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Complexation, Characterization and Toxicology Studies of the Crude Extract of A*nacardium Occidentale L*eaves and its Copper (Ii) Complex in Alloxan-Induced Diabetic Rats

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ABSTRACT

Aim and objective: The search for effective therapy for diabetes mellitus is still unattainable. This study investigated anti-diabetic effect of *Anacardium Occidentale* Ethanolic leaves extract and Ethanolic leaves extract complex copper (II) in alloxan-induced diabetic rats.

Method: Fifty six matured male Wistar rats (200-250g) were used. Alloxan monohydrate (65 mg/kgb.wt) was singly injected intraperitoneally to induced diabetes. The animals were randomly alienated into 7groups, 8rats/group. Group I: normal control; Group II: diabetic; Group III & IV: diabetic rats + 400 mg/kgb.wt and 600 mg/kgb.wt *A. Occidentale* Ethanolic crude leaves extract; Group V and VI: diabetic rats + 400 mg/kgb.wt and 600 mg/kgb.wt Ethanolic leaves extract complex copper (II); Group VII: diabetic rats + 500 mg/kgb.wt metformin for 15 days. Body weight and fasting blood glucose (FBG) were recorded at day 0, 5th, 10th, and 15th of the experiment. The animals were sacrificed at the end of the experiment and fasting blood samples were collected for biochemical parameters assay.

Results: Malondialdehyde (MDA), FBG, lipid profile, hepatic and kidneys function biomarkers levels were significantly ($p<0.05$) increased in the diabetic rats while the body weight and antioxidant activities were significantly ($p<0.05$) reduced. Administrations of *A. Occidentale* Ethanolic leaves extract, Ethanolic leaves extract complex copper (II) and metformin significantly ($p<0.05$) diminished the FBG, MDA, lipid profile, hepatic and kidney function biomarkers levels and elevated the body weight and antioxidant activities.

Conclusion: The Ethanolic leaves extract complex copper (II) possesses more anti-diabetic efficacies. This could be explored as effective complementary therapy for diabetes mellitus.

Keywords: *A. Occidentale* leaves; Diabetes mellitus; Dyslipidemia; Leaves extract complex copper (II); Oxidative stress

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INTRODUCTION

Diabetes mellitus is considered as one of the three non-communicable metabolic diseases with an alarming increase in prevalence globally. According to the International Diabetes Federation (IDF) estimation, the number of diabetes patient is projected to rise from 425 million to 629 million by 2045.

Diabetes mellitus is endocrine metabolic disorder characterized by chronic hyperglycemia resulting from deficient insulin secretion from beta-cells (β-cell) of pancreas (type-1) or irresponsiveness of target cells to insulin action (type-2) or both, thereby affecting metabolism of carbohydrates, proteins and fats. Diabetic patients suffered from type -2 diabetes mellitus is about 95 percent. Clinical symptoms and diagnosis of diabetes mellitus include body weight loss, fatique, polyphagia, ployuria, and polydipsia manifested with hyperglycemia.

Chronic hyperglycemia in diabetic state increases the risk and progression of micro and macro-vascular diabetic complications such as nephropathy, neuropathy, retinopathy, atherosclerosis, cardiovascular disease and stroke, reducing life quality and expectancy.

Hyperglycemia-induced hyperlipidemia and oxidative stress in terms of abnormal lipid profile and generation of excessive free radicals with suppression in endogenous antioxidants defense system play a central role in the pathogenesis of diabetes mellitus complications [1].

Generally, the available anti-diabetic drugs have many drawbacks owing to their numerous adverse toxicities, leading to an increasing search for a safe and non-toxic therapy for diabetes. The use of natural product and their purified compounds from medicinal plants has now gain popularity as promising and novel therapy for diabetes mellitus as it is easily accessible and could be safe from toxic effect.

Anacardium Occidentale Linn (family *Anacardiaceae*) alternatively known as cashew tree originated from Brazil. Phytochemical screening analysis of various part of *A. Occidentale* revealed the presence of numerous bioactive compounds (tannins, flavonoids, phenols, saponins, alkaloids, and anacardic). *A. Occidentale* leaf, nut, root and stem bark are medicinally used for management of different maladies such as diabetes, infection, diarrhea, hemorrhage, and as well as antimicrobial activity. The nut has been reported to attenuate dyslipidemia in rats. Also, anti-hyperglycemic and anti-oxidative of *A. Occidentale* methanolic leaves extract and Ethanolic stem bark extract

has been reported from *in vivo* studies. Beside the utilization of plant extracts as therapeutic agent for diabetes, several studies reported the role of essential metals in glucose metabolism regulation and their deficiency associated with the risk of diabetes.

Copper, the third most abundant essential metal in humans involved in oxidative stress processes regulation. Evidence from literature showed that deficiency of this metal leading to many diabetes complications. However, research on complexation of *A. Occidentale* part with medicinal metal oxide for diabetes and its related complications progression therapy has never been reported. Therefore, this study investigates the antidiabetic activity of *A. Occidentale* Ethanolic leaves extract and extract complexed with copper (II) in alloxan-induced diabetic rats model [2].

