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ANTIDIABETIC POTENTIAL OF *Irvingia gabonensis* ON DIABETES INDUCED MOTOR IMPAIRMENT ON ALBINO RATS CEREBELLUM

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Hyperglycemia as a life threatening disease causes motor impairments which has been ignored by researchers and clinicians. The study investigated the antidiabetic potential of *Irvingia gabonensis (IG)* on diabetic induced motor disorder in albino rats. Thirty rats were assigned into 6 groups of 5 rats each. Diabetes was induced by a single intra-peritoneal injection of 60 mg/kg of Streptozotocin (STZ) and confirmed after 72 hours. Blood glucose was checked at interval of 5 days for sustained hyperglycemia. Groups C, D and E were treated with100, 200 and 300 mg/kg of IG while Group F received 500 mg/kg of metformin. Motor activities were tested using string method to ascertain the role of *IG* on motor impairment in diabetic rats. The supernatants of homogenates were used to assay for lipid profiles namely TChol, Trig, HDL and LDL. The result showed significant decrease in TChol, LDL, triglyceride and HDL across the treated groups compared to group B ($P \leq 0.05$). Grip strength significantly decreased in group B while the extract significantly increased the grip strength in Groups C, D and E (Table 2). Limb impairment was significantly reduced in group B compared to A and increased in groups C, D and E ($P \leq 0.05$). Microscopically, group B showed structural alterations in the cerebellum with structural improvement in treated groups C, D, and E compared to group B. In conclusion, *Ig* have the potential to improve grip strength and limb impairment which may be useful in addressing motor complications arising from diabetes.

Keywords: Antidiabetic; hyperglycemia; grip strength; limb impairment; *Irvingia gabonensis*; motor activities.

1. INTRODUCTION

Diabetes is one of the debilitating ailments associated with hyperglycemia and shows characteristics of dyslipidemia which serves as a risk factor for several diseases including heart diseases [1]. According to Muramatsu et al. [2]; diabetes is also classified as a group of metabolic disorders characterized by either deficiency or resistanceto insulin leading to hyperglycemia. Chronic hyperglycemia may be associated with long-term failure of many organs includingeyes, kidneys, heartas well as brain [3]. Usually, it is accompanied by peripheral neuropathy, nephropathy, retinopathy and angiopathy as reported by Cheng et al. [4]. According to Radan et al., 2009 [5]; hyperglycemia leads to increased cerebellar

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glucose concentration to about 3.0mmol/l and 35% more in the thalamus while in the white matter it increases to about 173%. The cerebellum has long been recognized as the primary center for motor coordination in the central nervous system [6]. If the glucose level reduces as a result of diabetes, it will lead to malfunctioning of the organs and tissues including cerebellum thereby affecting the cells [7]. Disorder in cerebellar structure by high blood sugar is due to type 1 diabetes mellitus [8,9]. It also causes a decrease in the number of neuron and cortical thickness as well as the white matter in neonatal rats [10]. Several studies have reported the adverse effects of hyperglycemia on developing brain to includeloss of sensory-cognitive and psychomotor functions and altered auditory recognition; reduce visual, memory performance [11]. Diabetic neuropathy increases the risk of motor dysfunction to risk of falling, increased body sway, altered gait and balance [12]. Hyperglycemia has been implicated significantly in increased risk of movement disability [13]. The body achieves movement and balance via sensory-motor control as a result of interaction from PNS and CNS, motor dysfunction are very prominent each time there is an alteration on the interaction [14]. Much attention have been paid to cognitive, memory and learning as well as motor impairment in diabetic with little or no attention to motor activities involved in hyperglycemia [15].

Streptozotocin (STZ) is a naturally occurring alkylating antineoplastic agent that is particularly toxic to the insulin-producing beta cells of the pancreas and can reduce the tumor size due to excessive insulin secretion [16]. *Irvingia gabonensis*is an edible tree and can be referred to as bush mango and African mango [17]. French named it mangue sauvage, Yourba called it Oro, Hausa named it goron, while Igbos called it ogbono and is an edible fruit tree indigenous to Africa [18]. It consists of root, stem, leaves and fruits. The fruit consists of a fleshy part and nut. The nut itself is made up of a hard shell and kernel (seed). The seeds of *Irvingia gabonensis* are used for treatment of dysentery, wound dressing and it reduces fasting blood glucose levels in Wistar rats [19]. The study was designed to assess the effect of *Irvingia gabonensis* on the cerebellum of diabetic Wistar rats and to investigate the antidiabetic potential of *Irvingia Gabonensis* on streptozotocin induced diabetes on cerebellum of adult Wistar rats.

