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Alstonia boonei Leaf Mitigated Deleterious Effects of Experimental Type-II-Diabetes-Mellitus against Pancreatic and Neurocognitive Functions

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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

The search for prompt, less toxic and economically affordable medical therapy has facilitated the increased investigation into the therapeutic potentials and applications of plants samples against common tropical ailments. This study was designed to investigate the reported link between experimental type II diabetes mellitus (T2DM) and neurocognitive decline and the justification for the use *Alstonia boonei* leaf in similar treatments. Experimental T2DM were treated with 100, 1000 and 1600 mg/kg BWT of the *Alstonia boonei* leaf and glibenclamide (100 mg/kg BWT). Biochemical analyses were used to determine effects on the pancreatic and neuronal indices of tissues functions; oxidative stress; excitation; and inflammation; blood glucose and insulin concentrations. The study revealed that T2DM and *Alstonia boonei* leaf affected the activity of tyrosine hydroxylase which is the regulating enzyme for the biosynthesis of dopamine. The oxidative and inflammatory stress exacerbated by T2DM was mitigated by a significant reduction of TNF-α and an increase of GSH, NP-SH, GPx, SOD and GST levels. *Alstonia boonei* leaf reversed the insulin resistance by the cells, with effective transduction of insulin signal and a corresponding reduction of circulating

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glucose. *Alstonia boonei* leaf demonstrated hypoglycaemic effect, and mitigated the neurodegeneration that ensued from the diabetic induction.

Keywords: Neurocognition; Alstonia boonei leaf; inflammation; oxidative stress; pancreas; high fat diet; streptozotocin.

1. INTRODUCTION

Diabetes mellitus (DM) is an autoimmune disease which has claimed millions of lives all over the world and its complications had exceeded hyperglycaemia and/or insulin resistance, it has been implicated in cognitive defects as well as many other clinical issues. Diabetes mellitus is a chronic, complex metabolic derangement characterized by hyperglycaemia, insulin resistance and oxidative stress resulting from the acquired glycation end-products metabolism resulting from high circulatory glucose [1]. Some studies have suggested that insulin accelerates Alzheimer-related pathology through its effect on amyloid-beta (AB) metabolism, tau phosphorylation [2,3,4] and methyl glyoxal (advance glycation end-product-AGE), which is a promoter of neuronal cell death via AGE formation which in turn leads to neurofibrillary tangle [5]. A post-mortem report on the brain of Alzheimer's disease (AD) patients showed that AD may be a neuroendocrine disease associated with insulin signalling. The team termed it type 3 diabetes because it harbours elements of both types 1 and 2 diabetes, since there is both a decrease in the production of insulin and a resistance to insulin receptors [6].

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is synthesized by *Streptomycetes achromogenes* and it is a diabetogenic drug which induces both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM is known as type 1 DM and NIDDM is known as type 2 DM). The frequently used single intravenous dose in adult rats to induce IDDM is between 40 and 60 mg/kg body weight (BWT) [7]. STZ impairs glucose oxidation [8] and decreases insulin biosynthesis and secretion [9,10]. It was revealed that STZ nullifies the β-cell response to glucose. A non-permanent reappearance of responsiveness was later manifested, followed by the permanent loss of the responsiveness and cellular damage [11]. STZ is absorbed by β-cells of the pancreas through glucose transporter GLUT2. Diabetogenic effect of STZ is inhibited by reduced expression of GLUT2 [12]. The expression of GLUT2 can be restricted by STZ, this was discovered in a study on the *in vivo* and *in vitro* multiple doses/concentration effects of STZ. Intracellularly STZ acts by causing mutational changes of DNA in pancreatic β-cells resulting into its fragmentation [13,14]. Experiments have proven that the main reason for the STZ-induced B cell death is alkylation of DNA [15,16]. The ability of STZ to alkylate is related to the nitrosourea moiety. The injections of STZ into rats caused the elevation of different methylated purines in tissues of these animals [17].

The common anti-diabetic therapies are insulin and various oral drugs such as thiazolidinediones, sulfonylureas and α-glucosidase inhibitors, these drugs are used independently or dependently to achieve improvement in the diabetic status of the patient [18]. The aforementioned drugs controls glycaemic load, thus delaying the prognosis of a diabetic condition, but not completely restore diabetic complications. These diabetic drugs are characterized by serious complications resulting from prolonged use [18]. Therapeutic options consulted should possess anti-hyperglycaemic, anti-hyperlipidaemia and antioxidant properties, with proven long-term safety [19]. The quest for these features in a drug has caused the shift of focus to medicinal plants to offer new and promising efficiency in drugs with minimal side effects and economical [20]. The dependence of human on herbs for pharmacological interventions could result into toxic outcomes, this outcomes had persisted before the advent of orthodox medicine [21]. Antioxidants such as flavonoids, coumarins, curcuminoids, xanthons, phenolics, and terpenoids are present in various plant products [22]. This is the justification for a growing interest in characterizing and isolating natural antioxidant compounds isolated from the plants, which have proven as therapeutic in modern medicine [23]. *Alstonia boonei* leaf has been used remarkably for the treatment of several ailments ranging from malaria, fever [24], intestinal helminths, rheumatism and hypertension [25-27]. It has also been reported as an anti-inflammatory and analgesic agent [28]. There are folkloric evidence that the leaf is

therapeutic against ailments with complications such as oxidative stress, inflammation vis-a-vis cellular degeneration.

This study investigated the anti-diabetic effects of *Alstonia boonei* leaf against high dose of streptozotocin intraperitoneal induction in Wistar rats with emphasis on the effects on pancreatic and neurocognitive functions, and oxidative status.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Biochemistry laboratory of the Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Nigeria from January, 2019 to June, 2019).

2.2 Collection of *Alstonia boonei* Leaf

Alstonia boonei leaf was harvested from the plant garden within the premises of the Rufus Giwa Polytechnic, Owo, Nigeria. The leaves were rinsed in running water, wiped and air-dried. The dried leaves were pulverized with an industrial blender Beltone Luinohun Blender/Miller III (model MS-223, Taipei, Taiwan) and stored before extraction of phytochemicals.