MATERIALS AND METHODS

Chemicals and drugs: Alloxan monohydrate, glucose solution, ketamine, Xylazine, Ethanol

Plant material: *Anacardium Occidentale* leaves were collected from the Agricultural Research Farm, Ladoke Akintola University, Ogbomoso, and Oyo State, Nigeria. The plant was identified, authenticated and given a voucher number LH0533 by Dr. A. T. J. Ogunkunle at Biology Department, Ladoke Akintola University of Technology.

Preparation of *A. occidentale* crude ethanolic leaves extract and ethanolic leaves extract complex copper (ii)

The leaves of *A. occidentale* were washed thoroughly and air-dried at room temperature. The leaves were grinded into a fine-powdered form using an electric blender and stored in air-tight container. 500 g of the fine powdered form was extracted in a Soxhlet apparatus using ethanol solvent (95%).The filtrate was then evaporated to dryness under reduced pressure in a rotary evaporator and the ethanolic extract obtained was kept at 4°C in an air-tight container until used.

The ethanolic leaves extract copper (II) complex was prepared by dissolving 0.5 g ($2.75x10^{-3}$ mole) of copper (II) acetate in 42 cm^3 of distilled water. The copper acetate solution was added drop wisely to a solution of 1 g *A. occidentale* crude extract in 50 cm^3ethanol. The solution was rigorously stirred using a magnetic stirrer at 3500 rpm for 1hr. The precipitated complex was filtered and washed with water distilled water then dried in vacuum desiccators over calcium chloride ($Cacl_2$). A pure green solid was obtained and weighed [3].

Experimental animals: Fifty six matured male Wistar albino rats weighing (200-250 g) were procured and housed at Department of Physiology Animal Research House, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. The animals were kept in a well-clean ventilated plastic cage and allotted into seven groups, eight rats each. The animals were fed with a standard feed and water *ad libitum* under pathogen-free environment of temperature (25 ± 2°C), relative humidity (40 ± 5°C) and natural 12:12 light/dark cycle one week acclimatization prior the initiation of the experiment. The experimental procedures were conducted according to the National Guide for the Care and Use of Laboratory Animals and approved by Ladoke Akintola University of Technology Research Committee

Diabetes induction: The animals were fasted overnight (12hrs) before diabetes induction. After an overnight fasting, forty eight animals were injected intraperitoneally with a single dose (65 mg/kgb.wt) alloxan monohydrate dissolved in a normal saline (Nacl) to induced diabetes and allow to drink 5% glucose solution to prevent drug-induced hypoglycemic death. After 72hrs of alloxan injection, fasting blood glucose levels of the animals were checked with glucometer (Accu-check) using tail vein blood dropped wisely on test stripe to authenticate diabetes induction. Animals with fasting blood glucose levels ≥ 250 mg/dl were chosen as diabetic rats and used for the study. The fasting blood sugar levels of the animals were recorded prior the injection of alloxan monohydrate [4].

Experimental design and treatment

The animals were randomly divided into seven groups, 8 rats each.

Group I: Normal control (non-diabetic)

Group II: Diabetic (untreated) rats.

Group III: Diabetic rats + 400 mg/kgb.wt *A. occidentale* ethanolic leaves extract

Group IV: Diabetic rats + 600 mg/kgb.wt *A. occidentale* ethanolic leaves extract

Group V: Diabetic rats + 400 mg/kgb.wt *A. occidentale* ethanolic leaves extract complex copper (II)

Group VI: Diabetic rats + 600 mg/kgb.wt *A. occidentale* ethanolic leaves extract complex copper (II)

Group VII: Diabetic rats + 500 mg/kgb.wt metformin.

The extracts and metformin were given orally with the aid of cannula for 15 consecutive days with feed and water *ad libitum*.

The body weight and fasting blood glucose levels of the animals were measured at day 0, 5th, 10th, and 15th of the experimental period. Fasting blood glucose levels of the animals were measured by glucose oxidase-peroxidase (GOD-POD) method using one-touch digital glucometer (Accu-chek) by dropping tail vein blood on glucose test stripes.

Biochemical assay: At the end of the 15 days treatment, the animals were subjected to overnight fasting and sacrificed by cervical dislocation after anesthetized with (40 mg/kgb. wt) and xylazine (15 mg/kgb.wt) injected intraperitoneally. Fasting blood samples were collected from the animals apex beat heart via cardiac punctured into heparinized tubes and centrifuged at 3500 rpm for 5 minutes. The clear supernatant plasma collected after centrifugation was used for biochemical parameters estimations.

Triglycerides (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL) levels were estimated based on enzymatic colorimetric method with a commercial Diagnostic Kit (Genzyme Diagnostics, MA, USA) according to the manufacturers' protocol. Low-density lipoprotein-cholesterol (LDL) level was calculated using Friedewald *et al.* formula; LDL = TC – (HDL - TG/5) [5]. Oxidative stress marker malondialdehyde (MDA) level and activities of antioxidants superoxide dismutase (SOD),

and catalase (CAT) were measured by enzyme linked Immunosorbent assay (ELISA) methods using specific Rat MDA, SOD and CAT commercial Elisa Kit (Elabscience, China) follow manufacturers' instruction. Glutathione reductase (GSH) was measured based on the Gupta and Gupta method.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) hepatic enzymes biomarkers levels were measured spectrophotometrically using standard automated techniques based on the instruction of the manufacturer.