2. MATERIALS AND METHODS

2.1 Extraction of Plant Seeds

The fresh *Ig* fruits gotten from Ikwo Local Government Area of Ebonyi state were carefully washed with clean water, broken and the seeds sun dried. The dried seeds weighing 1.2kg were grounded and transfer into conical flask and extracted by cold maceration with distilled water at room temperature (25^0C) for 48hours with intermittent shaking using Iwu's, technique [20]. The sludge was filtered and the filtrate concentrated to dryness using a water bath set at 40^oC to obtained cream colour which was stored at room temperature (25^0C) for further analysis.

2.2 Chemicals

Streptozotocin was purchased sigma Aldrich and was manufactured on May, 2018 and expires on May, 2023. Metformin was obtained from Octovia Pharmacy at Abakaliki, Ebonyi State. Buffer solution of PH 4.5 was freshly prepared in the laboratory and used to dissolve STZ.

2.3 Experimental Design

Thirty adult male Wistar rats were gotten from the animal house of AE-FUNAI and randomly assigned into 6 groups of 5 rats each after 7 days of acclimatization. The fasting blood glucose level of each rats were checked using Accu-chek glucometer to confirm that they were not diabetic. All the animals received 60 mg/kg of STZ except the control animals which received only feed and water *ad libitum.* The low, medium and high groups received 100 mg/kg, 200 mg/kg and 300mg/kg respectively while the standard drug group was given 500 mg/kg to compare with *Ig* and all treatment given orally which lasted for a period of 7 days [21,22].

2.4 Diabetes Induction

The animals after acclimatization was fasted for at least 12 hours and their weight recorded prior to the induction ofdiabetes.STZ was weighed out and dissolved in 0.1M of citrate buffer solution of PH 4.5. The buffer was freshly prepared and adjusted using PH meter. Diabetes was induced by single intra-peritoneal injection of 60 mg/kg of STZ. The rats were took neither feed nor water until after 30 minutes of induction. The animals were given 5% dextrose solution at an interval of 6 hours for 24 hours after induction of diabetes. The rats were confirmed diabetic 72 hours at fasting glucose level of 200 mg/dl.

2.5 Assessment of Grip Strength

Grip strength as a motor activity was assessed by the use of 2mm by 60cm steel wire in diameter and length respectively. The wire was hung on two poles at 50 cm high from a cushion support. The rats were placed

on the wire with it forelimbs and allowed to hold-onto it a maximum of 180 seconds. The time (latency) taken for the rat to release the paws and fall was recorded in seconds. The present experiment used the method of Tariq et al. [23] for measuring grip strength in rodents.

2.6 Assessment of Limb Impairment

The apparatus used in measuring limb impairment is same as that used for grip strength in the present experiment. It was accessed based on the techniques developed by Yoom et al. [24] where rats were scored 3 for holding on to the wire with both hind paws, 2 for gripping the wire with one hind paw and 0 for not gripping the wire with either of the hind paws.

2.7 Training

The pre-test (training) was conducted during acclimatization period of the animals. The pre-test lasted for a period of 3 days. This was aimed at training the rats on the apparatus and to get them acquainted with the apparatus and the procedure. The rats were allowed to hold the wire stretched between two poles and foam placed under it for a maximum of 180 seconds.

2.8 Animal Sacrifice

The animals were sacrifice by cervical dislocation after 24 hours of fasting; then collect blood samples from the apex of the heart for biochemical analysis. The animal was decapitated, skinned, and the skull fixed in Bouin's fluid. After 48 hours, the skull is excised and the cerebellum is harvested and fixed again in 10% formalin for histological studies.