2.3 Extraction of Phytochemicals from *Alstonia boonei* Leaf

The powdered material was stocked in a sealed plastic container from which 1000 g was mixed with 2.5 L of distilled 80% methanol and stirred for 48 h at room temperature in a shaking water bathe. The filtrate was collected initially through a fine linen cloth and finally through Whatman filter paper (Whatman Clifton, NJ, USA). The filtrate was concentrated and the solvent removed under reduced pressure in a rotary film evaporator and freeze-dried using a freeze-drying machine to give dried residue. The extract was stored in an air-tight container in a desiccator at 4◦C until needed.

2.4 GC-MS Characterisation

The extract was concentrated to 1ml of which 1µl was injected into the injection port of the gas chromatography. The GC equipment used was HP 6890 powered with HP chemstation Rev. A09.01 (1206) software. The split ratio will be 20:1, the carrier gas was nitrogen at inlet temperature of 250 ^{o}C with a column type of HP INNOWax and column dimensions of 30 m x 0.25mm x 0.25µm. The oven program parameters include initial temperature at 60 ^{o}C, first ramping at 12 ^{o}C/min for 20 min, maintain for 2 min and second ramping at 15 ^{o}C/min for 3 min, maintained for 8 min. The detector used FID at 320 ^{o}C at hydrogen pressure 22psi and compressed air of 35psi.

2.5 Experimental Design

2.5.1 Animals

Adult male Wistar albino rats, weighing 180±20 g were received from experimental Animal Care Center (University of Ilorin, Kwara State. Nigeria). All animals were maintained under controlled conditions of temperature (22±1°C), humidity (50-55%) and light (12 h light/12 h dark cycle). They were acclimatized to the laboratory conditions for 14 days before the start of the experiment. Animals had free access to rat chow and drinking water.

2.5.2 Diabetic induction

Male albino rats were subjected to a high fat diet, total kcal value of 40 kJ/kg (20% fat, 45% carbohydrate, 22% protein) for 4 weeks Experimental diabetes was induced by a single dose of STZ (35 mg/kg, i.p.) in overnight fasted rats by dissolving in freshly prepared 5 mmol/L citrate buffer, pH 4.5. After STZ injection, the rats had free access to glucose solution (5%) for 24 h to avoid and/or attenuate subsequent inevitable hyperinsulinemia and hypoglycaemic shock. Forty-eight hours after the STZ injection, animals fasted overnight and a drop of blood samples were analysed for glucose levels (mg/dl) by using strips on glucometer (ACCU-CHEK ACTIVE, Roche, Germany). Individual glucose levels reached above 250 mg/dl are considered as diabetic and screened for use in the study which lasted for 28 days of therapeutic oral administration of *Alstonia boonei* leaf extract and glibenclamide.

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution;
Group 2: 4 weeks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of *Alstonia boonei* leaf extract;
Group 3: 4 weeks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of *Alstonia boonei* leaf extract;
Group 4: 4 weeks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of *Alstonia boonei* leaf extract;
Group 5: 4 weeks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and

Group 6: 4 weeks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control).

2.6 Necropsy, Blood Collection and Preparation

Eighteen hours after the last administration, rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture into non-anticoagulant serum tubes and allowed to stand for 1h. The clotted blood was centrifuged at 4^{o}C for 10min at 3000g. The serum was transferred into clean tubes for the evaluation of biochemical indices.

2.7 Tissue Parameters

The animals were quickly dissected and the brain and pancreas were removed and rinsed in ice-cold 1.15% potassium chloride solution. These were weighed and homogenized in 0.1 M potassium phosphate buffer (pH 7.4) by using a Teflon homogenizer (MP Biomedicals India Pvt Limited). The homogenized tissues were centrifuged at 3000 g for 10 min at 4ºC, and the supernatant was collected and stored in clean tissue supernatant collecting bottles and stored at 4^{o}C until when required for biochemical studies.

2.8 Determination of Blood Glucose and Serum Insulin Concentration

Blood samples of the individual animal were read using strips on glucometer (ACCU-CHEK ACTIVE, Roche, Germany) before and after treatment. The subtraction of these values produced the differences in blood glucose concentration. The concentration of serum insulin was determined using Abnova Insulin CLIA kit; CAT Number: KA2801

2.9 Effects on Neurocognitive Indices

2.9.1` Determination of dopamine concentration

The concentration of dopamine was evaluated [29].

2.9.2 Determination of tyrosine hydroxylase activity

The activity of tyrosine hydroxylase in the brain was determined with slight modification of spectrophotometric method described by Shiman et al [30].

2.10 Effects on Antioxidant and Excitatory Indices

2.10.1 Determination of Na^{+}/K^{+} ATPase activity

The activity of Na^{+}/K^{+} ATPase was determined as described and illustrated by Sunil et al. [31].

2.10.2 Determination of glutathione peroxidase (GPx) activity

GPx activity was determined by the change in the absorbance value at 340nm and expressed as micromoles of NADPH/minute/µg protein [32].

2.10.3 Determination of glutathione transferase activity

The activity of GST was determined by the method described by Habig et al. [33].

2.10.4 Determination of Reduced Glutathione (GSH) Concentration

The method of Jollow et al. [34] was followed in estimating the level of reduced glutathione (GSH).

2.10.5 Determination of superoxide dismutase (SOD) activity

SOD activity was determined by the method of Misra and Fridovich [35].

2.10.6 Determination of TBARS

A thiobarbituric acid reactive substances (TBARS) assay kit manufactured by Elabscience limited (USA) was adopted to determine the lipid peroxidation product in MDA equivalent.

2.10.7 Determination of NP-SH concentration

NP-SH was measured using the method of Jain and Jangir [36].