Urea and creatinine kidney function markers levels were determined using a commercial assay kit obtained from Siemens Health Care Diagnostics.

Statistical analysis: Statistical Package for Social Sciences (SPSS version, 21.0) was used to analyze the data. The data values were expressed as standard error of mean (mean ± SEM) and data statistical significant was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoctest. Data at P<0.05 were considered statistically significant [6].

RESULTS

Effect of *A. occidentale* ethanolic leaves extract and complex extract on body weight change in alloxan-induced diabetic rats

The body weight of untreated diabetic rats significantly (p<0.05) decrease throughout the experiment compared with the control group. However, administration of the *A. occidentale* ethanolic leaves extract and complex extract significantly increase the body weight compared with untreated groups (**Tab. 1a.**).

Effect of *A. occidentale* ethanolic leaves extract and complex extract on fasting blood glucose levels in alloxan-induced diabetic rats

Fasting blood glucose levels of untreated diabetic rats significantly (p<0.05) elevated compared with the control group. Supplementation of *A. occidentale* ethanolic leaves extract and complexed extract as well as metformin significantly reduced the fasting blood glucose level in comparison with the untreated diabetic group. Better hypoglycemia result was obtained in the diabetic group treated with high dose of the complex extract (600 mg/kgb.wt) (**Tab. 1b.**).

Effect of *A. occidentale* ethanolic leaves extract and complex extract on lipid profile in alloxan-induced diabetic rats

The untreated diabetic rats showed significant (p<0.05) elevation in triglyceride (TG), total cholesterol (TC) and low-density lipoprotein (LDL) levels with significant decrease (p<0.05) in high-density lipoprotein (HDL) level. Administration of *A. Occidentale* Ethanolic leaves extract, complex extract and metformin to alloxan-induced diabetic rats markedly decline TC, TG, LDL levels along with a marked increase in HDL level compared to the untreated group (p<0.05). The high dose of the complex extract (600 mg/kgb.wt) exhibited more significant lowering effect on TC, TG, LDL levels to the extent of approaching control level and elevates HDL-c level reaching the level of control group (**Fig.1.**).

Effect of *A. occidentale* ethanolic leaves extract and complex extract on oxidative stress marker and antioxidants enzymes activity and in alloxan-induced diabetic rats

Diabetic rats exhibited signification (p<0.05) reduction in the activities of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) with significant (p<0.05) increase in oxidative stress marker malondialdehyde (MDA) level in comparison with the control group. The antioxidants enzymes SOD, CAT, and GSH activities were up-regulated along with down-regulation in the MDA level after administration of crude extract of *A. Occidentale*, complexed extract and as well

Tab.1a. Effect of A. Occidentale Ethanolic leaves extract and complex extract on body weight change in alloxan-induced diabetic rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.

	PRE-TREATMENT	POST- TREATMENT		
GROUPS	DAY 0	DAY 5	DAY 10	DAY 15
Normal (non-diabetic)	239.67 ± 51.98	245.33 ± 52.60	246.00 ± 10.44	251.00 ± 8.54
Diabetic (untreated)	239.33 ± 45.39 *	227.67 ± 42.71*	214.00 ± 41.61*	195.67 ± 42.92 *
Metformin (500 mg/kg)	228.67 ± 21.73#	212.00 ± 20.30#	223 ± 19.00#	238.00 ± 17.06#
Crude extract (400 mg/kg)	223.67 ± 16.20#	229.00 ± 12.17#	234.00 ± 9.45#	247.00 ± 9.30#
Crude extract (600 mg/kg)	286.67 ± 14.57#	281 ± 12.77#	283.67 ± 12.90#	290.00 ± 13.45#
Complex extract (400 mg/kg)	216.00 ± 3.61#	221.67 ± 4.04#	226.33 ± 4.04#	232.67 ± 4.04#
Complex extract (600 mg/kg)	221.00 ± 3.61#	226.33 ± 4.51#	222.67 ± 4.51#	227.67 ± 4.16#

Tab.1b. Effect of A. Occidentale Ethanolic leaves extract and complex extract on plasma fasting blood glucose in alloxan-induced diabetic rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.

	PRE-TREATMENT	POST-TREATMENT		
GROUPS	DAY 0	DAY 5	DAY 10	DAY 15
Normal (non-diabetic)	100.33 ± 15.01	98.00 ± 14.53	101.00 ± 14.11	104.00 ± 14.01
Diabetic (untreated)	236.00 ± 10.58*	240.00 ± 10.54*	276.00 ± 15.28*	263.33 ± 6.51*
Metformin (500 mg/kg)	314.67 ± 11.68#	283.33 ± 21.73#	259.67 ± 44.80#	193.67 ± 12.12#
Crude extract (400 mg/kg)	324.67 ± 8.50#	329.00 ± 9.54#	191.33 ± 23.84#	151.00 ± 23.50#
Crude extract (600 mg/kg)	344.67 ± 18.82#	312.33 ± 9.61#	192.67 ± 7.51#	144.00 ± 32.36#
Complex extract (400 mg/kg)	331.33 ± 40.70#	343.00 ± 69.59#	272.00 ± 59.86#	220.00 ± 34.39#
Complex extract (600 mg/kg)	312.67 ± 3.06#	281.00 ± 36.67#	184.00 ± 45.62#	138.00 ± 20.82#

as metformin to the diabetic rats compared to untreated group. Low dose of the complex extract (400 mg/kgb. wt) offered best result in the up-regulation of antioxidant enzymes with value more than the control group and then other doses and metformin (**Tab. 2.**).

