

Full-Length Research Article

# Hypoglycemic, Hypolipidemic and Antioxidant Potentials of Ethanolic Stem Bark Extract of *Anacardium occidentale* in Streptozotocin-Induced Diabetic Rats

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**Summary:** Diabetes mellitus is one of the most widespread diseases affecting the world's population causing substantial morbidity, mortality and long-term complications. This study was designed to investigate possible hypoglycemic, hypolipidemic and antioxidant effect of ethanolic stem bark extract of *Anacardium occidentale* in streptozotocin (STZ)-induced diabetic rats. Twenty-eight STZ (60 mg/kg body weight)-induced diabetic, male albino rats were randomly distributed into Groups II-V (7 rats each) and orally administered with water, metformin (14.2 mg/kg), 200 mg/kg *Anacardium occidentale* extract and 400 mg/kg *Anacardium occidentale* extract respectively daily for 15 days. Group I rats were untreated with STZ and serves as control all under the same sham handling. Blood samples were taken for measurement of fasting blood glucose (FBG) and lipid profile. Liver and kidney tissue samples were taken for determination of glycemic indices (glucose and glycogen), as well as redox status markers such as malondialdehyde (MDA), total glutathione (GSH), activities of superoxide dismutase (SOD) and glutathione-s-transferase (GST). Results showed that treatment with 200 and 400 mg/kg *Anacardium occidentale* stem bark extract reversed hyperglycemia and hyperlipidemia induced by STZ similar to what was observed with the standard drug, metformin. Similarly, both extract concentration produced a significant reduction in MDA while the activity of SOD and GST, as well as concentration of GSH were elevated. This study suggested that ethanolic stem bark extract of *Anacardium occidentale* at 200 and 400 mg/kg can ameliorate diabetes and its associated complications via its hypoglycemic, hypolipidemic, antioxidant and free radical scavenging properties.

**Keywords:** *Anacardium occidentale*, Antioxidant, Diabetes, Hypoglycemic, Hypolipidemic

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Manuscript received- October 2021; Accepted- March 2022

DOI: <https://doi.org/10.54548/njps.v37i1.17>

## INTRODUCTION

Diabetes mellitus is one of the most widespread diseases affecting the world's population causing substantial morbidity, mortality and long-term complications. Available evidence indicated that diabetes currently affects an estimated 429 million people worldwide, and with the prevalence increasing alarmingly, the figure is projected to increase to around 625 million people by 2045 (IDF, 2017). In Nigeria, diabetes is becoming very common and currently rising to epidemic proportions. With an estimated 3.9 million people living with diabetes and a prevalence rate of 4.3% among adult populations, Nigeria has the highest incidence of diabetes in sub-Saharan Africa (Dahiru *et al.*, 2016). This upsurge in rate may be attributed to changing behavioral and lifestyle risk factors including bad eating habits, alcohol consumption, cigarette smoking and nonchalant attitude towards comprehensive and routine medical check-up.

Classified as either insulin-dependent diabetes mellitus (IDDM), also known as type 1 diabetes mellitus (T1DM) or non-insulin-dependent diabetes mellitus (NIDDM) or type 2

diabetes mellitus (T2DM). T1DM, characterized by inability to produce insulin because of autoimmune destruction of pancreatic  $\beta$  cells, accounts for about 5 to 10% of diabetes (Niedowicz and Daleke, 2005) while T2DM is characterized by a dysfunctional carbohydrate, lipid and protein metabolism resulting from a progressively impaired insulin secretion and/or insulin resistance (Ajuwon *et al.*, 2018). Whether resulting from defect in insulin production, insulin utilization or both, diabetes leads to decreased uptake and utilization of glucose in the skeletal muscle, liver and adipose tissues resulting in uncontrollable hyperglycemia.

Accumulating evidence has shown that reactive oxygen species (ROS) production and generation of oxidative stress are considered as key factors in the pathophysiology of diabetes and diabetic complications (Ajuwon *et al.*, 2018). Reports have shown that overproduction of reactive oxygen species (ROS) may result in  $\beta$ -cells dysfunction as a result of decrease in  $\beta$ -cells mass attributable to oxidative stress-induced apoptosis (Wali *et al.*, 2014). Furthermore, there are studies linking overproduction of ROS to insulin resistance,

impairment in insulin synthesis and  $\beta$ -cell insulin secretion (Robertson *et al.*, 1992; Kim *et al.*, 2008; Karunakaran *et al.*, 2013; Yang *et al.*, 2014).

Treatment for diabetes relied on pharmacological agents such as insulin, sulfonylureas, metformin and others to inhibit the hyperglycemic condition. However, the efficiency of these conventional antidiabetic agents is not completely guaranteed as indicated by increase in morbidity and mortality rate in diabetic patients (Asrafuzzaman *et al.*, 2017). Furthermore, the commonly used antidiabetic agents cause various side effects, such as weight gain, hypoglycemia, fluid retention, skin rash or itching, tiredness or dizziness, diarrhea, liver disease and heart failure (Akindele *et al.*, 2015; Xu *et al.*, 2018), thus limiting their use. These drawbacks, and the fact that about 80% of people affected by diabetes lives in low- and middle-income countries, where accessibility to adequate medical care may not be available, have made the development of new antidiabetic agents which can control hyperglycemia with fewer side effects very essential. Since hyperglycemia-induced oxidative stress has been fingered in the pathogenesis of diabetes and diabetic complications, it is therefore suggested at least in theory, that medicinal plants, which are known sources of natural antioxidants could be beneficial in the treatment and management of diabetes.

