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ANTI-DIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *ANACARDIUM OCCIDENTALE* (LINN) STEM BARK IN NORMAL AND ALLOXAN-INDUCED DIABETIC ALBINO RATS

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ABSTRACT

The present study was carried out to investigate the anti-diabetic activity of aqueous extract of *Anacardium occidentale* stem bark in both normal and alloxan induced diabetic rats. Alloxan-induced diabetic and non-diabetic rats were administered orally with aqueous extract of *Anacardium occidentale* stem bark at 400 mg/kg for 28 days, after which the blood glucose, total protein, albumin, marker enzymes, lipid profile and some haematological indices were determined and compared with the normal control. There was a significant ($p<0.05$) increase in the level of blood glucose, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol, low density lipoprotein (LDL), triglyceride (TG) and a significant ($p<0.05$) decrease in the level of high density lipoprotein (HDL), total protein, albumin, packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) of the diabetic untreated rats. Oral administration of aqueous extract of *Anacardium occidentale* stem bark at a dose of 400 mg/kg body weight for 28 days to diabetic rats resulted in a reversal of the above diabetic conditions. Phytochemical screening of the aqueous extract of *Anacardium occidentale* stem bark revealed the presence of alkaloid, balsam, cardiac glycoside, flavonoid, phenol and terpenes/steroids. The results from this study suggest that the aqueous extract of *Anacardium occidentale* stem bark used in the study possesses anti-diabetic activities and could be used for the management of diabetes and associated metabolic alterations.

Keywords: *Anacardium occidentale*, Stem bark, Phytochemicals, Diabetes, Haematological Indices.

INTRODUCTION

Diabetes mellitus (DM) is a disease of worldwide significance and increasing prevalence. It is a multifactorial disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health

care system [1, 2]. It is the most common endocrine disorder and the world's fastest growing metabolic disorder with an average annual growth of 1-2%. It has been shown that pathogenic course of both Type 1 and Type 2 DM involves alterations in the structures, organization and protein functions of membranes of cells and tissues (e.g. retina, glomerulli, erythrocyte, nerve), culminating in diabetic complications such as retinopathy, nephropathy and peripheral neuropathy [3-5]. Hyperglycemia is clinical hallmark of DM but etiology of this heterogeneous disorder likely involves multiple genetic and environmental interactions that ultimately result in alterations in insulin secretion, insulin action or both (American Diabetes Association, 2006). Several approaches like lifestyle changes, food intake modifications; lowering the fat content [6] or enhancing the fiber and magnesium content of the diet [7] and/or physical activity promoting weight loss [8], smoking status [9], moderate coffee [10], moderate alcohol consumption [11] and finally bariatric gastric surgery have been tried for the prevention of DM [12].

Complementary and alternative medicine applications have attracted special attention in recent research because they offer new promising opportunities for the development of efficient, side effect-free and lower cost alternatives to existing synthetic hypoglycemic agents [13, 14]. A wide range of medicinal plants have been used by various cultures to treat diabetes mellitus because of their hypoglycaemic properties [15].

Anacardium occidentale Linn is a tree in the family of the flowering plant *Anacardiaceae*. The plant is commonly referred to as Cashew in English, *Kashu* in Hausa and *kaju* in Yoruba. The family contains 73 genera and about 600 species. It is a multipurpose tree of the Amazon that grows up to 15m high. It has a thick and tortuous trunk with branches so winding that they frequently reach the ground [16]. The cashew tree produces many resources and products. All parts of the plant like leaves, false fruit and bark have been traditionally used to relieve variety of ailments. The cashew nut has international appeal and market value as food. Even the shell oil around the nut is used medicinally and has industrial applications in plastics and resin industries for its phenol content. The pseudo-fruit, a large pulpy and juicy part, have a fine sweet flavor and are commonly referred to as the "cashew fruit" or the "cashew apple" [17]. It is also used to treat diabetes, weakness, muscular debility, urinary disorders, asthma, eczema, psoriasis, scrofula, dyspepsia, genital problems, bronchitis, cough, intestinal colic, leishmaniasis, venereal diseases, as well as impotence, and