Effect of *A. occidentale* ethanolic leaves extract and complex extract on liver and kidney function markers in alloxan-induced diabetic rats

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, (ALP), albumin, globulin and total bilirubin of liver biomarkers and urea, and creatinine of kidney biomarkers were significantly ($p<0.05$) elevated in alloxan-induced diabetic rats compared to control group. AST, ALT, ALP, albumin, globulin, and total bilirubin of liver biomarkers, and urea and creatinine of kidney biomarkers were significant reduced after treatment with *A. Occidentale* Ethanolic leaves extract, complex extract and metformin compared with the untreated diabetic group. Complex extracts high dose (600 mg/kgb.wt) lowered the markers considerably (**Tab. 3a & 3b.**) [7-9].

The spectral are interpreted considering few main peaks observed. The results of the infrared spectra with those of complex and ligand (**Tab. 4.**). The absorption bands at 3641.6 cm^{-1} and 3641.6 cm^{-1} is due to O-H Stretching alcohol. The absorption band at 3391.9 cm^{-1} in complex due to (N-H) stretching vibration has been shifted to 3429.2 cm^{-1} in the ligand. The bands at 2288.6 cm^{-1} in complex, 2288.6 cm^{-1} were assigned to (C≡N) stretching

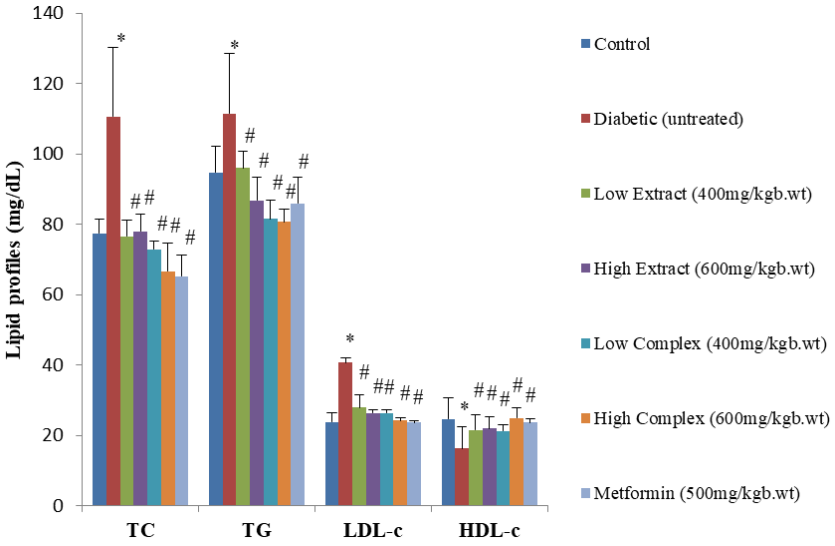

Fig. 1. Effect of A. occidentale ethanolic leaves extract and complex extract on lipid profiles in alloxan-induced diabetic rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.

Tab.2. Effect of A. Occidentale Ethanolic leaves extract and complex extract on oxidative stress marker and antioxidants enzymes activity and in alloxan-induced diabetic rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.

	GROUPS						
MARKERS	Control	Diabetic (untreated)	Low Extract (400mg/kgb. wt)	High Extract (600mg/kgb. wt)	Low Complex (400mg/kgb. wt)	High Complex (600mg/kgb. wt)	Metformin (500mg/kgb. wt)
MDA (µmol/g)	35.69 ±1.31	68.81 ± 3.75*	39.07 ± 5.01#	38.67 ± 2.60#	36.26 ± 2.93#	35.75± 1.10#	34.08 ± 1.72#
GSH (µmol/g)	0.60 ± 0.17	0.49 ± 0.19*	0.70 ±0.08#	0.60 ±0.004#	0.74 ±0.05#	0.57±0.01#	0.59 ± 0.03#
SOD (µmol/g)	1.29 ± 0.03	0.59 ± 0.08*	1.30 ±0.04#	1.26 ± 0.008#	1.34 ±0.08#	1.24± 0.01#	1.25 ± 0.01#
CAT (µmol/g)	7.29 ± 0.23	3.14 ± 0.34*	7.35 ±0.40#	7.12 ±0.28#	7.46 ±0.27#	7.28±0.19#	7.13 ±0.16#

Tab.3a. Effects of A. Occidentale Ethanolic leaves extract and complex extract on liver function markers in alloxan-induced diabetic rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.