2.11 Effects on Pro-Inflammatory Response

2.11.1 Determination of tumor necrosis factor-α (TNF-α) Concentration

One hundred (100) µL of standard or sample was put into each well for 90 min at 37°C, 100µL Biotin-detection antibody working solution was added to each well for 60 min at 37°C. The mixture was aspirated and washed 3 times. Then 100µL of SABC working solution was added to

each well and incubated for 30 min at 37°C. The mixture was aspirated, washed 5 times and 90 µL TMB substrate was added and then incubated for 30 min at 37°C. The volume of 50 µL Stop Solution. The absorbance was read at 450 nm immediately and results were calculated.

> The relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well).

The standard curve was plotted as the relative O.D.450 of each standard solution (Y) against the respective concentration of the standard solution (X). y=0.0032x

2.12 Statistical Analysis

All values are expressed as mean±standard deviation. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The significance level was set at $p \leq 0.05$.

3. RESULT AND DISCUSSION

3.1 Phytochemical Composition of an Extract of *Alstonia boonei* Leaf

The methanolic extract of *Alstonia boonei* leaf (MEABL) was characterized (Fig. 1) to comprise eugenol, caryophyllene, phenol, bicycle (3.1.1) heptane, 1-heneicosanol, phytol and 1-docosene. Eugenol was recorded as the most prominent phytochemical present in MEABL, it was reported as a bioactive phytochemical which had demonstrated positive effects against cancer [37] and oxidative stress as an antioxidant agent via inhibition of lipid peroxidation [38]. Eugenol was known to destabilize the lipid membrane of bacteria by the inhibition of ATPase, increasing the permeability and facilitating the uptake of toxic materials into the cell [39], it has also been reported to facilitate the blockage of octopamine receptor binding site causing neurotoxicity as insecticide [40]. Phytol was discovered in a small amount in the methanol extract, it is a terpenoid that was discovered to be a strong antioxidant, possessing anti-microbial, anti-inflammatory and anti-proliferative properties [41]. Phytol is a precursor of synthetic tocopherol and vitamin K. Phytol was reported as cytotoxic against breast cancer cell lines MCF7 [42]. These phytochemicals detected in MEABL may be responsible for the hypoglycaemic effects as well as protection of pancreatic and neuronal tissues from free radicals generated from the metabolism of streptozotocin.

3.2 Effects of Treatment on the Expression, Secretion and Resistance of Insulin, and Glucose Concentration

Fig. 2 revealed the changes in blood glucose as a result of the treatments, the HFD and intraperitoneal intoxication of streptozotocin-induced hyperglycaemia as expected, raised the blood glucose of the animals, indicating offset of hyperglycaemic condition. The untreated animals suffered further increase in the blood glucose concentration at 28th day of hyperglycaemic assault. The differences in the blood glucose concentration as revealed in Fig. 2 reflected a dose dependent effects, defined as decrease in the difference in blood glucose concentration as the doses decreased from 1600 to 100 mg/kg BWT. Similar hypoglycaemic effect was recorded for Olea European leaves extract [43] and quail egg yolk [1].

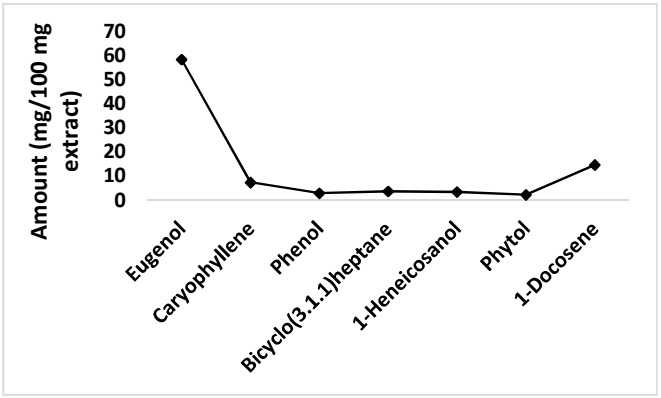

Fig. 1. Phytochemical characteristics of the methanolic extract of *Alstonia boonei* leaf

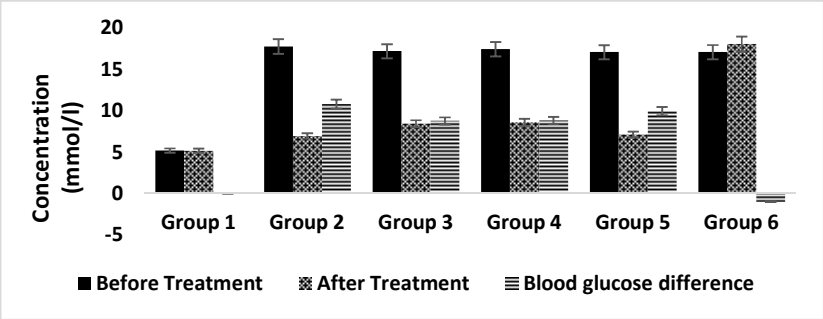

Fig. 2. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the change in blood glucose concentration in albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600 mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000 mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100 mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100 mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

Fig. 3 revealed the effect of the HFD/STZ treatments on serum insulin, HFD/STZ initiated hyperinsulinemia in the animals, and this was evident from the result, the rats in the negative control group exhibited the high insulin concentration than the treatment group but significantly lower than average concentration of the positive control group. The treatments with the doses of MEABL reversed the deleterious effects caused by STZ in the pancreas and the insulin resistance supposedly initiated by the HFD at the insulin receptor points of cells, regulated insulin concentration was restored in a dose treatment with MEABL, the experimental groups reduced the amount of serum insulin significantly. The result on serum insulin revealed a confirmation of several studies that defined hyperinsulinemia as key feature of type 2 diabetes mellitus [44,1]. A high fat diet and a low dose of STZ model of type 2 diabetes mellitus increased the concentration of serum insulin in circulation. This is contrary to type 1 DM condition where the concentration of insulin is depleted as a result of the complete degeneration of the β-cells of the Langerhans [53,1].