*Anacardium occidentale*, commonly known as Cashew, is a member of the Anacardiaceae family, and it is found all over Nigeria. The tree is rich in anthocyanins, carotenoids, ascorbic acid (vitamin C), flavonoids and other polyphenols, as well as mineral components. Reports from Nigerian folk medicine indicated that the stem bark extract of *A. occidentale* is used in the treatment of malaria fever, asthma, dysentery, toothache, sore gum, dyspepsia, impotence, urinary disorders and syphilis (Saidu *et al.*, 2012; Agedah *et al.*, 2010). Similarly, fruit, juice and the nut oil derived from *A. occidentale* are used in folk remedies for cancerous ulcers and elephantiasis (Saidu *et al.*, 2012). Evidence has shown that *A. occidentale* displayed a broad spectrum of biological effects, with reports indicating antioxidants, anti-inflammatory (Olajide *et al.*, 2004), antimutagenic (Melo-Cavalcante *et al.*, 2011), antiulcerogenic, antibacterial, antifungal and larvicidal (Behravan *et al.*, 2012) effects among others. Though, the various parts of *A. occidentale* are widely used in folk medicine in the treatment of various ailments including diabetes, however, scientific reports of the use of its stem bark extract for diabetes treatment is still limited. Therefore, in this study, we report on the antioxidant, hypoglycemic and hypolipidemic activities/potentials of the ethanolic stem bark extract of *A. occidentale*.

## MATERIALS AND METHODS

### Plant materials and authentication

The stem bark of *A. occidentale* was obtained in the vicinity of Kwara State University, Malete. The plant was identified and authenticated at the Department of Plant and Environmental Biology, College of Pure and Applied Sciences, Kwara State University, Malete, Nigeria.

**Chemicals and Reagents:** Kits for lipid profile assays were obtained from Randox Limited, London. All other chemicals and reagents used are of analytical grades and obtained from Sigma Aldrich, Johannesburg, South Africa.

**Preparation of extract:** The stem bark of *A. occidentale* was harvested, cut into small pieces and oven-dried at 40°C until constant weight was obtained. The dried stem bark (200 g) was powdered, soaked with 2000 ml of absolute ethanol for 72 hours with intermittent shaking. It was filtered with micelles cloth and then concentrated to dryness with water bath at 45°C. A yield of 46.5 g (23.25%) was obtained. The dried powdered filtrate extract was stored in an air tight container at 4°C in the refrigerator. A calculated amount of residue was weighed and re-constituted in distilled water to give the required doses of 200 and 400 mg/kg body weight of the extract used to treat the animals in this experiment.

**Animals:** Male Wistar rats (6 weeks old) weighing 135 - 150 g were obtained and housed at the Animal Unit of the Department of Biochemistry, Kwara State University, Malete, Kwara State. The animals were housed under controlled environment, maintained at a temperature of between 22-24°C, with a 12 h light-dark cycle and 50-54% humidity. The rats were fed standard rat pellet *ad libitum* and had free access to tap water. Animals were acclimatized under this condition for 10 days before the commencement of the experiment. All rats used received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

**Induction of diabetes mellitus:** Male Wistar rats (135 - 150 g) were injected intramuscularly with freshly prepared STZ (60 mg/kg body weight (Sigma Aldrich, South Africa) in 0.1 M citrate buffer (pH 4.5). Blood samples were taken from the tail tip after 72 h of STZ injection and glucose levels determined using an AccuChek glucometer (Roche, Cape Town, South Africa). Rats with blood glucose levels above 180 mg/dL were selected as diabetic and used in this study.

**Study design:** The rats were randomized into 5 experimental groups of 7 rats each and treated accordingly as shown below:

- i. **Control group:** Received a single intramuscular injection of citrate buffer and have access to food and drinking water for 15 days.
- ii. **Diabetes Mellitus (DM) group:** Received a single intramuscular injection of STZ (60 mg/kg body weight) and have access to food and drinking water for 15 days.
- iii. **Diabetes + Metformin (DM + MTF) group:** Received a single intramuscular injection of STZ (60 mg/kg body weight) and treated with 14.2 mg/kg body weight of a standard drug, metformin orally for 15 days. Rats also have free access to food and drinking water.
- iv. **Diabetes + 200 mg/kg extract (DM +200 mg.kg) group:** Received a single intramuscular injection of STZ (60 mg/kg body weight) and treated with 200 mg/kg body weight of *A. occidentale* stem bark ethanolic extract orally for 15 days. Rats also have access to food and drinking water.

v. **Diabetes + 400 mg/kg extract (DM +400 mg.kg) group:** Received a single intramuscular injection of STZ (60 mg/kg body weight) and treated with 400 mg/kg body weight of *A. occidentale* stem bark ethanolic extract orally for 15 days. Rats also have access to food and drinking water.

The general conditions of the rats were monitored daily throughout the study. Fasting blood glucose (Accu-Chek, Roche, South Africa) and body weight of the rats were recorded on day 1, 3, 5, 10 and 15. At the end of the experimental period, fasted (16 h) animals in all the groups were sacrificed under mild ether anesthesia. About 5 ml of blood was collected via the abdominal aorta into collection tubes with EDTA (1.5 mg/dL of blood) to obtain plasma. Plasma was separated immediately by centrifugation at 3,000 g for 10 min, transferred into properly labelled vials and stored frozen (-20° C) until used for analysis. The liver and kidney were removed, washed twice with ice-cold PBS (10 mM phosphate buffered saline pH 7.2) to remove residual blood, blotted to dry, weighed and stored frozen (-20° C).

**Preparation of liver and kidney homogenates:** Liver and kidney tissues were separately homogenized on ice in 5 volumes of cold 0.25 M sucrose solution. The homogenate of each was transferred into tubes and centrifuged at 10,000 g for 10 min. The supernatant of each was collected, divided into aliquots, and stored at -20° C until used for analyses of lipid peroxidation, antioxidant enzymes activity and glutathione status.

### Biochemical assays

**Plasma lipid Profiles:** Plasma samples were used for the determination of triglyceride (TG), total cholesterol (TC) and high density lipoprotein (HDL)-cholesterol (HDL-C). TG, TC and HDL-C were measured using commercially available kits (Randox Laboratories, UK) according to manufacturer's instructions.