	GROUPS						
Liver markers Parameters	Control	Diabetic (untreated)	Low Extract (400mg/kgb.wt)	High Extract (600mg/kgb.wt)	Low Complex (400mg/kgb.wt)	High Complex (600mg/kgb.wt)	Metformin (500mg/kgb. wt)
Albumin (g/dl)	1.74 ± 0.07	2.24 ± 0.81*	2.02 ± 0.20#	1.72 ± 0.05#	1.64 ± 0.06#	1.62 ± 0.06#	1.59 ± 0.06#
Globulin (g/dl)	1.31 ± 0.11	2.23 ± 0.09*	1.47 ± 0.09#	1.39 ± 0.15#	1.36 ± 0.02#	1.24 ± 0.07#	1.32 ± 0.12#
ALP (U/L)	24.66 ± 0.74	36.06 ± 5.59*	26.16 ± 2.03#	23.89 ± 2.16#	24.90 ±1.06#	24.70 ± 1.91#	24.18 ± 0.92#
AST (U/L)	35.26 ± 2.64	75.09 ± 3.52*	39.82 ± 1.15#	35.39 ± 3.38#	37.79 ±7.05#	29.30 ± 2.30#	29.82 ± 0.76#
ALT (U/L)	31.59 ± 1.55	56.62 ± 6.26*	37.06 ±1.56#	35.36 ± 2.56#	37.62 ±3.10#	27.81±2.28#	28.63 ±4.28#
Total bilirubin (mg/dl)	1.55 ± 0.07	2.68 ± 0.38*	2.06 ±0.20#	1.59 ± 0.18#	2.01 ±0.29#	1.41±0.08#	1.56 ±0.23#

Tab. 3b. Effects of *A. occidentale* ethanolic leaves extract and complex extract on kidney function markers in alloxan-induced diabetic rats.

Kidney markers Parameters	GROUPS						
	Control	Diabetic (untreated)	Low Extract (400mg/kgb. wt)	High Extract (600mg/kgb. wt)	Low Complex (400mg/kgb. wt)	High Complex (600mg/kgb.wt)	Metformin (500mg/kgb. wt)
Urea (mg/dl)	3.96 ± 0.21	6.33 ± 1.47*	4.10 ± 0.29#	4.02 ± 0.19#	4.15 ± 0.09#	3.81 ± 0.25#	3.84 ± 0.26#
Creatinine (mg/dl)	0.57 ± 0.02	1.23 ± 0.08*	0.67 ± 0.03#	0.63 ± 0.05#	0.59 ± 0.09#	0.52 ± 0.23#	0.53 ± 0.05#

*Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with untreated diabetic group.*

Tab. 4. Infrared spectral data of ligands and their metal complexes.

Peak number	Complex (wave number cm^{-1})	Ligand (wave number cm^{-1})	Band assignments
1	3641.6	3641.6	O-H stretching alcohol
2	3391.9	3429.2	N-H stretching primary amine
3	2288.6	2288.6	C≡N stretching of nitrile
4	2109.7	2210.2	C≡N stretching of alkyne
5	1979.2	1982.9	C≡N stretching of allene
6	1852.5	1889.8	C-H bending of aromatic
7	1781.7	1798.6	C=O stretching of phenyl esters
8	1654.9	0	C=C stretching of alkene
9	1476.0	1397.8	C-H bending of alkene
10	1416.4	0	O-H bending of alcohol
11	1062.3	1088.4	C-O stretching of primary alcohol
12	857.3	0	C-Cl stretching of an halo compound

vibration. The absorption bands at 2109.7 cm^{-1} increase to 2210.2 cm^{-1} due to C≡C Stretching of alkyne vibration, The absorption band at 1979.2 cm^{-1} in complex shifted to 1982.9 in the ligand due to (C=C) Stretching of allene vibration. The bands at 1852.5 cm^{-1} in the complex increases to 1889.8 cm^{-1} in C-H Bending of aromatic vibration. The band in the complex at 1781.7 cm^{-1} shifted to 1798.6 cm^{-1} at (C=O) Stretching of phenyl esters. There is an absence of absorption band in the ligand, complex with 1654.9 cm^{-1} in the (C=C) Stretching of alkene. The absorption of band in the complex which decreases from 1476.0cm^{-1} to 1397.8 cm^{-1} in the (C-H) Bending of alkene vibration. There is an absence of absorption band in the ligand, complex with 1416.48 cm^{-1} in the (O-H) Bending of alcohol. The absorption band at 1062.3 cm^{-1} in complex due to (C-O) Stretching of primary alcohol vibration has been shifted to1088.4 cm^{-1} in the ligand. There is an absence of absorption band in the ligand, complex with 857.3 cm^{-1} in the (C-Cl) Stretching of a halo compound [10-14].

DISCUSSION

In this study, anti-diabetic efficacies of *A. Occidentale* Ethanolic leaves extract and extract complex copper (II) in alloxan monohydrate-induced diabetic rats model were investigated.

Severe body weight loss is a critical symptom of diabetes mellitus. The disparity in energy utilization and expenditure leads to body weight alteration. The loss of body weight might be the result of a decline in glucose metabolism, elevated lipolysis, or structural proteolysis that generates an alternate source of energy. The findings of this study came in line with the result of Faried and El-Mehi, the diabetic rats exhibited a noticeably decrease in body weight. However, administration of the *A. Occidentale* Ethanolic leave extract and the complex extract caused increase in body weight of the diabetic rats. The observed obvious increase in body weight of diabetic rats treated with the highest Ethanolic extract dose (600 mg/kgb.wt) possibly resulted from its efficient hypoglycemic properties via inhibition of hyperglycemia-induced proteolysis and stimulation of insulin secretion and production to activate fat storage hormone which could be attributed to its bioactive compounds and agreed with the report of Bekara et al [15-19].