2.9 Assay of Lipid Profile

The cerebellar were homogenized and collected in an experimental sample bottles before centrifuge at 4000 rmp for 10mins. The supernatants were used to access Total Cholesterol (TChol), Triglyceride (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL). TChol, TG, and HDL concentrations were determined using procedures modified by Wasan et al. [25] while LDL concentration level was calculated by the use of Friedewald equation.

2.10 Data Analysis

The data was analyzed with Statistical Package for Social Sciences (SPSS) and expressed as Mean ± Standard Error of Mean and significant difference was established using One Way Analysis of Variance (ANOVA) at $p \leq 0.05$.

3. RESULTS

3.1 Concentration of Blood Glucose level (mg/dL)

The result showed that group B had glucose level increased from 170.60±43.80 after three days of induction to 290.40±71.36on the third week compared to 89.80±3.46 and 89.00±10.05 in Group A ($P \leq 0.05$). The result of treatment of the rats with the extract of Irvingia gabonensis on the third week, Groups C, D, E and F showed a significant decrease in glucose level compared to the second week increment as presented in Table 1.

3.2 Grip Strength

Group B significantly increased in latency (81.19±10.75) compared to group A (72.46±9.33) at $P \leq 0.05$. The latency results of treatment groups (C, D, E and F) significantly decreased in compared to group B as shown in Table 2. The reduction was very high in the high dose group (43.31±9.46) during the test period.

Table 1. Effect of aqueous extract of *Ig* seed on blood glucose level (mg/dL) in streptozotocin induced diabetic rats

Groups/ Time	0 Hr (mg/dL)	72 Hrs (mg/dL)	1st Week (mg/dL)	2nd Week (mg/dL)	3rd Week (mg/dL)
A	94.40±8.03	89.80±3.46	72.80±5.00	60.80±2.20	89.00±10.05
B	89.60±5.10	170.60±43.80a	177.80±45.12^	281.40±74.04ô	290.40±71.36*
C	102.20±7.69	220.60±7.71a	228.40±7.26^	309.80±40.85 ô	238.60±41.57**
D	92.60±6.52	201.40±7.07a	214.00±5.79^	365.80±35.07 ô	297.20±27.07**
E	95.00±5.64	218.60±9.08a	226.20±8.51^	295.60±16.12 ô	246.60±20.26**
F	79.00±3.70	223.60±12.90a	235.20±10.25^	321.00±47.36 ô	244.20±47.52**

*Values represent mean ± SEM; n = 5. Group A is control; group B is diabetic untreated; group C received100 mg/kg of Ig; group D treated with 200 mg/kg of Ig, group E treated received 300 mg/kg of Ig while group F treated with 500 mg/kg of metformin. a Significant increase compared to 0 Hr at 0.01; ^ Significant increase compared to 72 Hrs at 0.01; ô Significant increase compared to 1st week at 0.01 and ** Significant decrease compared to 2nd week at 0.01*

Table 2. The effect of aqueous extract of *Invirngia gabonensis* (Ig) on the grip strength of streptozotocin induced diabetic rats

Group	Pre-Test (s)	Test (s)
A	51.23±10.30	72.46±9.33
B	73.81±9.73	81.19±10.75**
C	37.44±10.55	65.86±9.03*
D	103.26±67.52	63.54±5.48*
E	70.81±9.81	43.31±9.46*
F	48.81±4.29	46.40±8.07*

*N=5(number of samples); Mean± Standard Error of mean; *Significant decrease compared to group B test at P≤0.01; ** Significant increase compared to group A test at P≤0.01*

3.3 Limb Impairment

The results of the limb impairment assessment showed a decrease in Group B (2.50±0.11 and 2.29±0.14) compared to Group A (2.57±0.13 and 2.57±0.13) in both pre-test and test respectively (P≤ 0.01). When the results of after treatment in groups C, D, E and F were compared with group B, there was reduction arithmetically as presented in Table 3.

3.4 Lipid Profile

There was a significant increase in the level of TChol in Group B (3.23±0.065) compared to Group A (2.35 ± 0.013) (P≤0.05). The result of the administration of the *Ig*, showed that the level of TChol was significantly decreased in Groups C, D, E and F compared to Group B. The concentration of Triglyceride group B was significantly decreased (0.65±0.017) compared to Group A (0.81 ± 0.026) at P≤0.05. Triglyceride concentration significantly decreased in groups C and E compared to group B while in showed non-significant increase in groups D and F compared to group B. HDL increased in group B(0.87±0.033) compared to A (0.84 ± 0.041)while it gradually decreased in treated groups (C, D and E) compared to group B at P≤0.05, see Table 4.