**Hepatic glucose and glycogen:** Hepatic glucose was estimated using the glucose oxidase-peroxidase method (Trinder, 1969). Hepatic glycogen was determined by a method previously described by Musabayane *et al.* (2005). Briefly, about 1 g of liver tissue was homogenized in 2 ml of 30% potassium hydroxide (300 g/L) and boiled at 100 °C for 30 min, and then cooled in ice saturated sodium sulphate. Glycogen was precipitated with ethanol, pelleted, and resolubilized in distilled water. Glycogen content was determined by treatment with anthrone reagent and the absorbance measured at 620 nm.

**Lipid Peroxidation:** The extent of lipid peroxidation was assessed in terms of thiobarbituric acid reactive substances (TBARS), using malondialdehyde (MDA) as standard by method of Beuge and Aust (1978). Briefly, 1 ml of liver or kidney homogenate was mixed with 2 ml of the TCA-TBA-HCl reagent [15% (w/v) TCA, 0.375% (w/v) TBA and 0.25 N HCl]. The mixture was heated in a boiling water bath for 15 minutes, cooled on ice and centrifuged at 3,500 x g for 3 min. at 4° C. The absorbance of the supernatant was read at 535 nm and the MDA concentration of the sample calculated using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$  and expressed as  $\mu\text{mol MDA/g tissue}$ .

**Superoxide dismutase activity:** The activity of superoxide dismutase (SOD) was determined according to method described by Das *et al.* (2000). Briefly, 1.4 ml aliquot of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer, pH 7.4, 0.075 ml of 20 mM L-Methionine, 0.04 ml of 1% (v/v) Triton X-100, 0.075 ml of 10 mM hydroxylamine hydrochloride and 0.1 ml of 50 mM EDTA) was added to 100  $\mu\text{l}$  of liver or kidney homogenate and incubated at 30° C for 5 min. Then 80  $\mu\text{l}$  of riboflavin was added and the tubes were exposed to two 20W-Philips fluorescent lamps arranged parallel to each other for 10 min. After the exposure time, 1 ml of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and absorbance of the colour formed measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay condition.

**Glutathione-S-Transferase Activity:** Glutathione-S-Transferase (GST) activity was determined according to a modified method of Habig *et al.* (1974). This method is based on the reaction between 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH), catalysed by GST. To 900  $\mu\text{l}$  of reaction mixture (comprising of 700  $\mu\text{l}$  of 0.1 mol/L potassium phosphate buffer (pH 5.5), 100  $\mu\text{l}$  of 25 mmol/L CDNB and 100  $\mu\text{l}$  of 50 mmol/L GSH) was added 100  $\mu\text{l}$  of liver or kidney homogenate. After adding the sample, the formation of the product was monitored for 5 min. at 340 nm. The results were reported as micromole per milligram protein ( $\mu\text{mol/mg protein}$ ).

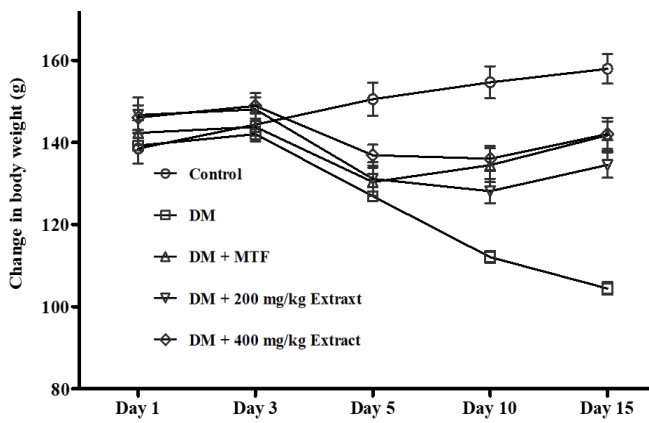
**Total glutathione:** Total glutathione (GSH) concentration was determined in samples according to the method of Boyne and Ellman (1972). Briefly, 1 ml of homogenate was treated with 4.0 ml of metaphosphoric acid precipitating solution (1.67 g of glacial metaphosphoric acid, 0.2 g EDTA and 30 g NaCl dissolved in 100 ml of distilled water). After centrifugation, 2 ml of the supernatant was mixed with 0.2 ml of 0.4 M  $\text{Na}_2\text{HPO}_4$  and 1 ml of DTNB (5, 5' -Dithio-bis-(2-nitrobenzoic acid) reagent (40 mg DTNB in 100 ml of aqueous 1% trisodium citrate). Absorbance was read at 412 nm within 2 min. GSH concentration was expressed as  $\mu\text{mol/g tissue}$ .

### Statistical analysis

Values were expressed as mean  $\pm$  standard deviation (SD). Differences between group mean were estimated using one-way analysis of variance (ANOVA) followed by Tukey's test for all pairwise comparison. Results were considered statistically significant at  $p < 0.05$ . All the statistics were carried out using MedCalc v 12.2.1 software (MedCalc software bvba, Mariakerke, Belgium).

## RESULTS

Figure 1 shows the effect of stem bark extract of *A. occidentale* on body weight in control and STZ-induced diabetic rats. The average body weight of all the animals in the five experimental groups did not differ significantly ( $p < 0.05$ ) before the administration of STZ. Rats in the control group showed a consistent gain in body weight over the period of the experiment.

