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RESEARCH

Protective Effects of *Spondias mombin* Leaves extract on the Cerebellar Architecture of Diabetic Wistar Rats

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Abstract

Background: *Spondias mombin*'s anti-inflammatory, antimicrobial, antibacterial, antifungal, and antiviral properties have been reported in wounds healing, treatment of dysentery, haemorrhoids, gonorrhea, leucorrhea and infections. The cerebellum however play central roles in the coordination of muscle activities, this research work is aimed at investigating the histological changes in the cerebellum of the hyperglycemic wistar rats treated with aqueous leave extract of *Spondias mombin*.

Methods: Twenty (20) wistar rats weighing between 75-120 g were employed, the animals were randomly selected into four (4) groups, Group A; made up of five (5) normoglycemic wistar rats treated with 2mls of distilled water (Negative control), Group B; made up of five (5) hyperglycemic wistar rats, given water only (positive control), Group C; made up of five (5) normoglycemic wistar rats treated with 475 mg/kg of *Spondias mombin* leaves extract and Group D; made up of five (5) hyperglycemic rats treated with 475 mg/kg of *S. mombin* leaves extract. Administration was done oro-gastrically and daily for period of 21 days, the animals were euthanized twenty-four (24) hours after the last administration by cervical dislocation, and the brain was excised following skull fracture, cerebellum was carefully dissected out and fixed in 10% formal saline for routine histological techniques.

Results: Degeneration and loss of purkinje cells, cellular hyper-trophy associated with intercellular vacuolation in the stroma of the cerebellar cortex of the induced hyperglycemic rat that were not exposed to the *S. mombin*. Cerebellar cortex in hyperglycemic rats treated with S. mombin aqueous extracts showed evidence of cell division and the pyramidal cells were well defined and the integrity of the cerebellar histoarchitecture maintained in relation to the control animals.

Conclusion: Treatment with *S. mombin* aqueous leaves extract protects the cerebellar architecture in hyperglycemic condition and promotes the cerebellar nuclei renewal. .

Keywords: Diabetes, Wistar rats, Cerebellum, *Spondias mombin*, antidiabetic, and antioxidant

1.0 INTRODUCTION

Diabetes mellitus is a descriptive term for a family of disorders that are characterized by chronic carbohydrate intolerance (fasting or postprandial hyperglycemia) and the development of long-term medical complications [1]. There are four main types of diabetes mellitus: type-1, type-2, gestational Diabetes mellitus, and "other specific types of diabetes mellitus" [2]. Inadequate management or uncontrolled hyperglycaemia manifests into signs and symptoms that can also be referred to as acute complications that could lead to development of chronic complications such as hypertension, stroke, blindness, erectile dysfunction, and kidney malfunction [2]. This metabolic disorder, is a progressive one that is found among all age groups. The prevalence of diabetes mellitus is estimated to rise to 592 million by the year 2035 [3]. Chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, pancreas, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action [4].

Extensive vascular supply is required to meet the demands of the cerebellum's neural activity and increased in blood glucose concentrations (define diabetes and impaired fasting glucose) is associated with high risk of macrovascular and microvascular disease. Untreated diabetes leads to ketoacidosis, the accumulation of ketones and acid in the blood, these products of disordered carbohydrate and fat metabolism result brain injury and consequent diabetic coma. Medicinal plants are important in traditional medicine and have been reported to posses; antimicrobial, antibacterial, antifungal, and the antiviral properties that are used as treatment aids in disease conditions such as diuretic and febrifuge, diarrhoea, dysentery, haemorrhoids, gonorrhoea and leukorrhea.

Spondias mombin Linn (Anarcadiaceae) is known commonly as 'Hog plum', 'Iyeye' and 'Olosan' (Yoruba, South West Nigeria) [5]. In traditional folklore medicine, *S. mombin* is used in treating intestinal disorders, as an emetic, for treating gonorrhoea, diabetes, psychiatric disorders, for the expulsion of placenta in goats and women.

The plant is also useful as an antidiarrhoeal agent [6], as an antimicrobial agent, for the treatment of wounds, and as an astringent [7] and as an oxytocic [8]. The fruit juice is consumed as a diuretic and febrifuge and as a remedy for diarrhea, dysentery, haemorrhoids and a treatment for gonorrhoea and leukorrhea. A tea made from the flowers and leaves is taken to relieve, biliousness, urethritis, cystitis and eye and throat inflammations [9]. *S. mombin* has been scientifically proved to exhibit antimicrobial, antibacterial, antifungal, and the antiviral properties [10]. The plant extract has been demonstrated to contain mainly phenolic derivatives which have been shown to possess antioxidant and anti-inflammatory properties [11]. The present study focused on the effect of this plant extract on the cerebellum of diabetic Wistar rats.