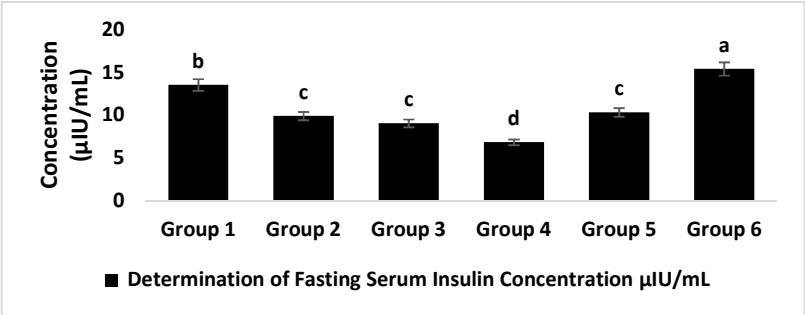

Fig. 3. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the concentration of plasma insulin in albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

3.3 Effects of Treatment on Oxidative Stress and Excitation

Fig. 4 revealed the effects of glibenclamide and Alstonia boonei leaf extract against glutathione depletion by experimental model of T2DM. Glutathione was depleted during diabetic state, this was revealed in Group 6 (positive control) which had significantly ($p \leq 0.05$) least concentrations of glutathione in the brain and in the pancreas. Alstonia boonei leaf extract demonstrated a dose-dependent effects through increased expression of glutathione in both the pancreas and the brain. The brain depends majorly on glutathione for its antioxidant feedback. Glibenclamide treatment resulted into increase in the concentration of glutathione in the pancreas than the outcome revealed by Alstonia boonei leaf extract, however, the effect of glibenclamide in the brain was contrariwise.

Superoxide dismutase (SOD) activity was inhibited in T2DM as revealed in Fig. 5. Treatment with 100, 1000 and 1600 mg/kg BWT of Alstonia boonei leaf extract reversed the inhibition of SOD significantly ($p \leq 0.05$) in a dose-dependent manner in the brain and the pancreas. Glibenclamide had significant effects than Alstonia boonei leaf extract in the pancreas but lesser effect in the brain.

Effect of T2DM on malondialdehyde (MDA) which is a product of lipid peroxidation was revealed in Fig. 6. The result confirmed that diabetes increased the rate of lipid peroxidation and the amount of MDA produced by the deleterious activities of free radicals. There was an elevated amount of MDA generated in the brain and pancreas, this was reduced dose-dependently by Alstonia boonei leaf extract. Glibenclamide significantly ($p \leq 0.05$) inhibited lipid peroxidation that yielded in both the brain and pancreas, however the effect on the brain was significantly ($p \leq 0.05$) lower compared to the effects manifested by Alstonia boonei leaf extract.

Effects of HFD/STZ experimental models of diabetes mellitus on brain and pancreatic GST as revealed in Fig. 7 showed a significant decrease in the enzyme activity of glutathione transferase (GST). Alstonia boonei leaf extract demonstrated a dose-dependent effects against the decrease in the activity of GST which resulted from depleted GSH by the overwhelming activity of the free radical resulting into oxidative stress. Alstonia boonei leaf extract demonstrated elevation of GST activity in the brain and the pancreas. Glibenclamide treatment also significantly ($p \leq 0.05$) increased the activity of GST in both brain and pancreas, however, the effects recorded by Alstonia boonei leaf extract was more overwhelming on the brain than pancreas.

Non-protein thiol (NP-SH) in both brain and pancreas was depleted by T2DM (Fig. 8), the concentration of NP-SH in both brain and pancreas was significantly ($p \leq 0.05$) increased by treatment with Alstonia boonei leaf extract. Glibenclamide had significant ($p \leq 0.05$) effect on the brain and pancreas, however, it did not have higher effect as Alstonia boonei leaf extract on the brain.

The activity of glutathione peroxidase (GPx) in both brain and pancreas was evaluated after HFD/STZ induction of T2DM vis a vis Alstonia boonei leaf extract and glibenclamide (Fig. 9). The effects of Alstonia boonei leaf extract was dose-dependent with significant ($p \leq 0.05$) increase in the activity of GPx in both brain and pancreas. Glibenclamide had similar single dose effects which increased the activity of the antioxidant enzyme in the brain and pancreas.

Experimental evidences have revealed a direct connection between free radical-mediated oxidative stress and diabetes mellitus by the evaluation of oxidative stress bio-indices in both diabetic human and rodents [45]. A hyperglycaemic state can increase the progression oxidative stress, vis-a-vis elevation of DNA damage markers such as 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-oxo-7, 8-dihydro-2'-deoxyguanosine; lipid peroxidation products measured as thiobarbituric acid-reactive substances (TBARS); protein oxidation products such as nitrotyrosine and carbonyl levels coupled with reduced endogenous antioxidant enzymes levels [45]. The increasing deterioration by hyperglycaemia resulted from accumulated circulatory glucose which biosynthesized into methyl glyoxal (advance glycation end-product-AGE), generating free radicals that degenerate biological molecules. The progression of this deterioration by these deleterious molecules can be tamed through the elevation of the antioxidant status of the subjects, which could be derived from phytochemical-rich plants. Oxidative stress is strongly implicated in chronic hyperglycaemia resulting from insulin resistance [46]. Oxidative stress leads to protein or enzyme inactivation such as SOD, GPX, CAT and reduced glutathione and reduction in these proteins

promotes the progression of the stress [47]. *Alstonia boonei* leaf demonstrated these antioxidant effects by the elevation of the activities of endogenous antioxidant enzymes-glutathione peroxidase (GPx), glutathione transferase (GST), superoxide dismutase (SOD), and concentrations of non-protein thiol (NPSH), reduced glutathione (GSH; Fig. 4), and a corresponding reduction of MDA through the inhibition of peroxidation of lipid molecules in the brain and the pancreas. The functional stability in the brain and the pancreas is hinged on the maintenance of oxidative and homeostatic balance which is dependent on the bioactive compounds present in *Alstonia boonei* leaf.