syphilis-related skin disorders [17]. It is taken for syphilis and as a diuretic, stimulant and aphrodisiac. In addition to being delicious, cashew fruit is a rich source of vitamins, minerals, and other essential nutrients. It has up to five times vitamin C than oranges and contains a high amount of mineral salts. Because of its high amount of vitamin C and mineral salts, cashew fruit is used as a catalyst in the treatment of premature aging of the skin. Several clinical studies have shown that anacardic acids a component of cashew, with highest concentration in the nutshells curb the darkening effect of aging by inhibiting tyrosinase activity, and that they are toxic to certain cancer cells [18]. The present study was undertaken to investigate anti-diabetic activity of aqueous extract of *Anacardium occidentale* stem bark in normal and alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The stem barks of *Anacardium occidentale* were obtained from Farin-gada Jos North, Plateau State, Nigeria and were authenticated at the Herbarium of the Department of Botany, University of Jos, Nigeria, where a voucher specimen was deposited at the Herbarium of the Institute.

Chemicals

Aloxan monohydrate was obtained from Sigma-Aldrich Chemical Company, St. Louis, U.S.A. All the other chemicals used were of analytical grade and prepared in glass distilled water.

Experimental Animals

Adult Wistar (male and female) albino rats (20) weighing between 180-250g were obtained from the National Veterinary Research Institute (NVRI), Vom, Jos, Nigeria. The animals were housed in aluminum cages under standard conditions. They were maintained on standard animal pellets (purchased from Grand Cereal and Oil Mills Limited Jos, Nigeria) and water *ad libitum*. The animals were acclimatized for two weeks before the commencement of the experiment.

Preparation of Extract

The stem barks of *Anacardium occidentale* were washed and oven dried at 40°C for 72 hours to a constant weight. The dried pieces were then pulverized using a blender. The powdered material was stocked in a plastic container from which 200 g was extracted in 2000ml of cold distilled water for 48 hours at 37°C.

This was then filtered with Whatman No. 1 filter paper. The filtrate was concentrated on a steam bath to give the extract. The extract was then reconstituted in distilled water to give the required dose of 400 mg/kg body weight as used in this study.

Phytochemical Screening

The presence of Alkaloids, Balsam, Cardiac glycoside, Flavonoids, Resin, Saponins, Tannins, Phenols and Terpenes/steroids content of the extract were determined by the methods described by Sofowora [19], Trease and Evans [20] and Harborne [21].

Induction of Diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared Alloxan monohydrate (150 mg/kg body weight) in ice cold 0.9% NaCl solution. The animals were allowed 5% glucose solution overnight ad libitum to overcome the drug-induced hypoglycemia. Control (normal) rats were not injected with alloxan and were placed on normal saline alone. After 24 hours, rats with blood glucose level 7.0 mmol/L (126.13 mg/dL) were considered as diabetic and used for the experiment [22].

Experimental Design

After randomizing into various groups and before initiation of the experiment, the rats were acclimatized to the animal house conditions. The rats were maintained during the study on standard rat feed consisting of 67.20% carbohydrate, 14.50% protein, 7% fat, 10% fibre and 1.30% minerals. The animals were randomized into 4 groups of 5 animals each for the evaluation of anti-diabetic activity.

GROUP I: Control rats on 1.0 ml normal distilled water per day.

GROUP II: Diabetic control rats on 1.0 ml normal distilled water per day.

GROUP III: Diabetic treated rats on 1.0 ml of extract (equivalent to 400mg/kg aqueous *Anacardium occidentale* stem bark extract).

GROUP IV: Normal treated rats on 1.0 ml of extract (equivalent to 400mg/kg aqueous *Anacardium occidentale* stem bark extract).

All administered were done orally per day using cannula for 28 days.

Collection of Blood Sample

The rat was placed under diethyl ether anesthesia; the neck area was quickly shaved with scissor to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade and about 4cm^3 of blood was collected in an EDTA sample bottle for the haematological assay and also collected into clean, sterile sample bottles which were allowed to clot for 30 minutes. This was then centrifuged at 33.5 g for 15 minutes using a Centrifuge. The sera were aspirated with Pasteur pipettes and stored frozen until required for the biochemical analyses.