2.0 METHODOLOGY

2.1 Animal Care and Management

Twenty adult Wistar rats, weighing 130- 170g were procured from the College of Health Sciences Animal House, Ladoke Akintola University of Technology, Ogbomoso. The ethical approval on animal act right was obtained from the Institutional Animal Care Committee of the same Institution. The animals were randomly divided into Four groups with each group comprising of five (n=5) rats. They were kept in the Laboratory for two weeks for acclimatization and were fed on a standard diet (Vital Feeds and Grand Cereals Ltd); water was given ad libitum and maintained under standard conditions. The animal room was well ventilated with a temperature range of 25-27°C under day/night in a 12-12 hour photoperiodicity. All the experimental procedures were done following the experimental guidelines of the Institutional Animal Ethics Committee (IAEC) of the Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

2.2 Sample Collection and Preparation of Plant Extract

Fresh hog plum leaves were collected in Ita Alasa, Ogbomoso North Local Government and authenticated at Department of Pure and Applied Biology, Ladoke Akintola University of Technology. The hog plum leaves were air-dried at room temperature for a week and were grinded using grinding machine at WAZO market and weighed at 600g. Extraction process was carried out

in the Department of Food Science and Engineering (FSE), LAUTECH, Ogbomoso. The pulverised hog plum leaves were soaked in 2000mls of distilled water for 48hours and thoroughly stirred for proper mixing. The mixture was filtered with muslin cloth for the purpose of separating the residue from the filtrate. The filtrate was condensed and subjected to evaporation through water bath at temperature between 45-57°C for one (1) week. The extract weighed 475g after evaporation.

2.3 Acclimatization and Animal Management

After acclimatization for a period of two (2) weeks, the rats were weighed using the weighing balance and were randomly divided into four groups. Experimental animals were maintained on clean water and were fed with standard diets. The rats were kept in standard room temperature throughout the process of the experiment which spanned for 3weeks.

2.4 Determination of LD$_{50}$

LD$_{50}$ of the extract was done at the Pharmacology Department of LAUTECH Ogbomoso. The acute toxicity tests of *S. mombin* aqueous leaves extract were carried out according to the Organization of Economic Co-operation and Development (OECD) test guidelines (OECD 423- Limit test procedure). The LD$_{50}$ was found to be 800mg/kg.

2.5 Induction of Diabetes mellitus

Hyperglycemia was induced in forty Wistar rats fasted overnight and randomly selected by a single intraperitoneal administration of Streptozotocin (STZ) at 100mg/kg [12]. STZ was dissolved in citrate buffer (0.1M, pH 4.5) just before the injection. Hyperglycemia was allowed to develop for 72hrs [13]. Animals with Fasting Blood Glucose ≥250mg/dL were considered hyperglycemic and were included in this study. Control animals received a single intraperitoneal injection of 0.1M citrate buffer (1ml/kg bw; pH 4.5)

Twenty rats with an average weight of 130g were used in this experiment and were subdivided into 4 groups: A, B, C, and D, of five animals. The animals in group A were administered only distilled water and tagged the control group, Group B-induced hyperglycaemic wistar rats, Group C-Normoglycemic Wistar rats treated with 475mg/kg b.w of Spondias mombin aqueous leaves extract, Group D-induced hyperglycaemic Wistar rats treated with 475mg/kg b.w of *S. mombin* extract. All

these animals were given feed and water. The animals were treated daily at around 8am daily for 21days. The animals were weighed on alternate days using weighing scale. Blood glucose of the Wistar rats was checked weekly. The extract was administered orally, using a metal oral cannula. The treatment lasted for twenty-one days and was carried out at 07:00 hours daily.

2.7 Animal Sacrifice and Collection of Organs

All the Wistar rats were weighed and sacrificed twenty-four hours after the last administration by cervical dislocation method. The cranium of each animal was opened up using brain forceps and the whole brain was carefully removed and weighed, the cerebellum was then excised. Tissues were then fixed in formol calcium, and processed for histological observation using routine Haematoxylin and Eosin staining techniques and Nissl stain (Nissl).