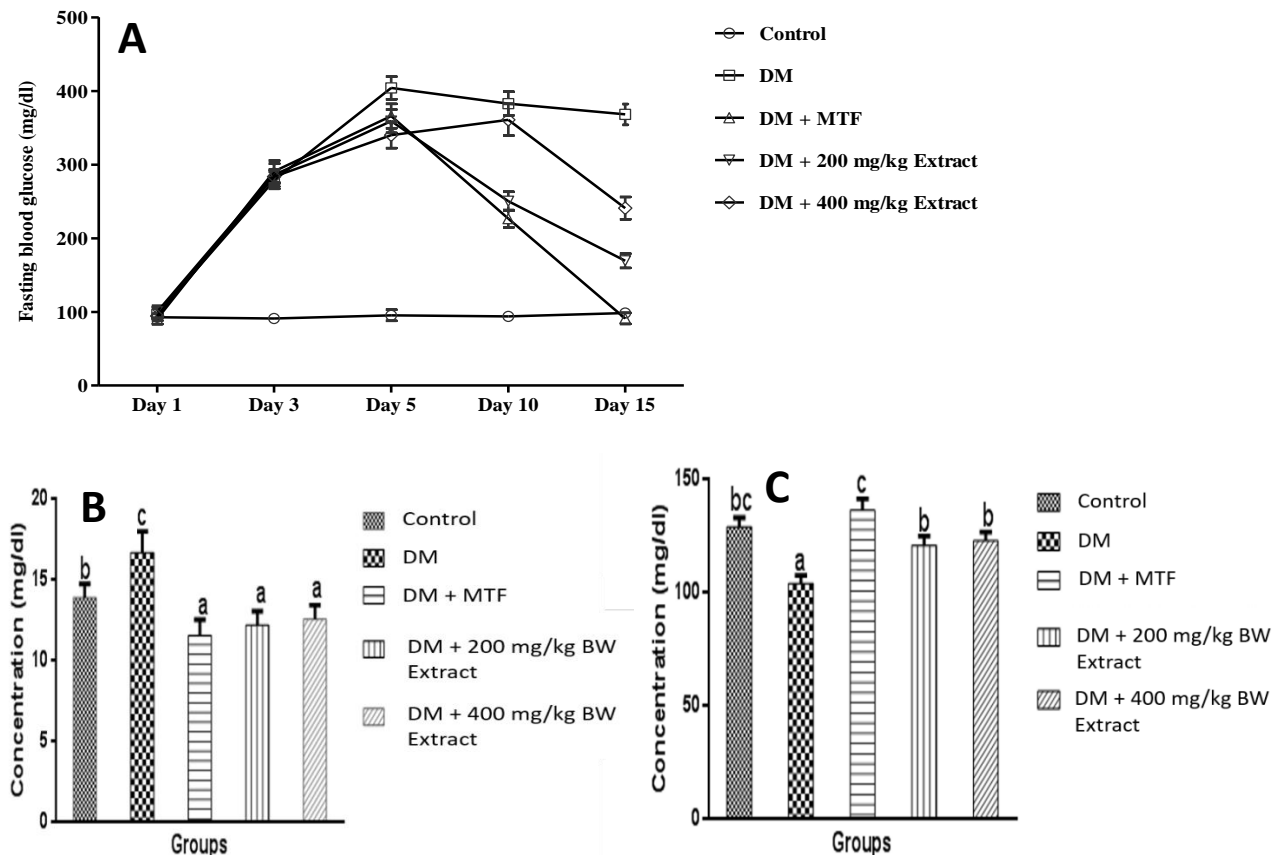


**Figure 1**  
Effect of ethanolic stem bark extract of *Anacardium occidentale* on body weight of STZ-induced diabetic rats. Data points represent mean  $\pm$  standard deviation. **Note**, DM = diabetic rats; MTF = metformin.

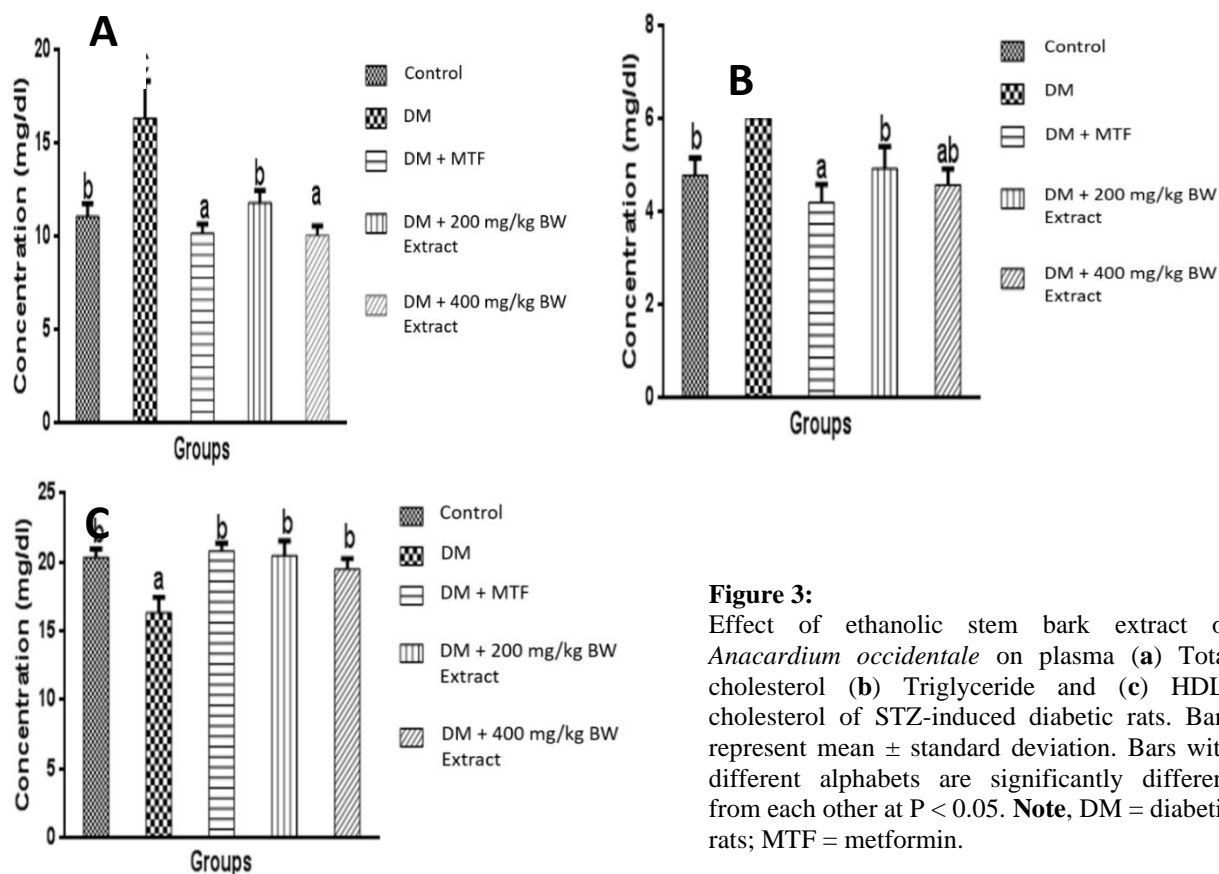
By the 5th day of the experiment, body weight of animals in the diabetes untreated (DM), diabetes + metformin (DM + MTF) and diabetes + extract (DM + 200 mg/kg and 400 + mg/kg) groups was significantly ( $p < 0.05$ ) reduced compared to control animals. This weight loss continued until the end of the experiment. At the end of the experiment, DM + 200 mg/kg and DM + 400 mg/kg extract treated rats showed sign of recovery from body weight loss ( $134.49 \pm 3.11$  vs  $104.40 \pm 3.99$  g and  $141.99 \pm 4.02$  vs

