ISSN: 2471-2663
Clinical & Medical Biochemistry

OPEN ACCESS Freely available online

Research Article

Ethanol Leaf Extract of *Anacardium occidentale* Ameliorates Alloxan Induced Changes on Blood Glucose Level and Lipid Profile of Wistar Rats

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ABSTRACT

Background: It is estimated that by the year 2030 the number of persons with Diabetes Mellitus (DM) would increase to 366 million. Diabetes Mellitus (DM) is a condition primarily defined by the level of hyperglycemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy).

Aim: To investigate the effect of aqueous leaf extract of *Anacardium occidentale* on blood glucose level and lipid profile of diabetic rats.

Methods: Adult male Wistar rats (n=30) weighing 150 g-200 g (mean weight=175 g ± 25 g) were randomly assigned to 6 groups (5 rats/group): Normal control, diabetic control and Dimethyl Sulfoxide (DMSO), metformin, extract and treatment groups. Diabetes Mellitus (DM) was induced *via* intraperitoneal injection of freshly prepared alloxan monohydrate solution at a dose of 120 mg/kg Body Weight (BWT). Fasting Blood Glucose (FBG) level was assayed using glucometer. Lipid profile parameters and activities of Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were determined using their respective kits.

Results: Treatment of diabetic rats with aqueous extract of *A. occidentale* leaves significantly reduced their body weights ($p<0.05$), but there were no significant differences in the relative weights of kidney, pancreas and liver among the groups ($p>0.05$). Treatment of diabetic rats with aqueous extract of *A. occidentale* leaves led to significant and time dependent reductions in their FBG levels, as well as activities of ALT and ALP ($p<0.05$). It also significantly reduced the circulating levels of Triacylglycerol (TG), Total Cholesterol (TC), Very Low Density Lipoprotein Cholesterol (VLDL-C) and Low Density Lipoprotein Cholesterol (LDL-C), but significantly increased High Density Lipoprotein Cholesterol (HDL-C) ($p<0.05$).

Conclusion: These results suggest that aqueous extract of *Anacardium occidentale* leaves mitigates diabetogenic action of alloxan in wistar rats.

Keywords: Alloxan; *Anacardium occidentale*; Diabetes mellitus; Fasting blood glucose; Lipid profile

INTRODUCTION

It is estimated that by the year 2030 the number of persons with Diabetes Mellitus (DM) would increase to 366 million. Diabetes Mellitus (DM) is a condition primarily defined by the level of hyperglycemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease), as well as diminished quality of life [1]. If blood glucose level remains high (hyperglycemia) over a

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Received: 22-Sep-2020, Manuscript No. CMBO-20-6561; **Editor assigned:** 26-Sep-2020, PreQC No. CMBO-20-6561 (PQ); **Reviewed:** 10-Oct-2020, QC No. CMBO-20-6561; **Revised:** 02-Jan-2023, Manuscript No. CMBO-20-6561 (R); **Published:** 30-Jan-2023, DOI: 10.35841/2471-2663.22.9.142

Citation: Abu ODO, Imafidon KE, Obayuwana O (2023) Ethanol Leaf Extract of *Anacardium occidentale* Ameliorates Alloxan Induced Changes on Blood Glucose Level and Lipid Profile of Wistar Rats. Clin Med Bio Chem. 9:142.

long period of time, it results in long term damage to organs such as kidney, liver, eye, nerves, heart and blood vessels. Type-2 Diabetes Mellitus (T2DM) often exhibits an atherogenic dyslipidemia characterized by elevation of TG, reduction of HDL-C, as well as residual cardiovascular risk. Insulin affects many aspects of mammalian lipid metabolism. Early interventions aimed at normalizing circulating lipids reduce cardiovascular complications and mortality. Many of the drugs currently used for the treatment of DM produce adverse effects. Sulfonylureas stimulate pancreatic islet cells to secrete insulin, while metformin slows down hepatic glucose production. All these therapies have limited effectiveness, thereby necessitating the search for novel plant based compounds that can effectively reduce blood glucose [2].