2.8 Histological Techniques

Histological examination was carried out on the tissues fixed in formol calcium. Tissue blocks were sectioned for routine Hematoxylin and Eosin (H/E) and Nissl Stain. The fixed organs were cut in slabs of about 0.5cm thick transversely and transferred to 70% alcohol for dehydration. The tissues were passed through 90% and absolute alcohol and xylene for different durations before they were transferred into two changes of molten paraffin wax for 1 hour each in an oven at 65°C for infiltration. They were subsequently embedded and serially sectioned using a rotary microtome at six microns (6μ). The tissues were transferred onto albumenized slides and allowed to dry on a hot plate for 2 minutes. The slides were dewaxed with xylene and passed through absolute alcohol (2 changes); 70% alcohol, 50% alcohol and then to water for 5 minutes. The slides were then stained with hematoxylin and eosin.
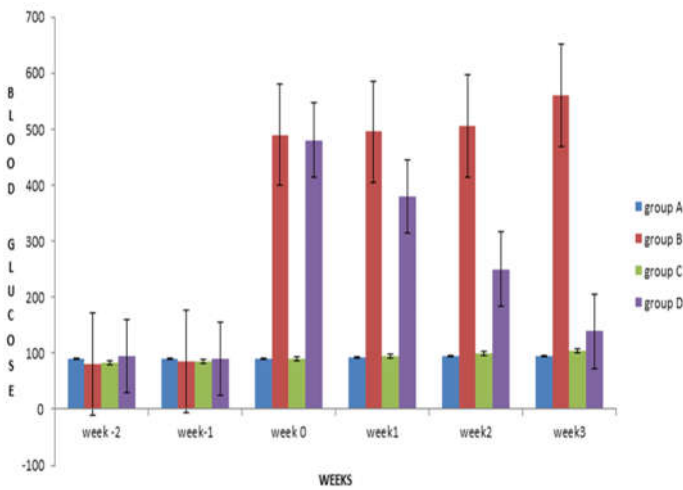
2.9 Data Analysis

The results were expressed as Mean ± standard error of Mean (SEM), and subjected to statistical analysis using the ANOVA Graph-Pad prism software package 6 for data analysis.
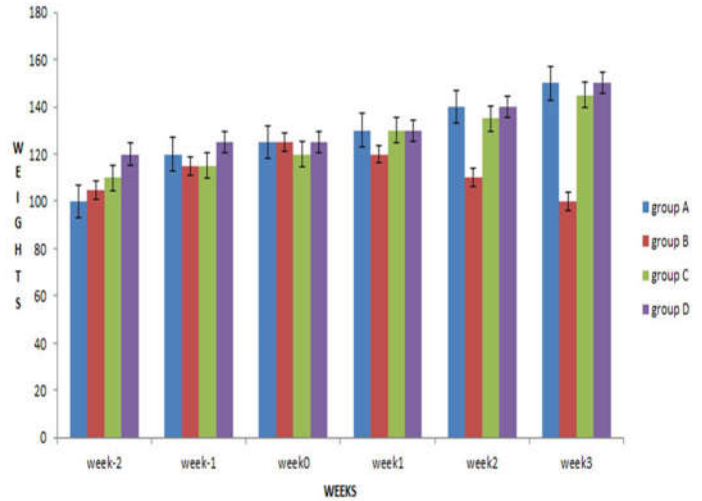
3.0 RESULTS

3.1 Blood Glucose on Weekly Basis (mg/dl)

Figure 1 showed the blood glucose of different groups on weekly basis. The untreated diabetic group rats were hyperglycaemic at week 0 until the end of the third week. The value was significantly different from normal control (P<0.05). Whereas the *S. mombin* aqueous leaves extract treated Diabetic groups had high blood glucose at week 0 up to week 2 which were significantly different from control (P<0.05). From the third week the blood glucose levels of Spondias mombin aqueous leaves extract treated groups were comparable to normal control and not significantly different at P>0.05. Figure 2 revealed average weight of the animals on weekly basis (Grams), increase in the weight was observed across the groups of the animal treated with *S. mombin* leaves extract and the control animals. However, a significant reduction in the weight among the hyperglycemic wistar rats that were not exposed to treatment with *S. mombin* leaves extract in relation to the hyperglycemic animal treated *S. mombin* leaves extract and the control animals that were given water *ad-libitum.*

Figure 2. Average Weight of the Animals on Weekly Basis (Grams)