$104.40 \pm 3.99$  g respectively) when compared with DM group rats. The improvement in body weight induced in the DM + 400 mg/kg extract group is similar to what was observed in the DM + MTF group ( $141.99 \pm 4.02$  vs  $141.75 \pm 3.33$  g).

The effect of the *A. occidentale* stem bark extract on markers of glycemic control in control and STZ-induced diabetic rats is shown in Figure 2. The fasting blood glucose of the control rats were within the range of 90-100mg/dL throughout the study period. Intraperitoneal injection of STZ resulted in a 3-fold (3.06 – 3.20) increase in fasting blood glucose (FBG) level across the treatment groups by day 3 of the study (Figure 2a), and reaches its peak by day 5 across all the groups. Starting from the 10<sup>th</sup> day of the study, administration of both 200 mg/kg and 400 mg/kg body weight of *A. occidentale* induced a significant ( $p < 0.05$ ) decrease in the FBG level of the diabetic rats to various degrees. The effect of the ethanolic stem bark extract of *A. occidentale* on hepatic glucose and glycogen concentration of STZ-induced diabetic rats was also assessed. STZ injection resulted in a significant ( $p < 0.05$ ) increase and decrease in hepatic glucose and glycogen concentration, respectively (Figure 2b and 2c). Both the 200 mg/kg and 400 mg/kg body weight extract reversed the increase in hepatic glucose concentration similar to what was observed with the standard drug, metformin. STZ-induced reduction in hepatic glycogen was also reversed in both extract treatment groups similar to the level observed in the control group.



**Figure 2:** Effect of ethanolic stem bark extract of *Anacardium occidentale* on change in (a) Fasting blood glucose (b) Hepatic glucose and (c) Hepatic glycogen concentration of STZ-induced diabetic rats. Data points and bars represent mean  $\pm$  standard deviation. Bars with different alphabets are significantly different from each other at  $P < 0.05$ . **Note**, DM = diabetic rats; MTF = metformin.



**Figure 3:** Effect of ethanolic stem bark extract of *Anacardium occidentale* on plasma (a) Total cholesterol (b) Triglyceride and (c) HDL-cholesterol of STZ-induced diabetic rats. Bars represent mean  $\pm$  standard deviation. Bars with different alphabets are significantly different from each other at  $P < 0.05$ . Note, DM = diabetic rats; MTF = metformin.

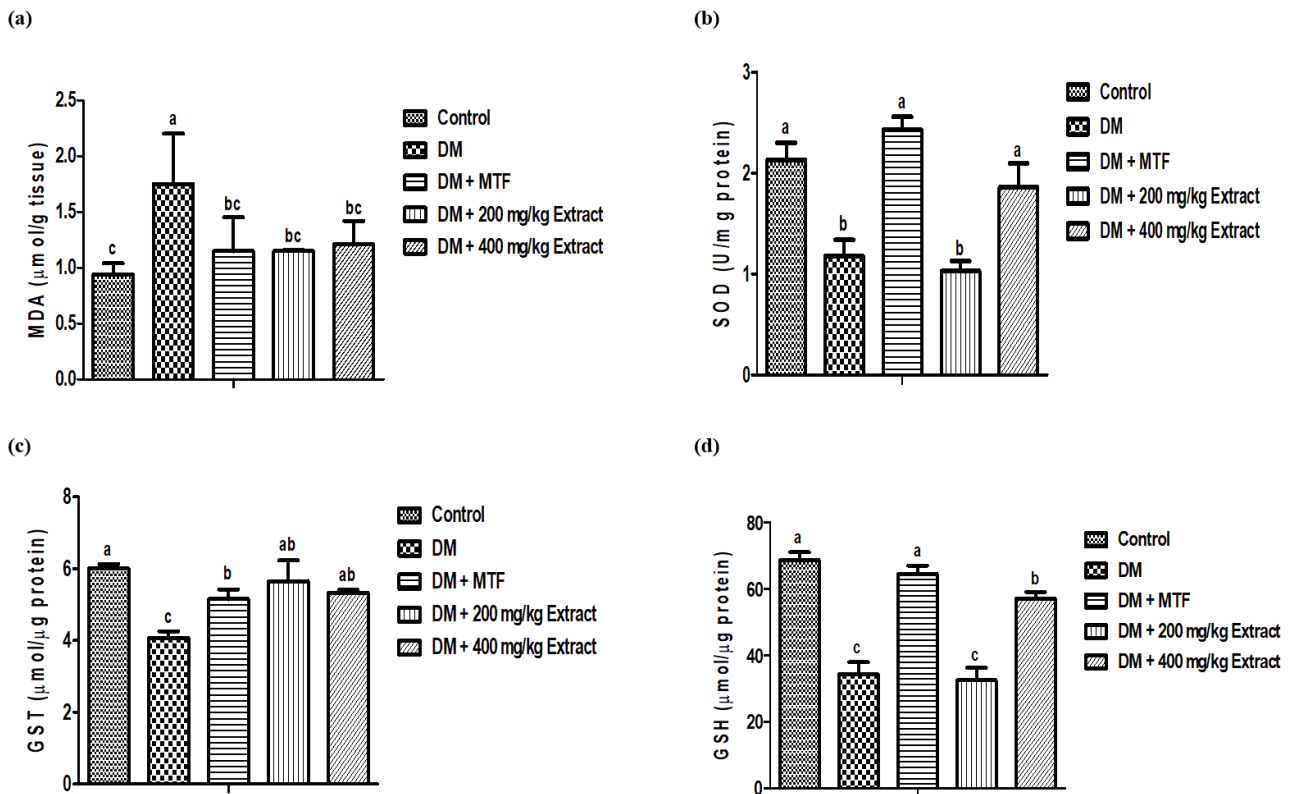
Next, we assessed the effect of the ethanolic stem bark extract of *A. occidentale* on plasma lipid profile in STZ-induced diabetic rats. The level of plasma total cholesterol (Figure 3a) and triglyceride (Figure 3b) was significantly ( $p < 0.05$ ) increased while HDL-cholesterol (Figure 3c) was reduced in diabetic untreated (DM) group when compared with control animals. Treatment with both 200 mg/kg and 400 mg/kg body weight extracts reversed the observed changes by significantly ( $p < 0.05$ ) reducing the increase in plasma total cholesterol and triglycerides, as well as increasing ( $p < 0.05$ ) plasma HDL-cholesterol to a level comparable with what was observed in either the standard drug (MTF) group or the control group.