Determination of Haematological Parameters

Packed cell volume (PCV) haemoglobin (Hb), white blood cell (WBC) and platelets (PLT) values were determined using Mindray Haematology Analyzer (Mindray BC-2300, Guangzhou Medical Equipment Co., Ltd, China).

Biochemical Assays

The total protein content of the serum was determined using the Biuret method [23]. Albumin (ALB) level was determined as described by Grant and Kacchman [24]. Serum glucose, total cholesterol (TC), low density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride (TG) were determined by the method of Trinder [25], Friedrickson *et al.* [26], Albers *et al.* [27], Assman *et al.* [28], Jacob and Van Demark [29] respectively. The activities of Aspartate transaminase (AST) (EC 2.6.1.2), Alanine transaminase (ALT) (EC 2.6.1.1) in serum were determined based on the method described by Schmidt and Schmidt [30], while the activity of Alkaline phosphatase (ALP) (EC 3.1.3.1) was determined as described by Wright *et al.* [31]. All measurements were done using spectrophotometer.

Statistical Analysis

The data were expressed as Mean ± Standard Error of Mean. Statistical analysis was performed using analysis of variance (ANOVA) and Duncan multiple range test at 5% level of confidence ($p<0.05$).

RESULTS

Phytochemical screening of the aqueous extract of *Anacardium occidentale* stems bark revealed the presence of alkaloid, balsam, cardiac glycoside,

flavonoid, phenol and terpenes/steroids. The presence of resin, saponin and tannins were not detected (Table 1).

Table 2 shows the effect of aqueous extract of *Anacardium occidentale* stem bark on selected marker enzymes (ALT, AST and ALP) in alloxan induced diabetic rats. There was a significant ($p<0.05$) increase in the level of ALT, AST and ALT of the untreated diabetic rats when compared with the control group and the other treated groups. Oral administration of aqueous extract of *Anacardium occidentale* stem bark at dose of 400 mg/kg body weight for 28 days to the diabetic rats significantly ($p<0.05$) decrease the level of the marker enzymes in diabetic rats which were comparable to control rats. The effect of aqueous extract of *Anacardium occidentale* stem bark on glucose, total protein and albumin of both normal and alloxan induced diabetic rats is as presented in Table 3. There was a significant ($p<0.05$) increase in the level of the glucose while a significant decrease in the level of total and albumin in the untreated diabetic rats when compared with the control and the treated rats. The administration of the aqueous extract of *Anacardium occidentale* stem bark at the dose of 400 mg/kg body weight significantly ($p<0.05$) decrease the level of glucose and significantly increase the level of total protein and albumin in the diabetic treated rats. A similar result was observed in the effect of aqueous extract of *Anacardium occidentale* stem bark on lipid profile of both normal and alloxan induced diabetic rats (Table 4). There was a significant ($p<0.05$) increase in the level of the total cholesterol, triglyceride, low density lipoprotein and a significant decrease in the level of high density lipoprotein in the untreated diabetic rats when compared with the control and the treated rats which was reversed by the administration of the aqueous extract of *Anacardium occidentale* stem bark at the dose of 400 mg/kg body weights to control levels in 28 days. Meanwhile, in the haematological parameters, a significant ($p<0.05$) increase was recorded in the levels of PCV, Hb, RBC and WBC of diabetic treated rats (Table 5).

Table 1: Qualitative Phytochemical Screening of Aqueous Extract of *Anacardium occidentale* Stems Bark

Phytochemicals	Status
Alkaloids	+
Balsam	+
Cardiac glycoside	+
Flavonoids	+
Resin	-
Saponin	-
Tannins	-
Phenols	+
Terpenes/steroids	+

Key = + present; - absent

Table 2: Effect of Aqueous extract of *Anacardium occidentale* stem bark on selected marker enzymes in alloxan induced diabetic rats

	Marker Enzymes (Iμ/L)		
Groups	**ALT**	**AST**	**ALP**
Control	57.10±1.51^{a}	78.40±1.50^{a}	188.30±1.45^{a}
Normal + extract	55.08±1.12^{a}	77.20±1.50^{a}	179.50±1.52^{a}
Diabetic control	103.60±1.38^{b}	107.40±1.46^{b}	232.40±1.34^{b}
Diabetic + extract	54.04±1.50^{a}	79.00±1.50^{a}	200.60±1.51^{ab}