The effect of T2DM on Na+/K+ ATPase activity was revealed in Fig. 10. Na+/K+ ATPase activity was depressed in the brain and pancreas as a result of the assault, and extract of *Alstonia boonei* leaf and glibenclamide demonstrated significant mitigation of the ionic disturbance caused by the inhibition of this enzyme. Na+/K+ ATPase is an ubiquitous membrane enzyme that allows the extrusion of three sodium ions from the cell and two potassium ion from the extracellular to maintain cellular physiology. The activity of this enzyme irrespective of the specificity of the cell is inhibited by STZ intoxication.

3.4 Effects of Treatments on Inflammatory Response

It was indicated that the enhanced proinflammatory phenotype in T2DM not only affected complications like cardiovascular disease [48] but also exacerbated other pathologies such as dementia and social withdrawal induced by activation of the innate immune system with lipopolysaccharide or hypoxia [49]. These research findings were confirmed in this present study. HFD/STZ model of type II diabetes mellitus caused elevation of tumour necrosis factor-α (TNF-α) in the brain and the pancreas (Fig. 11), extract of *Alstonia boonei* leaf mitigated and inhibited this inflammatory response in both the brain and the pancreas. *Alstonia boonei* leaf proved effective as an anti-inflammatory agent protecting the neuronal cells and pancreatic β-cells of Langerhans. Pickup et al. [50] suggested that T2DM was a proinflammatory disease involving activation of the innate immune system [50].

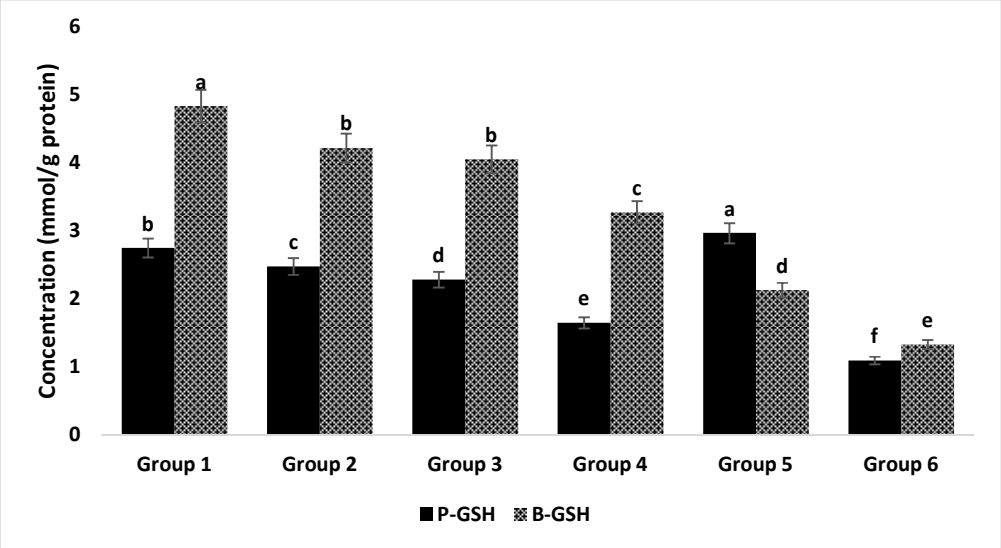

Fig. 4. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the concentration of reduced glutathione in the brain (B-GSH) and pancreas (P-GSH) of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

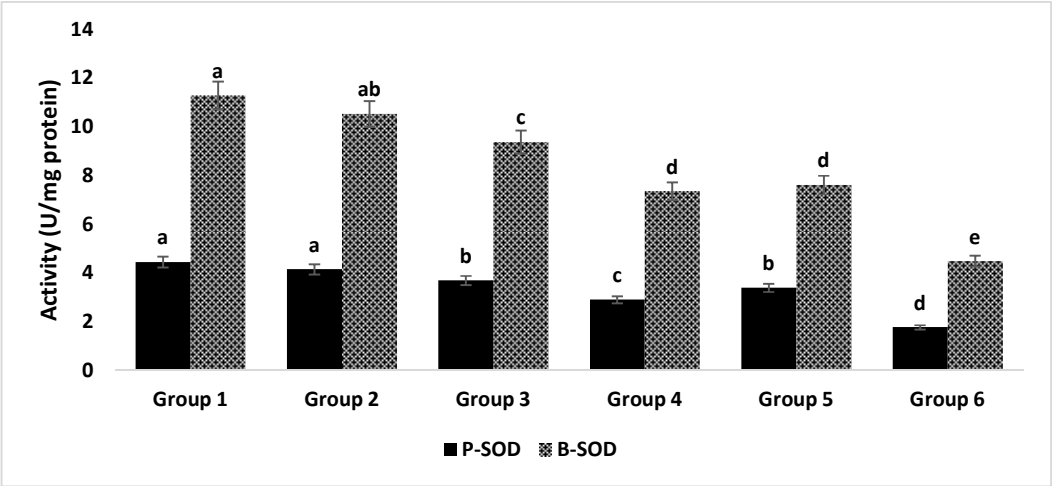

Fig. 5. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the activity of superoxide dismutase in the brain (B-SOD) and pancreas (P-SOD) of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

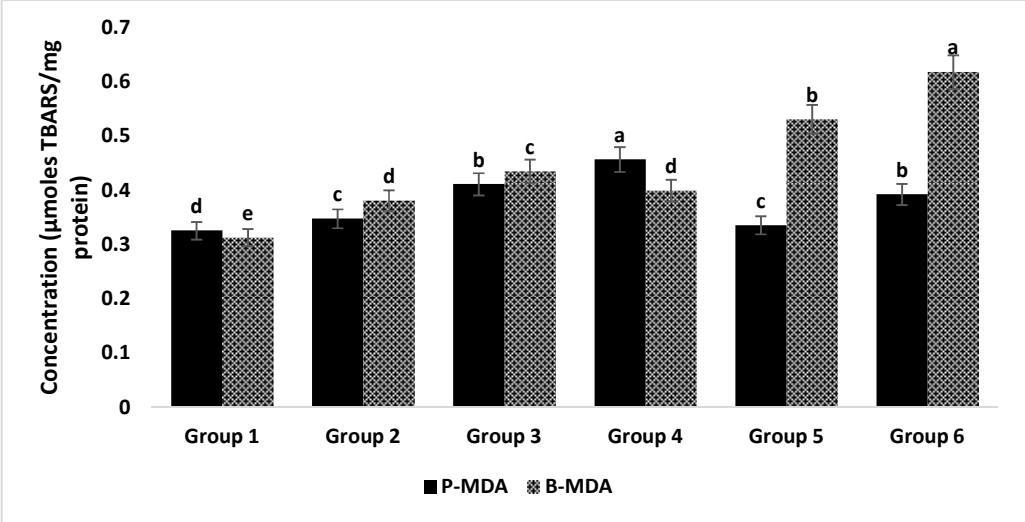

Fig. 6. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the concentration of malondialdehyde in the brain (B-MDA) and pancreas (P-MDA) of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