Group A indicated an increase in the body weight of the animals from the 1st week to the 3rd week., **Group B** showed a decline in the body weight of the animals during the 1st week, followed by a rapid decrease in the body weight of the animals from 2nd to 3rd week., **Group C** also showed a tremendous increase in the body weight of the animals from the 1st week to the 3rd week, **Group D** indicated an increase in the body weight of the animals throughout the 1st week to the 3rd week.
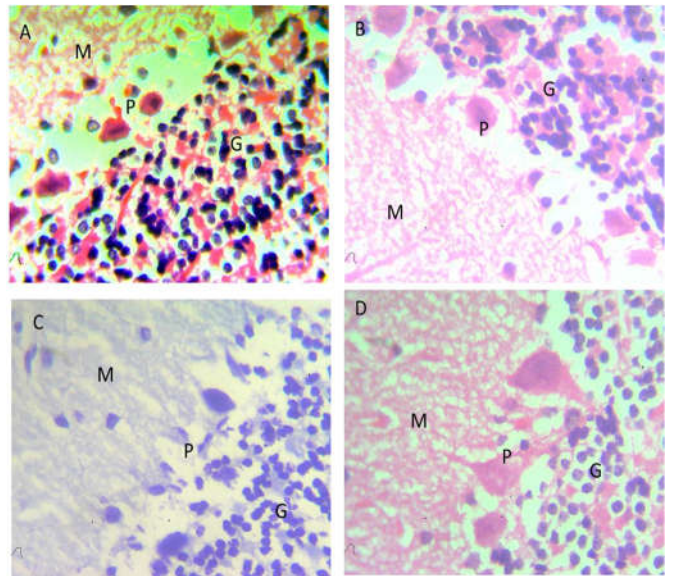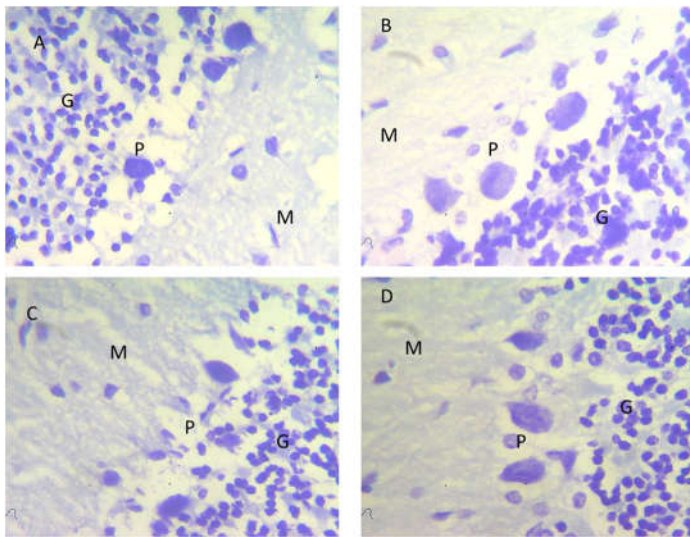

Figure 1. Blood Glucose on Weekly Basis (mg/dl).

Group A indicated an increase in blood glucose level of the animal after the induction of diabetes from the 1st week to 2nd week followed by a slight increase from 2nd week to 3rd week, **Group B** showed that there was an increase in the level of blood glucose level of the animal immediately after the induction of diabetes during the 1st week, followed by a slight increase in blood glucose during the 2nd week and a tremendous increase during the 3rd week., **Group C** showed an increase in the level of blood glucose of the animal throughout the weeks of administration, **Group D** showed a great increase in blood glucose level immediately after induction, followed by a tremendous decrease in the level of blood glucose from the 1st week to 3rd week

Plate 1. Detail histological session of the Cerebellum

Group A: (Normoglycemic Control), the molecular M, Purkinje P, and the Granular G layers expressed with the integrity of nuclei maintained across the layers, **Group B:** (Diabetic Control) Molecular layer M, the degenerating Purkinje layer with loss of the pyramidal cells, and the formation of vacuolation along the Granular layer, in **Group C:** (Normoglycemic treated *Spondias mombin*), Molecular layer, Purkinje layer, Granular layer, the nuclei integrity was maintained, however, pyramidal cells seen scattered along the purkinje layer, in **Group D:** (Diabetic treated with *Spondias mombin*, Molecular, Purkinje layer, and Granular layer showed increased and evidence of cell division and the pyramidal cells were well defined and expressed. H/E Stain, X400

Plate 2. Detail histological session of the Cerebellum

Group A: (Normoglycemic Control) the molecular M, Purkinje P, and the Granular G layers expressed with the integrity of nuclei maintained across the layers the Nissl substances expressed in the nuclei, **Group B:** (Diabetic Control) Molecular layer M, the degenerating Purkinje layer with loss of the pyramidal cells, and reduced Nissl substances stain along the Granular layer, **Group C:** (Normoglycemic treated *Spondias mombin*), Molecular layer, Purkinje layer, Granular layer, the nuclei integrity was maintained, however, pyramidal cells seen scattered along the purkinje layer, **Group D:** (Diabetic treated with *Spondias mombin*, Molecular, Purkinje layer, and Granular layer showed increased and evidence of cell division and the pyramidal cells were well defined and expressed and deeply stained for the Nissl granules. Nissl Stain, X400