Values are expressed as Mean ± SEM (n = 5). Values in each column with different superscript (a-b) are significantly different ($p<0.05$). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase

Table 3: Effect of Aqueous Extract of *Anacardium occidentale* Stem Bark on Blood Glucose, Protein, and Albumin in Alloxan Induced Diabetic Rats

Groups	Glucose (mg/dL)	Total Protein (g/L)	Albumin (g/L)
Control	105.20±1.46^{a}	72.00±1.28^{a}	37.90±1.34^{a}
Normal + extract	102.40±1.24^{a}	71.50±1.28^{a}	37.20±1.34^{a}
Diabetic control	177.40±1.56^{b}	64.10±1.50^{b}	32.00±1.24^{b}
Diabetic + extract	108.00±1.38^{c}	70.20±1.40^{a}	38.54±1.51^{a}

Values are expressed as Mean ± SEM (n = 5). Values in each column with different superscript (a-c) are significantly different ($p<0.05$).

Table 4: Effect of Aqueous Extract of *Anacardium occidentale* Stem Bark on Serum Lipid Profile of Alloxan Induced Diabetic Rats

	Lipid Profile (mg/dL)			
Groups	**TC**	**TG**	**LDL**	**HDL**
Control	171.60±1.50^{a}	114.60±1.28^{a}	127.11±1.10^{a}	132.42±2.10^{a}
Normal + extract	157.00±1.50^{a}	112.30±1.24^{a}	122.50±3.12^{a}	136.12±1.23^{a}
Diabetic control	374.40±1.50^{b}	258.50±1.38^{b}	254.12±1.51^{b}	84.23±1.32^{c}
Diabetic + extract	187.20±1.50^{c}	141.30±1.38^{c}	147.20±2.00^{c}	112.04±1.30^{b}

Values are expressed as Mean ± SEM (n = 5). Values in each column with different superscript (a-c) are significantly different ($p<0.05$). TC, total cholesterol; TG, triglyceride; LDL, low density lipoprotein; HDL, high density lipoprotein

Table 5: Effect of aqueous extract of *Anacardium occidentale* stem bark on some haematological parameters in alloxan induced diabetic rats

	Haematological Parameters			
Groups	**PCV (%)**	**Hb (g/dL)**	**RBC (10^{6}/µL^{3})**	**WBC (10^{x3}/µL)**
Control	45.00±1.58^{a}	14.14±0.42^{a}	6.02±0.41^{a}	12.02±0.41^{a}
Normal + extract	47.05±1.53^{a}	15.18±0.38^{a}	8.89±0.34^{a}	12.89±0.34^{a}
Diabetic control	38.04±1.54^{b}	9.12±0.40^{b}	3.21±0.45^{b}	6.21±0.45^{b}
Diabetic + extract	43.00±1.55^{c}	11.27±0.41^{c}	5.45±0.51^{a}	11.45±0.51^{c}

Values are expressed as Mean ± SEM (n = 5). Values in each column with different superscript (a-c) are significantly different ($p<0.05$). PCV, packed cell volume; Hb, haemoglobin; WBC, white blood cell; PLT, platelets

DISCUSSION

Prolonged exposure to hyperglycemia is now recognized as the primary causal factor in the pathogenesis of diabetic complications as well as induces a large number of alterations in vascular tissue that potentially promote or accelerated atherosclerosis [32]. In this study, there was a significant increase in the level of blood glucose of rats in the diabetic group. Oral administration of aqueous extract of *Anacardium occidentale* stem bark to the diabetic rats significantly reduced the blood glucose level compared with the control. Previous reports has indicated that plant extracts possess hypoglycemic properties, possible insulin release stimulatory effects and uptake of peripheral glucose, which in turn reversed alloxan induced hyperglycemia [33, 34]. In this study, the phytochemical screening of the aqueous extract revealed the presence of alkaloid, balsam, cardiac glycoside, flavonoid, phenol and terpenes/steroids. Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease [35]. Flavonoids of different plant origin showed a promising anti-diabetic

activity, as demonstrated in diabetic animal models [36-38]. Saponins are glycosides of triterpenes, steroids or alkaloids. Previous researchers have demonstrated the anti-diabetic activity of triterpenoid glycosides [39,40]. Therefore, the phytochemical constituents of *Anacardium occidentale* might be responsible for it's the anti-diabetic property.