Figures 4 and 5 showed the effect of ethanolic stem bark extract of *A. occidentale* on antioxidant indices and lipid peroxidation level in the liver and kidney of STZ-induced diabetic rats. Malondialdehyde, the marker of lipid peroxidation was significantly ( $p < 0.05$ ) increased in the liver (1.86-fold) and kidney (1.63-fold) of STZ-induced diabetic rats, as shown in figure 4a and figure 5a respectively. Administration of 200 mg/kg and 400 mg/kg body weight of *A. occidentale* extract was able to reverse the elevation in MDA level induced by STZ in both liver and kidney similar to what was obtained with the standard drug, metformin. Other indices of antioxidant status assessed indicated that in the liver, diabetes induction with STZ significantly ( $p < 0.05$ ) decreased the activity of SOD (1.80-fold) and GST (1.47-fold), as well as total glutathione level (2.00-fold). GST activity was restored to the level obtained in the control and metformin treated rats as a result of the administration of 200 mg/kg and 400 mg/kg body weight of *A. occidentale* extract (Figure 4c). The 400 mg/kg body weight extract, when administered to diabetic rats

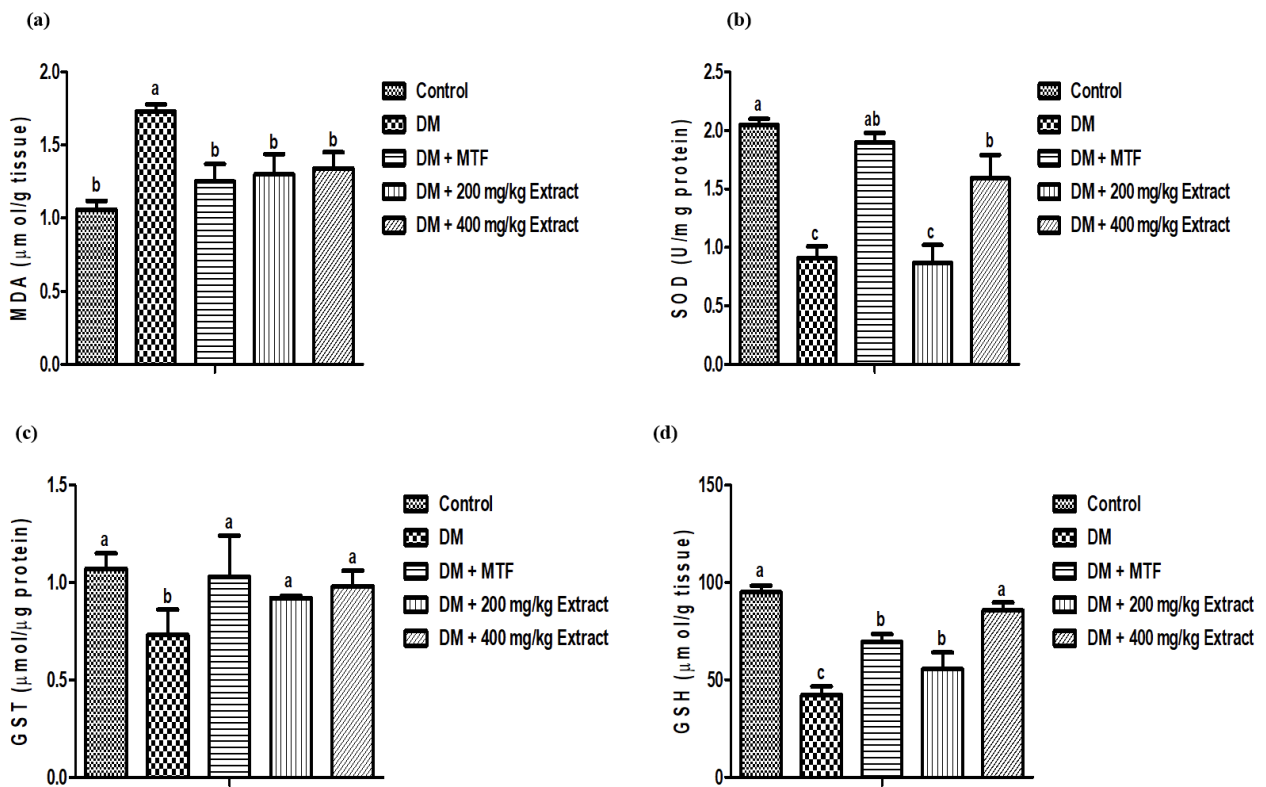
reversed the decrease in activity of SOD and glutathione concentration to a level comparable to what was obtained in either the control or metformin group (Figures 4b and 4d). Similarly, in the kidney, STZ injection resulted in a significant ( $p < 0.05$ ) reduction in the activity of SOD (2.25-fold), GST (1.47-fold), as well as in the concentration of total glutathione (2.26-fold) as shown in Figures 5b, 5c and 5d respectively. Only the 400 mg/kg body weight extract was able to reverse the reduction in SOD activity to the level obtained in the control and metformin group when administered to diabetic rats (Figure 5b). Both 200 mg/kg and 400 mg/kg body weight stem bark extract of *A. occidentale* ameliorated the pro-oxidant effect of STZ by significantly ( $p < 0.05$ ) improving the GST activity and total glutathione concentration when administered to diabetic rats (Figures 5c and 5d). Both extract concentrations restored the GST activity and glutathione concentration to the level observed in the control rats.