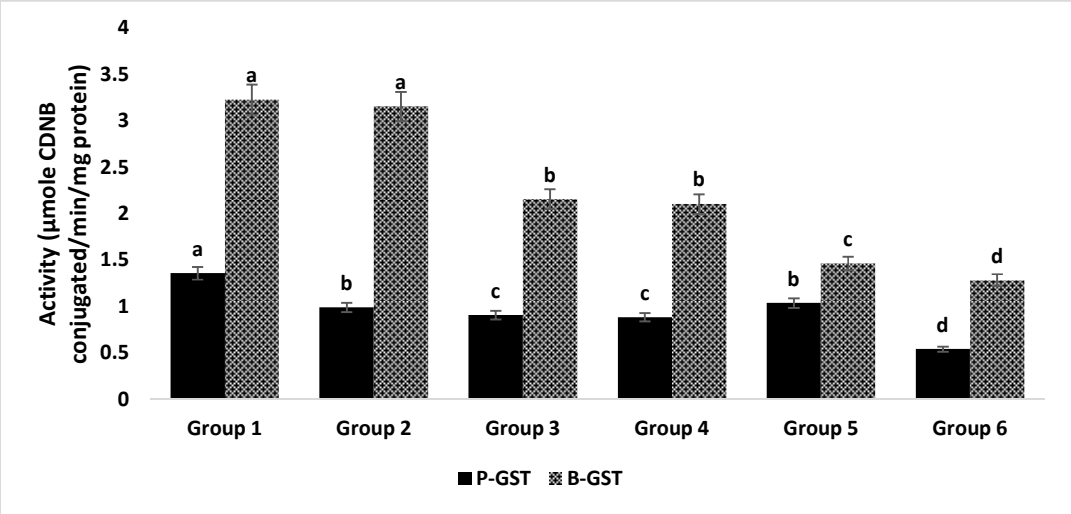

Fig. 7. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the activity of glutathione transferase in the brain (B-GST) and pancreas (P-GST) of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

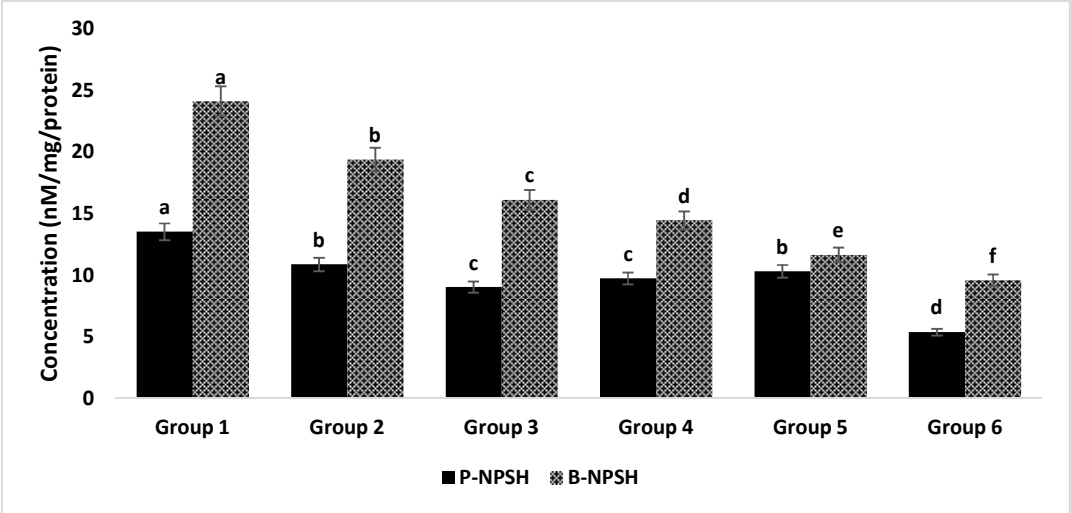

Fig. 8. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the concentration of non-protein thiol in the brain (B-NPSH) and pancreas (P-NPSH) of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

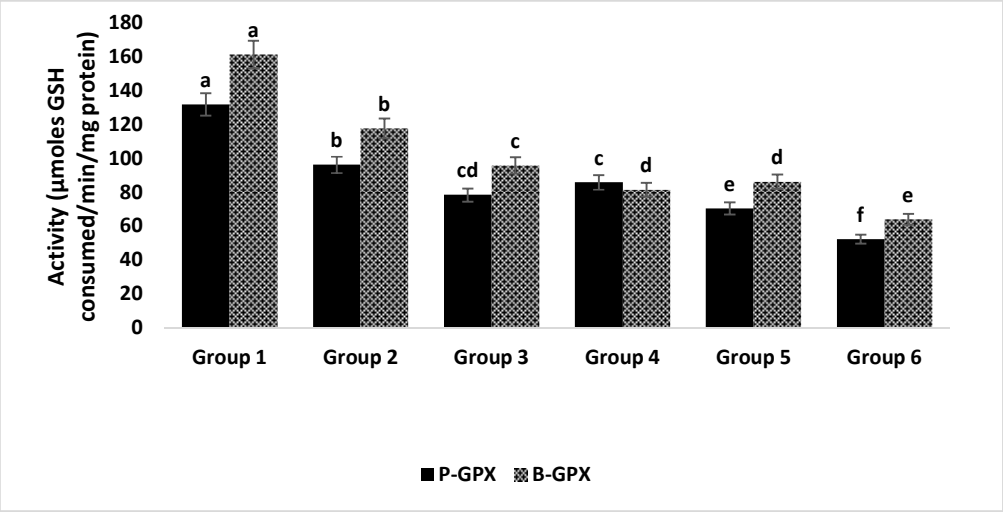

Fig. 9. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the activity of glutathione peroxidase in the brain (B-GPx) and pancreas (P-GPx) of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