3.5 Microscopical Examination of the Cerebellum

Microscopical examination of group A cerebellum showed normal layers, blood vessels and well-marked white and gray matters (Plate 1) while a section of group B showed severe vacuolation of purkinje cell layer, optical empty spaces due to cell necrosis, severe

Table 3. The effect of aqueous extract of *Invirngia gabonensis (Ig)* on the limb impairment of streptozotocin induced diabetic rats

Group	Pre-Test (s)	Test (s)
A	2.57±0.13	2.57±0.10
B	2.50±0.11	2.29±0.14
C	2.43±0.12	2.67±0.07
D	2.23±0.11	2.50±0.13
E	2.51±0.10	2.50±0.09
F	2.47±0.13	2.47±0.15

N=5(number of samples); Mean± Standard Error of mean. N/B: The increment and decrease ere not significant the limb impairment result

Table 4. The effect of aqueous extract of *Invirngia gabonensis (Ig)* on the lipid profile of streptozotocin induced diabetic rats

Groups	TChol (mg/dL)	Trig (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
A	2.35±0.013	0.81±0.026	0.84±0.041	1.13±0.022
B	3.23±0.065	0.65±0.017	0.87±0.033	1.85±0.016
C	2.48±0.019	0.58±0.010	0.65±0.023	1.58±0.011
D	2.84±0.032	0.97±0.014	0.73±0.042	1.72±0.011
E	2.27±0.011	0.52±0.013	0.58±0.025	1.51±0.021
F	2.42±0.012	0.88±0.013	0.87±0.027	1.39±0.018

N/B: TChol-Total Choleresterol; Trig-Triglyceride; HDL-High Density Lipoprotein; LDL-Low Density Lipoprotein; N=5; Mean ± SEM; P≤0.05

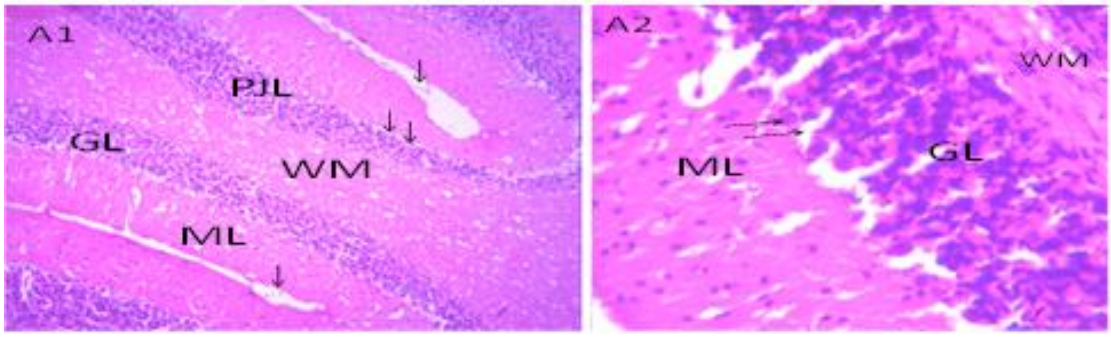

Plate 1. A section of group A cerebellum showing normal granular layer (GL), molecular layer (ML), purkinje cell layer (double arrows), blood vessels (arrow) as well as while matter (WM). (x100/400)(H/E)