Hyperglycemia, the common feature for both type-1 and type-2 diabetes mellitus leads to the pathogenesis of many diabetic complications. In the current study, alloxan-induced diabetic rats showed dramatic elevated blood glucose levels, which correspond with the findings of Arika et al. Elevated blood glucose in diabetic condition may be a consequence of insulin secretion deficiency from pancreatic beta-cells/insensitivity of peripheral tissue to insulin action for glucose uptake and increase hepatic gluconeogenesis. The Ethanolic extract and complex extract administrations effectively lowered the fasting blood glucose levels of the diabetic rats. The complex extract high dose (600 mg/kgb.wt) and Ethanolic leaves extract high dose (400 mg/kgb.wt) efficiently reduced the elevated fasting blood glucose levels than other extracts and metformin. Bioactive compounds in medicinal plants have been reported to play a significant role in lowering blood glucose. Hypoglycemic effect of these extracts may be attributable to the presences of bioactive compounds such Phenolics and flavonoids, capable of stimulating insulin secretion from pancreatic beta-cells, facilitate glucose uptake at peripheral tissues, inhibition of hepatic and muscle glucose production, and inhibition of intestinal α-amylase and α-glucosidase enzyme activities and is in line with the report of Shobha and Andallu, on hypoglycaemic effect of anise extract [20-24].

Dyslipidemia, elevated total cholesterol (TC), triglyceride (TG), and altered lipoprotein components noticed by high concentration of low-density lipoprotein-cholesterol (LDL) and low concentration of high-density lipoprotein-

cholesterol (HDL), is a recognized metabolic abnormality in diabetic condition. Elevated LDL concentration has been linked with progression of cardiovascular disease in diabetic patients. High TC, TG, LDL concentration with diminished HDL concentration remarkably observed in the diabetic rats, consistent with findings of Miaffo et al. Abnormal increment in lipid levels may likely due to deficiency in anti-lipolytic action of insulin to inhibit hormone-sensitive lipase leading to increased mobilization of free fatty acids and peripheral fat deposits. Treatment of diabetic rats in this study with *A. Occidentale* leaves Ethanolic extract and complex leaves extracts attenuated the dyslipidemia by lowering the TC, TG, LDL levels and ameliorate the HDL level and the complex extract high dose (600 mg/kgb.wt) produce considerable anti-dyslipidemic effect by raising the HDL level almost the same as control level, thereby prevent cardiovascular disease development. Several authors have reported the lipid lowering effect of some bioactive compounds include saponins, phenolic and flavonoids. Attenuation of dyslipidemia in the diabetic rats may also relate to the presence of these bioactive compounds, consistent with report of Limaye et al [25-29].
Diabetic related complications progression has been linked with overwhelming antioxidants enzymes by reactive oxygen species (ROS) generated by oxidative stress. The diabetic rats in this study demonstrated increased oxidative stress marker malondialdehyde (MDA) level and depletion in the activities of antioxidants enzymes superoxide dismutase (SOD), catalase and reduced glutathione (GSH), came in line with findings of Yazdanimehr and Mohammadi. The activities of antioxidants SOD, CAT and GSH were ameliorated with suppressed MDA level in the diabetic rats treated with Ethanolic leaves extract and complexed extract. This revealed its free radical scavenging efficacies via its antioxidant properties; support the findings of Hroob et al. Complex extract lowest dose (400 mg/kgb.wt) strongly boost the antioxidant enzymes activities. Also, phytochemicals flavonoids and phenolics have been identified with antioxidant properties for oxidative stress-related diabetic complications therapy. The presence of these phytochemicals in *A. Occidentale* leaves may responsible for its antioxidants property [30-34].
In diabetic condition, elevation in hepatic aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase, (ALP) are good indicators for hepatic damage. Also, rise in urea and creatinine signify renal malfunction. Hepatic biomarkers AST, ALT, ALP and renal urea and creatinine were elevated in the diabetic rats of current study, which corroborate with the findings of Wen et al. Oxidative stress, induced many organs complications in diabetic state. The hepatic and renal dysfunction observed might be consequence of excessive free radicals. Elevated AST, ALP, and ALP hepatic markers and renal urea and creatinine levels were restored after administration of A. *Occidentale* leaves Ethanolic extract and complexed extract to the diabetic rats. Complex extract high dose (600 mg/kgb.wt) appreciably restored the renal and hepatic functions. This suggests that the extracts prevent toxic effect of oxidative stress on organs via its antioxidant property from the bioactive components; concurrence with report of D'Souza et al on amelioration of diabetic nephropathy by *Acacia catechu* leaves extract [35-41].

CONCLUSION

From this finding, the complexed extract remarkably attenuate hyperglycemia, dyslipidemia and oxidative stress. It also ameliorates the antioxidants activities and protects kidney and liver damages. Complexation of *A. Occidentale* leaves with copper would improve its potency as novel therapeutic for diabetes mellitus.