3.2 Histological Observations

The Induction of hyperglycemia in animals model showed distortion in the molecular layer M of the cerebellum; the degenerating Purkinje layer with loss of the pyramidal cells, and the formation of vacuolation along the Granular layer, when compared with the histological features of the Cerebellar layers in control animals with the integrity of nuclei maintained across the layers. However, treatment with *S. mombin* aqueous leave extract maintained the nuclei integrity across the cerebellar layers however, pyramidal cells seen scattered along the purkinje layer. *S. mombin* leave extracts in hyperglycaemic animals showed increased and evidence of cell division in molecular, purkinje layer, and Granular layer as shown in Plate 1.

Increased neuronal density as shown by the stain intensity of the Nissl substances in the cerebellar layers among the hyperglycaemic animals treated with the *S. mombin* leave extracts demonstrates the anti-hyperglycaemic activities of the *S. mombin* leave extracts; Molecular, Purkinje layer, and Granular layer showed increased and evidence of

cell division and the pyramidal cells were well defined and expressed and deeply stained for the Nissl granules Diabetic/hyperglycaemic untreated animals revealed the Molecular layer M, the degenerating Purkinje layer with loss of the pyramidal cells, and reduced Nissl substances stain along the Granular layer as shown in Plate 2

4.0 DISCUSSION

The current study establishes the antidiabetic activity of *Spondias mombin* leaves using streptoxotozin-induced diabetic rats. The blood glucose level of groups A and C showed insignificant differences throughout the experimental period and there was no period it exceeded normal. The blood glucose of were in normal ranges throughout the experimental period indicating that water and *S. mombin* leaves extract have no effect on a normoglycermic condition.

The blood glucose level of the animals after the induction of Diabetes mellitus increased rapidly throughout the experimental period. Several studies have proven that distilled water has no effect on a diabetic condition [4] which is in agreement with this study. Induced diabetic animals showed a significant drop in blood glucose level and this proved that *S. mombin* leaves extract has some antihyperglycermic effects. Also, according to this study, the body weight of the experimental animals in groups A and C showed significant increase in contrast to what was observed in group B, with resultant reduction in the body weight throughout the period of observation. The observations are in agreement with the previously done related researches on the antiviral and antinflamation properties of *S. Mombin* [14, 15]. The antimicrobial, antibacterial, antifungal, and the antiviral properties of *S. mombin* have been reported in several studies [16, 17, 18, 19, 20].

The histological findings of the cerebellum of group B animals showed distorted histoarchitecture, neuropathological changes in some Purkinje cells including shrunken cells and darkly stained nuclei are clearly seen, suggesting apoptotic cell death compared to that of the Normal control group. This showed that diabetic condition had serious adverse effects on the histology of cerebellum and in accordance with our results, several studies had found that there were neuropathological changes of Purkinje cells which was induced by diabetes, including being smaller in size, denser cytoplasm, a darkly stained nucle-

us and more densely packed organelles in the cytoplasm [21].

The cerebellar sections of the animals treated with *S. mombin* extract revealed distortions in the histoarchitecture of the cerebellar cortex; cortical degenerative changes and vacoulations [22]. The granular layer showed neuronal degeneration, and Purkinje cell layer showed loss and degeneration of Purkinje cells which appeared as vacoulations and smaller sized Purkinje cells. The layers of the cerebellum show various stages of recovery Plate1 in line with the study by Gillespie *et. al.,* [23] showed that hexane extract of *S. mombin* showed beta lactamase inhibitory properties.

This proved that despite the fact that group D animals were diabetic, the treatment with *S. mombin* leaves extract was able to ameliorate the condition and slowed down the neuronal degeneration of the cerebellum [24]. From the present findings, it is evident that *S. mombin* leaves extract had some antihyperglemic ameliorative effects on the cerebellar architecture of diabetic wistar rats. The chemical study of leaf extracts from *Spondias* species shows the occurrence of quercetin, rutin and ellagic acid which indicates they have antioxidant and antimicrobial constituents [23]. The constituents' activities can be directly correlated to wound healing and anti- inflammatory characteristics [25, 26] hence, more researches should be carried out on the ethnopharmacological use of *S. mombin.*

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

AOA conceived and designed the study; **JBD** performed data analysis and wrote the manuscript ; **OFS, GVO** performed data collection and contributed to data analysis tools; **MTF** contributed to data analysis tools; **SAE** contributed to data analysis tools and analysis of data; **OOB** contributed to data collection and analysis of data

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