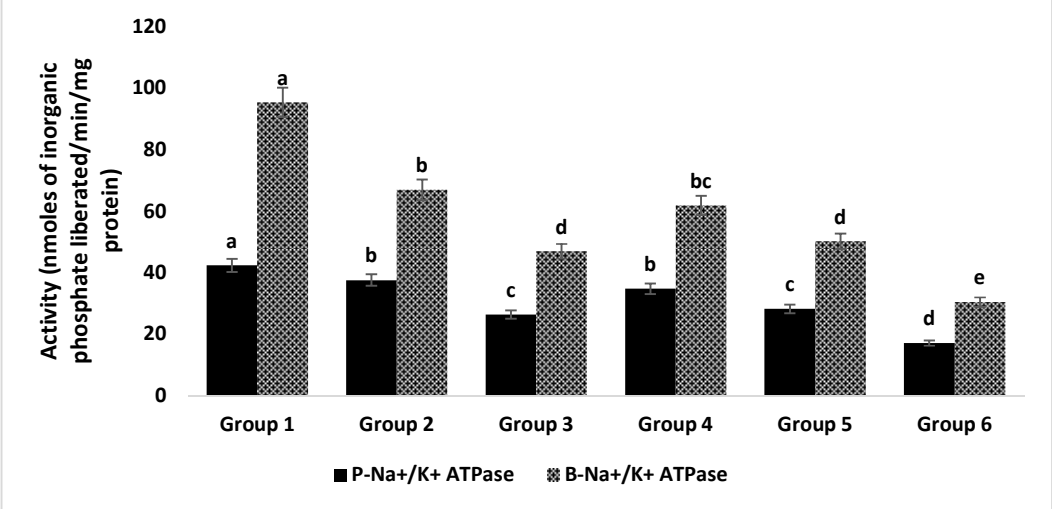

Fig. 10. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the activity of sodium/potassium ATPase in the brain (B-Na+/K+ ATPase) and pancreas (P-Na+/K+ ATPase) of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

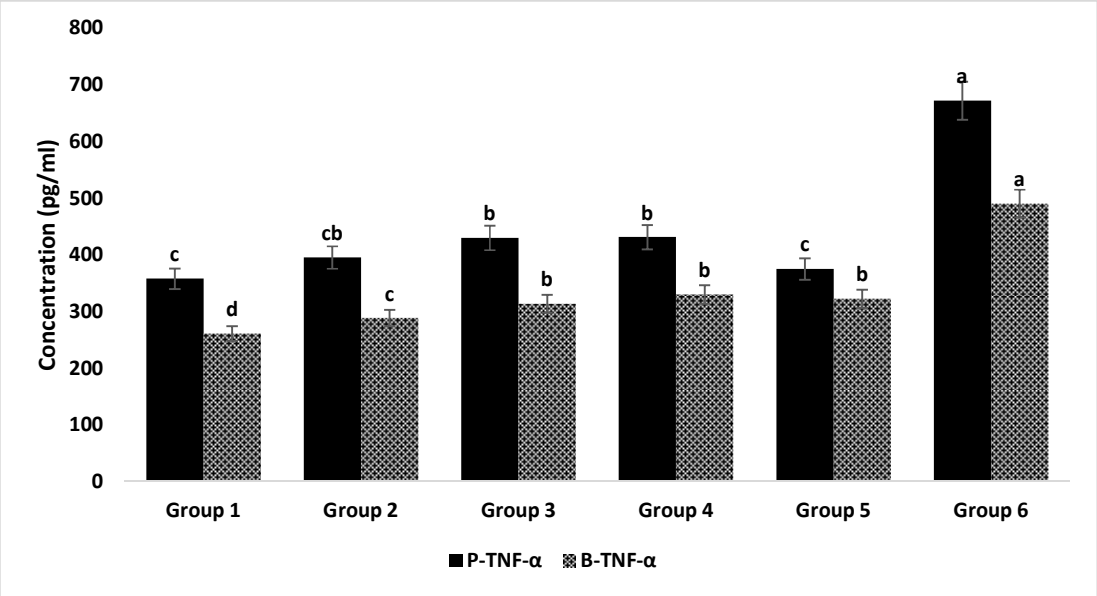

Fig. 11. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the concentration of tumor necrosis factor-α in the brain (B-TNF-α) and pancreas (P-TNF-α) of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

3.5 Effects of Treatment on Dopamine Synthesis through Tyrosine hydroxylase

A reduction in the activity of tyrosine hydroxylase and concentration of dopamine which are the hallmarks of Alzheimer's disease were detected in type 2 diabetes mellitus cases. Interestingly, a high-fat diet (providing a model for early-stage T2DM) has been described to disrupt nigrostriatal dopamine function, resulting in a decrease in dopamine release and clearance in experimental animals [51]. This suggests that T2DM may lead to a loss of dopamine function. Glibenclamide is currently as a first-line oral therapy for T2DM and is considered a prophylactic factor in the prevention of diabetic development. This study revealed that glibenclamide was able to mitigate cognitive decline assumed by type 2 diabetes mellitus. The *Alstonia boonei* leaf extracts demonstrated high effects than glibenclamide by increasing the activity of tyrosine hydroxylase (Fig. 12) through the control of oxygen and glucose toxicity resulting in an increase in the concentration of dopamine (Fig. 13) synthesize from tyrosine.