DECLARATIONS

Authors' Contributions

FO, AO conceived the original idea, designed and supervised the research. DE, MO performed the experiment with the support of FO and AM. DE collected the data. DE, FO analyzed the data and prepared the manuscript. FO, MO reviewed the manuscript. All authors' have read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

All data generated and analyzed during this study are included in this article.

COMPETING INTERESTS

No competing interests.

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REFERENCES

1. **Dianna J, Edward J**. IDF Diabetes Atlas. 7th edition 2015. *IDF*. 2016;13: 78-98.
2. **Roshanzamir N, Hassan-Zadeh V.** Methylation of Specific CpG Sites in IL-1β and IL1R1 Genes is Affected by Hyperglycaemia in Type 2 Diabetic Patients. *Immunol Invest*. 2020;49: 287-298.
3. **Khamchan A, Paseephol T, Hanchang W, et al.** Protective effect of wax apple (*Syzygiumsamarangense* (Blume) Merr. & LM. Perry) against streptozotocin-induced pancreatic β-cell damage in diabetic rats. *Biomed Pharmacother*. 2018;108: 634-645.
4. **Piero NM, Eliud NN, Susan KN, et al.** In vivo Antidiabetic Activity and Safety In Rats of *Cissampelospareira* Traditionally Used In He Management of Diabetes Mellitus In Embu County, Kenya. *Expert Opin Drug Metab Toxicol*. 2015; 6: 184.
5. **Chehade JM, Gladysz M, Mooradian AD, et al.** Dyslipidemia in type 2 diabetes: prevalence, pathophysiology, and management. *Drugs*. 2013;73: 327-39.
6. **Fernandes SM, Cordeiro PM, Watanabe M, et al.** The role of oxidative stress in streptozotocin-induced diabetic nephropathy in rats. *Arch EndocrinolMetab*. 2016; 60: 443-449.
7. **Mahmoodnia L, Aghadavod E, Beigrezaei S, et al**. An update on diabetic kidney disease, oxidative stress and antioxidant agents. *J Renallnj Prev*. 2017; 6: 153-157.
8. **Alam M, Meerza D, Naseem I, et al.** Protective effect of quercetin on hyperglycemia, oxidative stress and DNA damage in alloxan induced type 2 diabetic mice. *Life Sci*. 2014;109: 8-14.
9. **Salehi B, Gultekin-ozguven M, Kirkin C, et al.** Anacardium Plants: Chemical, Nutritional Composition and Biotechnological Applications. *Biomolecules*. 2019;9: 465.
10. **Silva RA, Liberio S, Amaral FM, et al.** Guerra RNM. Antimicrobial and antioxidant activity of *Anacardium occidentale L*. flowers in comparison to bark and leaves extracts. *J Biosci Med.* 2016; 4:87-99.
11. **Dias CCQ, Madruga MS, Pintado MME, et al.** Cashew nuts (*A. occidentale* L.) decrease visceral fat, yet augment glucose in dyslipidemic rats. *PLoS ONE*. 2019;14: 225-236.
12. **Ukwenya VO, Ashaolu JO, Adeyemi AO, et al.** Caxton-Martins EA. Anti-hyperglycemic activities of methanolic leaf extract of *Anacardium occidentale* (Linn.) on the pancreas of streptozotocin-induced diabetic rats. *J Cell Anim Biol.* 2012;6: 169-177.
13. **Singh R.** Anti-hyperglycemic effect of ethanolic extract and fractions of *Anacardium occidentale L.* stem bark in streptozotocin-induced diabetic rats. *J Basic Clin Pharm*. 2009;1: 16-9.
14. **Zhang C.** Diabetes-induced hepatic pathogenic damage, inflammation, oxidative stress, and insulin resistance was exacerbated in zinc deficient mouse model. *PLoS ONE*. 2012;7: 49-57.
15. **Crichton RR, Pierre JL**. Old iron, young copper: From Mars to Venus. *Biometals*. 2001;14: 99-112.
16. **Pizzino G, Irrera N, Cucinotta M, et al.** Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev.* 2017;8: 16-23.
17. **Uriu-Adams JY, Rucker RB, Commisso JF, et al.** Diabetes and dietary copper alter 67Cu metabolism and oxidant defense in the rat. *J Nutr Biochem.* 2005;16: 312-320.
18. **Friedewald WT, Levy RI, Fredrickson DS, et al.** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*. 1972;18: 499-502.
19. **Gupta R, Gupta RS.** Protective Role of *Pterocarpus marsupium* in Diabetes-Induced Hyperlipidemic Condition. *J Complement Integr Med*. 2009; 6: 21.
20. **Yakubu MT, Uwazie NJ, Igunnu A, et al**. Anti-diabetic activity of aqueous extract of *Senna alata* (Fabacea) flower in alloxan induced diabetic male rats Cameroon. *J Biol Biochem Sci*. 2016;24: 7-17.