Hyperglycemia, a characteristic of diabetes leads to long-term tissue damages and complications, such as liver dysfunctions, often associated with serious diseases. The levels of AST, ALT and ALP have been reported to increase in alloxan-induced diabetic rats [41] as also shown in this study. Aqueous extract of *Anacardium occidentale* stem bark significantly reduces ($p<0.05$) the serum levels of ALT, AST and ALP when compared with the alloxan-induced diabetic untreated rats (Table 2). Measurement of the activities of "marker" enzymes or biomarkers in body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organs/tissues [42, 43]. Such measurements can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques [44].

Albumin is a major protein of human plasma and represents about 25% of total hepatic protein synthesis and half its secreted proteins. Its synthesis is depressed in a variety of diseases, particularly those of the liver [45]. Table 3 shows that there was a significant decrease in the concentration of albumin and total serum protein of the untreated alloxan-induced diabetic rats when compared with the control and the treated groups. This observation may be attributed to numerous effects of hyperglycemia in alloxan-induced diabetes. Hyperglycemia increases gluconeogenesis and as such leads to excess protein breakdown as well as excess loss of nitrogen resulting in negative nitrogen balance [46]. A decline in the total protein level in diabetic rats has been attributed to inhibition of oxidative phosphorylation, which leads to decrease in protein synthesis, increase in catabolic processes and reduction in protein absorption [47]. Oral administration of the extracts at 400 mg/kg body weight caused a remarkable increase in the serum total protein and albumin levels in the diabetic rats.

Diabetes affects both glucose and lipid metabolism [48]. In the postprandial state, elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism [49]. The deficiency of insulin depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes [50]. Alloxan induced diabetic

untreated rats showed significantly increased serum lipid profiles except HDL compared with the control rats. The elevated TG, TC, LDL level and decreased HDL level in alloxan-induced diabetic rats observed in this study is in agreement with the previous reports regarding alteration of these parameters under diabetic condition [51]. This may be due to the increase in the mobilization of free fatty acids (FFA) from the peripheral depots, since insulin inhibits the hormone sensitive lipase [52]. Serum FFA concentration is a result of the balance between the release from lipolysis, neosythesis and disposal and represent the major determinant of insulin effect on FFA oxidation and non-oxidative metabolism [53]. Oral administration of the aqueous extract of *Anacardium occidentale* stem bark to the diabetic rats reverse the diabetic conditions as stated above. The results suggest that aqueous extract of *Anacardium occidentale* stem bark possesses hypolipidaemic agents in combating atherosclerosis, which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level.

The blood is an important body fluid, which contains the Red Blood Cells, White Blood Cells and platelets suspended in the serum in homeostatic concentrations. Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status of organisms [54,55]. Assessment of haematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal [56,57]. It can also be used to explain blood relating functions of chemical compounds/plant extract [58]. The present study thus revealed that alloxan-induced diabetic untreated rats showed some abnormalities in the haematological parameters (PCV, Hb, RBC and WBC) when compared to normal control rats (Table 5). Some of these abnormalities might be due to destruction of mature red blood cells, leading to the low Hb counts accompanied by the fall in the RBC and PCV [59,57]. Administration of the extracts elicits a positive change in the haematological parameters suggesting that it may not contribute further diabetic complications to heamatoligical parameters.

CONCLUSION

This study has shown that aqueous extract of *Anacardium occidentale* stem bark possesses hypoglycaemic, hypolipidaemic effects and ameliorates other adverse diabetic condition imposed by alloxan-induced diabetes on the experimental rats as indicated by the haematological and liver functional indices

assayed in diabetic rats at the dosage and duration of study. Further studies are needed to be carried out to isolate and identify the active principle(s) in the extract as well as elucidate its mode of action and toxicity for enhanced Phytomedicine.

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