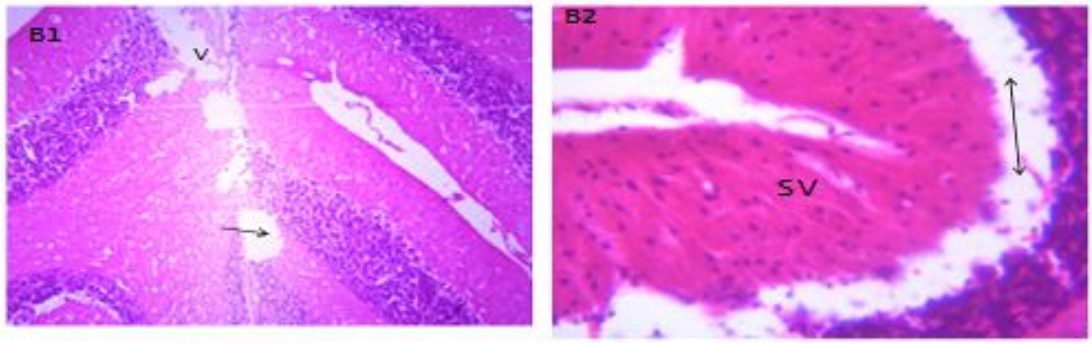

Plate 2. A section of group B cerebellum showing severe vacuolation of purkinje cell layer (v) , optical empty spaces due to necrosis (arrow) , severe separation of purkinje cell layer from granular layer (double head arrow) , severe hemorrhage of white matter (SV) . It also shows severe degeneration (x100 / 400) (H / E)

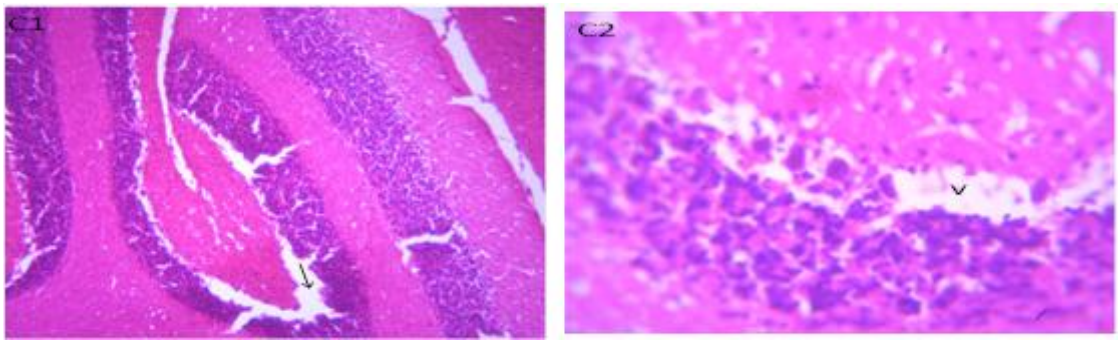

Plate 3. A section of group C cerebellum showing vacuclated purkinje cell layer (V), optical empty spaces due to necrosis (arrow), mild separation of purkinje cell layer layer (arrow). It also shows severe degeneration (x100 / 400)(H / E)

separation of purkinje cell layer from granular layer, severe hemorrhage and cell degeneration (Plate 2). Groups C, D and E showed recovery ranging from mild alterations to near normal of the cerebellar tissues. There were numerous large healthy nuclei, regenerated molecular layer as shown in Plates 3, 4 and 5. Group F showed features such as regenerated molecular cell layer, presence of blood vessel as well as moderate healing of cell layer (Plate 6).

4. DISCUSSION

In the present study, a significant elevation in glucose level was observed following diabetes induction in agreement with the works of Akbarzadeh, [26] and Abdulfatai et al., [27]. The glucose level in the rats had a sustained increase for two weeks before the commencement of treatment. On treatment of the animals with *Ig* extract, the low, medium and high

doses had a significant drop in the glucose levels during the third week as opposed to the diabetic untreated animals in which the glucose level was increasing. The significant reduction in the glucose levels observed during the administration of *Ig* aqueous extract was an indication of the antidiabetic potential of the Ig seed. The result was in agreement with the studies by Ogunwande et al., [28] which showed that *Ig* extract was capable of reducing blood glucose level.

