh and Somani, 2010; Zhang et al., 2020). Notably, the present findings provide evidence that *Annona muricata* may ameliorate hyperglycemia-associated motor deficits, as demonstrated by improved performance on the beam-walking test. To the best of our knowledge, this represents the first evidence of the potential beneficial effect of *A. muricata* on motor coordination and balance using this behavioural paradigm.

Hyperglycaemia associated with diabetes significantly decreased the levels of glutathione (GSH), and superoxide dismutase (SOD), while significantly increasing nitrite and malondialdehyde (MDA) levels in the cerebellar tissue homogenates of experimental rats. The excessive generation of reactive oxygen species (ROS), coupled with the depletion of endogenous antioxidant defence systems, contributes to increased oxidative stress, which has been associated with diabetes-related cognitive deterioration (Ceretta et al., 2012; Özsan et al., 2024). However, treatment with soursop fruit extract increased SOD, catalase, and GSH levels, suggesting an enhancement of the cerebellar antioxidant defence system and a potential mitigation of oxidative stress induced by the diabetic state.

Serotonin and dopamine levels were significantly reduced in the diabetic-only group when compared with the control group, the soursop-only group, and to some extent, the diabetic group treated with soursop. This reduction agrees with earlier reports that persistent hyperglycaemia disrupts monoaminergic neurotransmission in the brain, partly through increased oxidative stress and partly through impaired tryptophan and tyrosine metabolism, which are the precursors for serotonin and dopamine synthesis respectively (Vijayam et al., 2022; Gupta et al., 2023). The cerebellum receives and modulates serotonergic and dopaminergic projections, and depletion of these neurotransmitters in diabetic animals may account for some of the motor and non-motor deficits that have been documented by (Okoh et al., 2022; Ugwu & Afolabi, 2022) on diabetic encephalopathy.

The observed modulation of cerebellar dopamine and serotonin may be associated with the ability of *Annona muricata* (soursop) to influence monoaminergic neurotransmission. Previous studies have demonstrated that *A. muricata* leaf extracts modulate brain dopamine and norepinephrine levels, while isoquinoline alkaloids isolated from the fruit exhibit activity at serotonergic 5-HT_{1A} receptors (Souza et al., 2018).

Caspase-3 is a key executioner protease and an established marker of apoptosis (Akanbi et al., 2022). The significantly elevated caspase-3 level in the diabetic group compared with the control, soursop-only, and soursop-treated diabetic groups suggests that chronic hyperglycaemia promotes apoptotic cell death, possibly through oxidative stress, mitochondrial dysfunction, and activation of intrinsic apoptotic pathways (Akanbi et al., 2022; Potenza et al., 2024). Treatment with soursop markedly reduced caspase-3 levels in diabetic rats, suggesting a potential antiapoptotic and cytoprotective effect of the extract against diabetes-associated cerebellar injury. This finding is consistent with previous reports demonstrating the cytoprotective and antiapoptotic properties of soursop (*Annona muricata*), including suppression of caspase-3 activation under conditions of cellular stress (Kim et al., 2020).

Motor function relies on cortico-cerebellar circuits involving afferent inputs from the prefrontal, posterior parietal, and motor cortices, which contribute to motor and higher cognitive functions. Purkinje cells receive excitatory input from parallel fibers, the axons of cerebellar granule cells, which regulate Purkinje cell dendritic arborization (Goa et al., 2018).

Purkinje cells are metabolically specialized neurons that are particularly vulnerable to metabolic stress because they receive extensive synaptic input and provide the sole efferent output of the cerebellar cortex. Consequently, Purkinje cell degeneration is a common feature of inherited ataxias and may also occur in response to metabolic disturbances (Yang et al., 2014).

The reduced Nissl staining observed in the diabetic group suggests neuronal stress and chromatolysis associated with sustained hyperglycaemic insult, consistent with previous reports of cerebellar neuronal degeneration under diabetic conditions (Uchegbu & Ibekwe, 2022; Chen & Wang, 2023; Akanbi et al., 2022). This structural alteration was further

supported by the significant reduction in Purkinje cell number in the diabetic group compared with the control, soursop-only, and soursop-treated diabetic groups. The improvement in Nissl staining intensity following soursop treatment, together with the preservation of Purkinje cells, suggests that soursop mitigated diabetes-associated neuronal damage and preserved cerebellar neuronal integrity. The preservation of Purkinje cells and improved Nissl staining following soursop treatment is consistent with previous reports of the neuroprotective effects of *Annona muricata* on cerebellar neurons. Soursop administration has been shown to attenuate Purkinje-cell degeneration and preserve cerebellar cytoarchitecture in experimental models of neuronal injury (Astini et al., 2024).

Conclusion

Soursop fruit extract attenuated diabetes-induced cerebellar neurodegeneration and motor dysfunction, with improvements in glycaemic control, antioxidant status, neurotransmitter levels, apoptosis, and cerebellar architecture. These findings support its potential as a neuroprotective adjunct in diabetes-associated neurological complications. Future studies should clarify the underlying molecular mechanisms and establish its long-term safety and therapeutic efficacy.

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Authors contribution

Toheeb Adesumbo Ajibola: Conceptualization, methodology, investigation, data collection, data analysis, writing—original draft, and project administration. **Adeshina Oloruntoba Adekeye:** Methodology, investigation, data collection, and review and editing of the manuscript. **Abimbola Mercy Olutope:** Investigation, data collection, laboratory analysis, and review and editing of the manuscript. **Olusola Atilade Adeeyo:** Supervision, methodology, validation, review and editing of the manuscript, and project supervision.

All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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