Diabetes mellitus is a debilitating disease that this study has revealed to exacerbate the oxidative and apoptotic states in the neuron altering the cognitive status of individual on prolong suffering from the disease. Glibenclamide demonstrated hypoglycaemic effect but some non-pancreatic complications resulting from diabetes were not corrected, as revealed by the concentration of dopamine and the activity of tyrosine hydroxylase. *Alstonia boonei* leaf was potent against diabetes by mitigating apoptotic, oxidative and functional disruptions that ensued from the diabetic induction. The neurocognitive indices were positively resolved by the treatment using *Alstonia boonei* leaf. The contributions of the phytochemicals in *Alstonia boonei* leaf can be further investigated to evaluate the structural and signalling modifications that could be implicated in the amelioration of T2DM and the neurocognitive disruption that resulted.

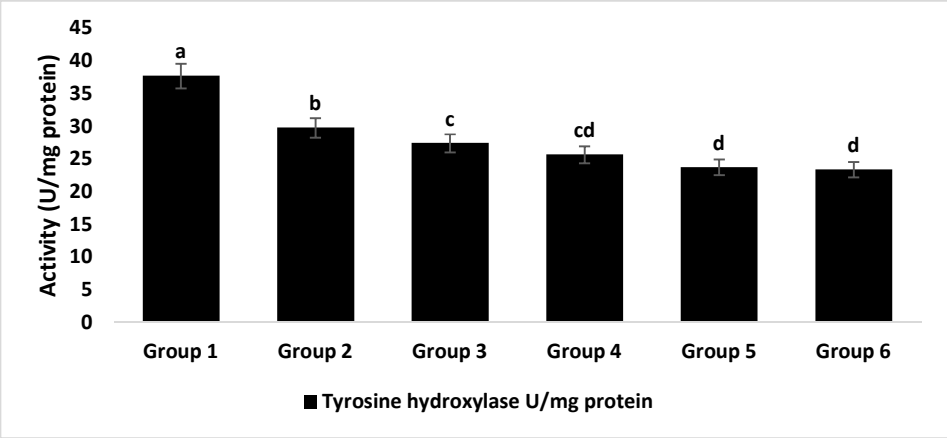

Fig. 12. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the activity of brain tyrosine hydroxylase of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

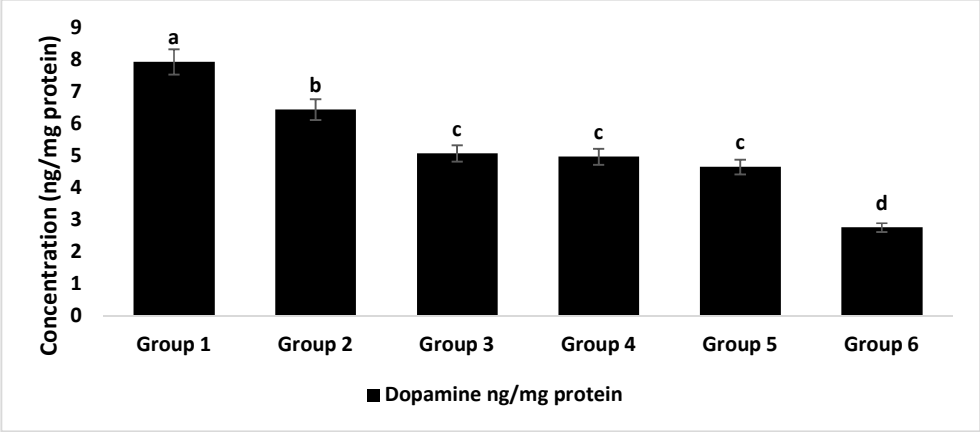

Fig. 13. Effects of HFD/STZ with and without glibenclamide or methanolic extract of *Alstonia boonei* leaf on the concentration of brain dopamine of albino rats. Results were presented as mean±standard deviation where n=5. Values with different superscript are significantly different (p≤0.05)

Group 1: Negative control with intraperitoneal administration of 0.9% saline solution; Group 2: 4 wks HFD/STZ (35 mg/kg BWT) induction and 1600mg/kg BWT of Alstonia boonei leaf extract; Group 3: 4 wks HFD/STZ (35 mg/kg BWT) induction and 10000mg/kg BWT of Alstonia boonei leaf extract; Group 4: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of Alstonia boonei leaf extract; Group 5: 4 wks HFD/STZ (35 mg/kg BWT) induction and 100mg/kg BWT of glibenclamide; and Group 6: 4 wks HFD/STZ (35 mg/kg BWT) induction without treatment (positive control)

4. CONCLUSION

The study has provided support for existing knowledge on the relationship between neurocognition and type II diabetes mellitus. The protective role played by *Alstonia boonei* leaf is a complement to the bioactive phytochemicals discovered therein. Extract from *Alstonia boonei* leaf demonstrated hypoglycaemic effect vis a vis inhibition of insulin resistance at the cells, as well

as scavenging of free radicals generated through highly oxidative processes ensuing from the glucose metabolism to methyl glyoxal (advance glycation end-products). *Alstonia boonei* leaf inhibition of lipid peroxidation preserved the cells in stable conformation that would facilitate molecular insulin-cell transduction for a glycaemic response. *Alstonia boonei* leaf restored the inhibited activity of tyrosine hydroxylase and initiated the corresponding elevation of dopamine which is essential for cognitive function.

SIGNIFICANCE STATEMENT

This study discovers that *Alstonia boonei* leaf contains phytochemicals which are antidiabetic agents as well as neurocognitive stimulants. The study will help researchers to unravel the mechanisms and indices that are essential in determination of antidiabetic effects of some other natural substances and be the basis for further research on the investigation into the synergy between phytochemicals present in *Alstonia boonei* leaf and some of the enzymes implicated in neurocognitive and pancreatic disruption ensuing from T2DM.

ETHICAL APPROVAL

All experimental procedure including euthanasia was conducted under the Ethical Regulation and Guide for the Care and Use of Laboratory Animals of the Centre for Research and Development, Federal University of Technology, Akure, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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