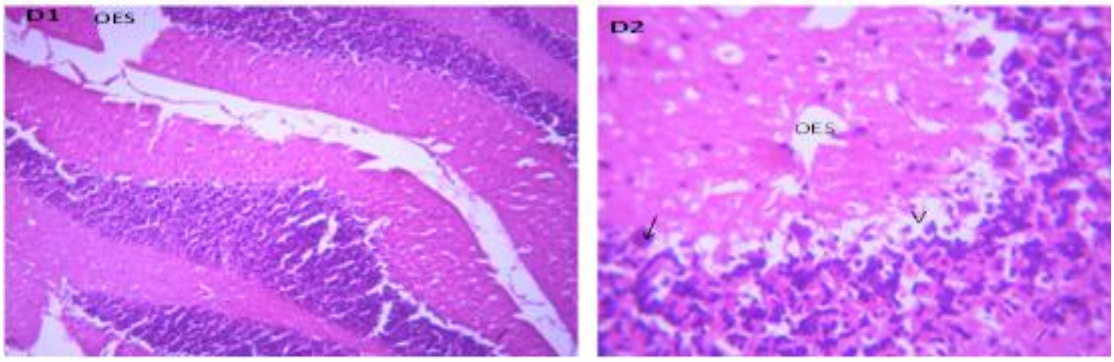

Plate 4. A section of group D cerebellum showing vacuolated purkinje cell layer (V), optical empty spaces due to necrosis (OES), neuron with large nucleus (arrow). (x100 / 400) (H/E)

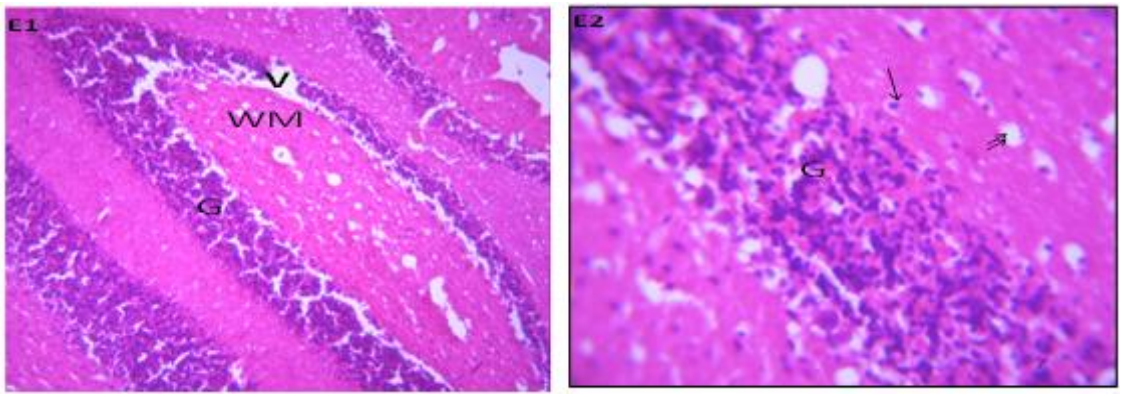

Plate 5. Photomicrograph of E section of cerebelhum showing mild vacuolation of granular layer (V) , white matter (WM) , granular layer (G) , regeneration molecular layer cells (arrows), blood vessel (double arrows) . Moderate healing with mild cystic space otherwise normal

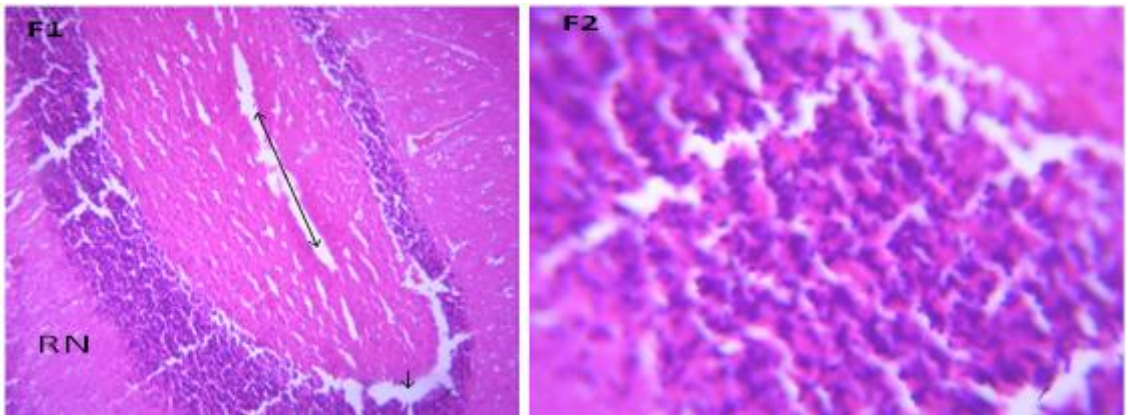

Plate 6. Photomicrograph of E section of cerebellum showing mild vacuolation purkinje cell layer (arrow) , regenerated molecular cells layer (RN), blood vessel (double arrows). (X100 / X40) (H / E) show moderate healing