## DISCUSSION

Diabetes is a metabolic disease resulting from defective insulin secretion and/or resistance to the action of insulin in the peripheral tissues. Characterized by sustained hyperglycemia as a result of decrease in uptake and utilization of glucose in the skeletal muscle, liver and adipose tissues, diabetes is rising to epidemic proportions globally. Unfortunately, the efficacy of orthodox drugs for the treatment of diabetes cannot be completely guaranteed and majority of them are known to have serious side effects, thereby limiting their use.



**Figure 4:** Effect of ethanolic stem bark extract of *Anacardium occidentale* on liver (a) Lipid peroxidation (b) SOD activity (c) GST activity and (d) Total GSH concentration of STZ-induced diabetic rats. Bars represent mean ± standard deviation. Bars with different alphabets are significantly different from each other at P < 0.05. **Note:** GSH = total glutathione; GST = glutathione-s-transferase; SOD = superoxide dismutase; DM = diabetic rats; MTF = metformin.



**Figure 5:** Effect of ethanolic stem bark extract of *Anacardium occidentale* on kidney (a) Lipid peroxidation (b) SOD activity (c) GST activity and (d) Total GSH concentration of STZ-induced diabetic rats. Bars represent mean ± standard deviation. Bars with different alphabets are significantly different from each other at P < 0.05. **Note:** GSH = total glutathione; GST = glutathione-s-transferase; SOD = superoxide dismutase; DM = diabetic rats; MTF = metformin.

Therefore, the development of alternative antidiabetic agents is of utmost importance. Recently, the pharmacological benefits of natural products from plants has been gaining a lot of attention, as these plants are believed to be important sources of new chemical substances with therapeutic potentials. In Nigeria, several hundreds of plants are used traditionally in the management of diabetes, however, there are very limited scientific data to back up this therapeutic claims. Therefore, this study was designed to investigate the potential hypoglycemic, hypolipidemic and antioxidant effects of ethanolic stem bark extract of *Anacardium occidentale*.

The diabetic state has been associated with severe body weight loss, and we observed a significant reduction in the body weight of STZ-induced diabetic animals compared with control animals in this study. Evidence has shown this to be due to degradation of fats and structural proteins, as well as to decrease in protein content in muscular tissue by proteolysis induced by insulin deficiency (Subash Babu *et al.*, 2007; Ahmad *et al.*, 2014). When diabetic animals were treated with both 200 mg/kg and 400 mg/kg body weight of ethanolic stem bark extract of *A. occidentale*, they showed an improvement in body weight loss. The reversal of body weight loss in the extract-treated diabetic animals may be due to the ability of phytochemicals in the extract to reverse proteolysis, gluconeogenesis and glycogenolysis (Ahmed *et al.*, 2014).

The streptozotocin diabetic model is a widely used and accepted model of diabetes that is comparable to the human diabetic state. STZ is a pancreatic  $\beta$ -cells toxic agents that enters the  $\beta$ -cells via the glucose transporter 2 (GLUT 2) and can induce sustained hyperglycemia within few days of administration (King and Bowe, 2016). STZ causes alkylation of DNA and subsequently, PARP activation, NAD<sup>+</sup> depletion, cellular ATP content reduction and inhibition of insulin production follows (Asrafuzzaman *et al.*, 2017). We observed sustained elevated levels of fasting blood glucose in STZ-induced diabetic animals when compared with control animals throughout the experimental period. Similarly, STZ administration resulted in elevated level of glucose and lowered level of glycogen in the hepatic tissue of diabetic animals. Results from our study also showed that the stem bark extract of *A. occidentale* showed hypoglycemic effect by reversing the alteration in carbohydrate metabolism associated with the diabetic state as reported earlier (Saidu *et al.*, 2012). The hypoglycemic effect shown by *A. occidentale* stem bark extract may be due to its ability to stimulate key enzymes involved in carbohydrate metabolism, thus increasing glycogen storage in the liver, glucose uptake by the muscle and/or inhibition of gluconeogenesis (Miranda-Osorio *et al.*, 2016). Lower dose of STZ (60 mg/kg body weight) as used in our study has been reported to produce an incomplete destruction of the  $\beta$ -cells despite the animals having stable, long-lasting hyperglycemia (Asrafuzzaman *et al.*, 2017), thus there could be many surviving  $\beta$ -cells, making regeneration possible. Although we did not measure plasma insulin level in our study, however we are tempted to speculate that the hypoglycemic activity of *A. occidentale* stem bark extract observed in this study may be due to its ability to enhance the regeneration of pancreatic  $\beta$ -cells as previously reported (Basse *et al.*, 2012), and hence potentiate insulin release from the regenerated  $\beta$ -cells. Furthermore, the

hypoglycemic action of the ethanolic stem bark extract of *A. occidentale* observed in our study is comparable to that of the conventional drug, metformin, which mode of action involved reduction of the hepatic glucose production as well as potentiation of insulin sensitivity (Rena *et al.*, 2017).