21. **Faried MA, El-Mehi AES.** Aqueous anise extract alleviated the pancreatic changes in streptozotocin-induced diabetic rat model via modulation of hyperglycaemia, oxidative stress, apoptosis and autophagy. *Folia Morphol.* 2020;79: 3.
22. **Bekara A, Hamadouche NA, Kahloula K, et al.** Effect of *Pimpinellaanisum L* (Aniseed) Aqueous Extract against Lead (Pb) Neurotoxicity: Neurobehavioral Study. *Int J Neurosci Behav Scien.* 2015;3: 32 -40.
23. **Kawasaki E.** Type 1 diabetes and autoimmunity. *Clin Pediatr Endocrinol*. 2014;23: 99-105.
24. **Arika WM, Nyamai DW, Agyirifo DS, et al.** *In vivo* Antidiabetic Effect of Aqueous Leaf Extract of *Azardirachtaindica, A*. juss in Alloxan Induced Diabetic Mice. *J Diabetic Complications Med.*2016; 1: 2-6.
25. **Ibrahim MA, Koorbanally NA, Islam MS, et al.** Antioxidative activity and inhibition of key enzymes linked to type-2 diabetes (alpha-glucosidase and alpha-amylase) by Khayasenegalensis. *Acta Pharm*. 2014;64: 311-324.
26. **Shobha R, Andallu B.** Antioxidant, anti-diabetic and hypolipidemic effects of aniseeds *(pimpinellaanisum L*.) In vitro and In vivo studies. *J Complement Med Alt Healthcare*. 2018; 5: 2.
27. **Abdulmalek SA, Balbaa M.** Synergistic effect of nano-selenium and metformin on type 2 diabetic rat model: Diabetic complications alleviation through insulin sensitivity, oxidative mediators and inflammatory markers. *PLoS ONE*. 2019;14: 70-79.
28. **Balbaa M, Abdulmalek SA, Khalil S, et al.** Oxidative stress and expression of insulin signaling proteins in the brain of diabetic rats: Role of *Nigella sativa* oil and antidiabetic drugs. *PLoS ONE*. 2017;12: 172-179.
29. **Oloyede OB, Ajiboye TO, Abdussalam AF, et al.** *Blighiasapida* leaves halt elevated blood glucose, dyslipidemia and oxidative stress in alloxan-induced diabetic rats. *J Ethnopharmacol*. 2014;157: 309-319.
30. **Miaffo D, Kamgue OG, Tebou NL, et al.** Antidiabetic and antioxidant potentials of *Vitellariaparadoxa* barks in alloxan-induced diabetic rats. *Clinical Phytoscience*. 2019;5: 44.
31. **Ference BA, Kastelein JJ, Ray KK, *et al.*** Association of triglyceride-lowering LPL variants and LDL-C–lowering LDLR variants with risk of coronary heart disease. *JAMA.* 2019;321: 364-73.
32. **Limaye PV, Raghuram N, Sivakami S, et al**. Oxidative stress and gene ion of antioxidant enzymes in the renal cortex of streptozotocin induced diabetic rats. *Mol Cell Biol*. 2003;243: 147-52.
33. **Réus GZ, Carlessi AS, Silva RH, *et al.*** Relationship of oxidative stress as a link between diabetes mellitus and major depressive disorder. *Oxid Med Cell Longev*. 2019;2019: 863-870.
34. **Yazdanimehr S, Mohammadi MT.** Protective effects of rosuvastatin against hyperglycemia induced oxidative damage in the pancreas of streptozotocin-induced diabetic rats. *Physiology and Pharmacology*. 2018;22: 19-27.
35. **Al Hroob AM, Abukhalil MH, Alghonmeen RD, et al.** Mahmoud AM Ginger alleviates hyperglycemia-induced oxidative stress, inflammation and apoptosis and protects rats against diabetic nephropathy. *Biomed Pharmacother*. 2018;106: 381-389.
36. **Babu PVA, Liu D, Gilbert ER, et al.** Recent advances in understanding the anti-diabetic actions of dietary flavonoids. *J Nutr Biochem.* 2013;24: 1777-1789.
37. **Dkhil MA.** The potential protective role of *Physalisperuviana L.* fruit in cadmium-induced hepatotoxicity and nephrotoxicity. *Food Chem Toxicol.* 2014;74: 98-106.
38. **Anders HJ, Huber TB, Isermann B, et al.** CKD in diabetes: Diabetic kidney disease versus non-diabetic kidney disease. *Nat Rev Nephrol.* 2018;14: 361-377.
39. **Wen W, Lin Y, Ti Z, et al.** Anti-diabetic, Antihyperlipidemic, Antioxidant, Anti-inflammatory Activities of Ethanolic Seed Extract of *Annona reticulata L.* in Streptozotocin Induced Diabetic Rats. *Front Endocrinol*. 2019;10: 716.
40. **Panigrahy SK, Bhatt R, Kumar A, et al.** Reactive oxygen species: sources, consequences and targeted therapy in type 2 diabetes. *J Drug Target*. 2017;25: 93-101.
41. **D'souza P, Holla R, Swamy G, et al**. Amelioration of Diabetic Nephropathy in Streptozotocin-Induced Diabetic Rats by *Acacia catechu* Leaves Extract. *J Health Allied Sci*. 2019; 9: 116-120.