Typically, string test is used in measuring grip strength and as traction apparatus to assess limb impairment in rodents [29]. Reduced latency in grip loss was an indication of compromised muscle strength and the ability to grasp and hold onto objects while the reduced scores on limb impairment indicated compromised limb function and strength [30]. The present study showed an increased latency in the diabetic untreated animals during the test period compared to the Control which was in disagreement with Kalyani et al., [31]. The increase in latency might be a pointer that hyperglycemic animals were more agitated and tends to hold unto objects better with an increased muscle tone. It may also be a prediction of increased and non-compromised muscle strength. All the treated rats showed a reduced latency of the grip strength compared to the untreated rats. The treatment with the *Ig* extract and the standard drug showed lowered latency as a measure of grip strength below the Control. In measuring limb impairment, there was a non-significant decrease in the scores in the diabetic untreated Group compared to the Control group which was in agreement with the findings of Kalyani et al., [31]. The administration of the Ig extract and the standard drug used in the treatment of diabetes, the result showed an elevation as a sign of improvement in the limb functions brought about by through the activities of the extract.

An increase in the concentration of total cholesterol in the lipid study of the hyperglycemic rats was always accompanied by elevated levels of triglyceride which triggered the activation of the enzyme, lipoprotein lipase that has been identified as an artherogenic risk factor with a low level of HDL which agrees with the present study [32]. When the concentration of LDL is high, it is a precursor for coronary activity depending on the other risk factors which can lead to brain damage that may hamper cerebellar motor activities [33]. According to Arvill et al., [34]; the extract has the capacity of binding to bile in the gut to take them out of the body in the feces, which requires the body to convert more cholesterol into bile acids. The process results in lowering blood cholesterol and other lipids in the blood stream. This agrees with the present study where the administration of the extract resulted in the reduction of total cholesterol leading to the decrease in the other blood lipids. Controlled double-blind studies have shown that the administration of several grams of *Irvingia gabonensis* extract significantly reduced Tchol, LDL and trig and in some cases raised HDL [35]. The results agreed with the present study on reducing Tchol, LDL and trig while it disagreed with the increased HDL. In the present study, total cholesterol was increased in the diabetic untreated animals compared to the Control group which may have led to the cerebellar damage as it is capable of hampering blood supply which was in agreement with the work of Kou et al., [36].

Streptozotocin induction of diabetes in rodents caused distortions of different types in the cerebellar histo-structure compared to the Control which was normal showing all the underlying cerebellar layers. The histological distortion caused by diabetes in the cerebellum was an indication of its ability in altering motor activities of the rodents as well as motor learning. The features of the cerebellum of the untreated rats showed a correlation with the increased total cholesterol concentration compared to the Control which depicts increased cellular damage within the brain [37]. The administration of the extract showed to have reduced the effects of the diabetes in the cerebellar structure to near normal in dose dependent manner. The decreased cerebellar damage was evident from the structures of the cerebellum of the treated rats. Furthermore, the standard drug group (metformin) also played a vital role in returning the histology of the cerebellum to near normal by repairing the alterations to normal.

5. CONCLUSION

Irvingia gabonensis seed has been identified as a natural antidiabetic agent capable of lowering blood glucose level. It also reduced total cholesterol, low density lipoprotein, Triglycerides and high density lipoprotein thereby protecting the arteries that supply the body at large and brain in particular. It can serve a remedial purpose for people with hyperglycemia for the improvement of functions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The research adhered strictly to the approved guidelines by National Guide for the Care and Use of Laboratory Animals. The ethical clearance was obtained from Alex Ekwueme Federal University Ndufu-Alike Nigeria (AE-FUNAI) Animal Use and Research Ethical Committee with Reference Number AE-FUNAI-2021/0022352 and all efforts was geared towards minimizing animal suffering during sample collections and sacrifice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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