Dyslipidemia is one of the main risk factors for coronary artery disease and cerebrovascular disease (Baena-Diez *et al.*, 2011). Hypercholesterolemia and hypertriglyceridemia are the two most common lipid abnormalities in the pathogenesis of diabetes. In this study, we observed an increased level of total cholesterol and triglyceride, with a concomitant reduction in HDL-cholesterol level in STZ-induced diabetic animals. This observed STZ-induced dyslipidemia may be due to increased intestinal absorption and increased cholesterol biosynthesis, as well as increased fatty acid mobilization from adipose tissue in the diabetic animals (Subash Babu *et al.*, 2007; Watcho *et al.*, 2012; Ahmed *et al.*, 2014). Administration of ethanolic stem bark extract of *A. occidentale* lowers total cholesterol and triglyceride while HDL-cholesterol level was increased in diabetic rats. This result is consistent with previous studies that have demonstrated hypolipidemic effect for *A. occidentale* (Olatunji *et al.*, 2005; Anyaegbu *et al.*, 2017). Hyperglycemia-induced oxidative stress and its resultant cellular effects are well established in several studies (Fiorentino *et al.*, 2013). In the diabetic state, ROS production overwhelms the cell antioxidant defense resulting in damage to macromolecules. Peroxidation to membrane lipids is one of the manifestation of oxidative stress in STZ-induced diabetic animals. In the current study, diabetes-induced lipid peroxidation was assessed by determining the hepatic and kidney malondialdehyde (MDA) levels, and these were found to be significantly elevated in the diabetic rats. Our result is similar to several studies that have previously shown that biomarkers of oxidative damage including MDA were elevated in the liver and kidney of chemical-induced diabetic rats (Palmer *et al.*, 1998; Van Dam *et al.*, 2001; Nain *et al.*, 2012; Akindele *et al.*, 2015). We also observed that administration of *A. occidentale* stem bark extract at 200 mg/kg and 400 mg/kg of body weight, decreases MDA level in the liver and kidney of the diabetic rats in a manner comparable to that of the antidiabetic drug, metformin. This finding suggests that administration of ethanol stem bark extract of *A. occidentale* prevents lipid peroxidation in STZ-induced diabetic rats. This is similar to earlier reports where methanol stem bark extract of *A. occidentale* reduced MDA level in triton X-100 induced hyperlipidemic and fructose-induced diabetic rats (Olatunji *et al.*, 2005; Anyaegbu *et al.*, 2017).

Antioxidant enzymes such as SOD, CAT and GST produced by mammalian cells as defense against ROS generation are closely related to lipid peroxidation. Evidence abound of association between change in antioxidant enzymes activity and oxidative stress-mediated injury in STZ-induced diabetic rats (Palsamy *et al.*, 2010; Alkan and Celik, 2018; Uddand Rao *et al.*, 2019). Similar to those reports, we observed a reduction in the activities of SOD and GST in the liver and kidney of diabetic rats in our study. The superoxide anion ( $O_2^{\cdot-}$ ) is the chief free radical that has been implicated as one of the toxic mediators responsible for STZ-induced oxidative damage to macromolecules. SOD, a metalloprotein, is the key enzyme involved in protection of cells which spontaneously dismutate  $O_2^{\cdot-}$  to hydrogen

peroxide (H<sub>2</sub>O<sub>2</sub>). The H<sub>2</sub>O<sub>2</sub> produced by SOD is then decomposed to water and oxygen by CAT, a hemoprotein localized in the peroxisomes. GST is a family of cytosolic isoenzymes that catalyzes the conjugation of glutathione with a variety of reactive electrophilic compounds, thereby neutralizing their active electrophilic sites. Therefore, the observed reduction in the activities of SOD and GST may be as a result of glycation (Yan and Harding, 1997) and ROS-induced inactivation of the enzymes (Kono and Fridovich, 1982). GSH is the predominant, non-enzymatic intracellular redox buffer which functions as a direct free radical scavenger, as cofactor for many enzymes, including GST and in maintenance of membrane protein thiol. In agreement with previous studies (Palsamy *et al.*, 2010; Alkan and Celik, 2018; Uddand Rao *et al.*, 2019), we also observed a decline in the concentration of total glutathione (GSH) in the liver and kidney of the STZ-induced diabetic rats compared to control. The reduction in tissue GSH concentrations might be alluded to the ability of GSH to directly scavenge free radicals and other peroxidation products generated in the diabetic rats. Furthermore, the lower levels of GSH could be as a result of decreased synthesis and/or increased degradation of GSH (Loven *et al.*, 1986; Uddand Rao *et al.*, 2019). Results from our study further showed that administration of *A. occidentale* ethanolic stem bark extract at 200 mg/kg and 400 mg/kg body weight was able to reverse the decrease in the activities of SOD and GST, as well as the reduction in GSH concentration in both the liver and kidney. This ameliorative effect, especially with the 400 mg/kg body weight extract is also observed to be similar to what was obtained with the antidiabetic drug, metformin. The increase in GSH and improvement in antioxidant enzymes activities under *A. occidentale* extract supplementation had been reported previously in the liver and kidney of Triton X-100 induced hyperlipidemic rats (Anyaeibu *et al.*, 2017). These results clearly showed that ethanolic extract of *A. occidentale* possess potent antioxidant activity. Evidence has shown that *A. occidentale* stem bark extract is rich in total phenolic and total flavonoid contents (Anyaeibu *et al.*, 2017), and there are reports of presence of several phenolic compounds in the stem bark and leaves extract of *A. occidentale* (Encarnacao *et al.*, 2014; 2016). Therefore, the antioxidant effects shown by *A. occidentale* stem bark extract could be ascribed to the ability of its individual bioactive components to scavenge and/or quench free radicals generated due to diabetic induction, and boost the synthesis of GSH and other key enzymes.

In conclusion, results of the present investigation indicate that ethanolic stem bark extract of *A. occidentale* showed ameliorative effect in STZ-induced diabetic rats. This is demonstrated by its ability to (1) reverse hyperglycemia and dyslipidemia induced by diabetes and (2) prevent lipid peroxidation and maintaining redox status in the liver and kidney of the diabetic rats. This suggests that ethanol extract of *A. occidentale* may be of benefit as an adjuvant therapy in the management of diabetes. However, future studies are necessary to fully understand the specific mechanisms of the ameliorative effect shown by this extract.

#### Acknowledgement

The authors are grateful to Kwara State University of Science and Technology and Federal University, Oye-Ekiti, Nigeria for support and use of facilities.

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