The antidiabetic effect of plant derived compounds is due to their capacity to alter carbohydrate digestion/absorption, stimulate beta cell function, mimic insulin action and mop up Reactive Oxygen Species (ROS). *Anacardium occidentale* (cashew tree) is a tropical plant indigenous to Brazil, Portugal, India, Southeast Asia and Africa. Extracts of the plant have been reported to possess hypoglycemic effect [3]. The young and tender leaves of *A. occidentale* are consumed raw or sometimes blanched to reduce the stringent taste. In traditional medicine, the leaves are used for the treatment of dysentery, diarrhea, piles, toothache, sore gums, rheumatism and hypertension. This study investigated the effect of aqueous leaf extract of *A. occidentale* on blood glucose level and lipid profile of diabetic rats [4].

MATERIALS AND METHODS

Chemicals and reagents

All reagents used were of analytical grade. Lipid profile, ALT and ALP assay kits were products of Randox laboratories limited (UK). All other chemicals were obtained from British Drug House (BDH) (England), Merck (Germany) and Sigma-Aldrich (USA).

Plant sample collection

The plant leaves were obtained from dentistry quarters, university of Benin, Benin city and authenticated at the herbarium of the department of plant biology and biotechnology, university of Benin, Benin city, Nigeria [5].

Plant preparation and extraction

The leaves were washed and shade dried at room temperature for a period of two weeks and pulverized using mechanical blender. Aqueous extract of the leaves was obtained using cold maceration method [6].

Experimental rats

Adult male Wistar rats (n=30) weighing 150 g-200 g (mean weight=175 g ± 25 g) were obtained from the department of anatomy, university of Benin, Benin city. The rats were housed in metal cages under standard laboratory conditions: Average temperature of 25°C, 55% 65% humidity and 12 h light/12 h dark cycles. They were allowed access to rat feed (pelletized growers mash) and clean drinking water [7]. Prior to commencement of the study, the rats were acclimatized to the laboratory environment for one week. The study protocol was approved by the faculty of life sciences ethical committee on animal use.

Experimental design

The rats were randomly assigned to 6 groups (5 rats/group): Normal control, diabetic control and DMSO, metformin, extract and treatment groups. Diabetes Mellitus (DM) was induced *via* intraperitoneal injection of freshly prepared alloxan monohydrate solution at a dose of 120 mg/kg bwt. Rats in normal control, DMSO and extract groups were not induced [8]. Rats in treatment and extract groups received aqueous leaf extract of *A. Occidentale* at a dose of 100 mg/kg bwt orally for 4 days. Metformin served as standard drug and was administered orally at a dose of 50 mg/kg bwt. Dimethylsulfoxide (DMSO) was used to solubilize the extract.

Blood sample collection and plasma preparation

At the end of the treatment period peripheral venous blood was drawn from retro orbital plexus of each rat after mild anesthesia and centrifuged at 3000 rpm for 10 min to obtain plasma which was used for biochemical analysis [9,10].

Determination of blood glucose level

The rats were fasted overnight and their FBG level was measured with a glucometer (Accu-Chek).

Measurement of activities of ALT and ALP

Alanine Aminotransferase (ALT) and ALP activities were assayed using their respective commercial kits [11].

Determination of lipid profile

Lipid profile parameters were determined using randox kits. Only LDL-C and VLDL-C were determined *via* calculation using the frieldwald equation as shown below:

VLDL-C=TG/5

LDL-C=TC-(TG/5)-HDL-C

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using SPSS (21.0). Groups were compared using student t test [12]. Statistical significance was assumed at $p<0.05$.

RESULTS

Effect of aqueous extract of *A. occidentale* leaves on body and organ weights of diabetic rats: Treatment of diabetic rats with aqueous extract of *A. occidentale* leaves significantly reduced their body weights, but there were no significant differences in the relative weights of kidney, pancreas and liver among the groups (Tables 1 and 2) [13].

Table 1: Body and organ weights of diabetic rats.

Group	Change in body weight (g)	Organ kidney	Weight (g) pancreas	Liver
Normal control	18.50 ± 1.17	0.49 ± 0.01	0.46 ± 0.04	6.17 ± 0.41
Diabetic control	11.75 ± 0.36	0.56 ± 0.02	0.44 ± 0.05	5.13 ± 0.24
Dmso	18.00 ± 3.08	0.50 ± 0.02	0.48 ± 0.07	5.26 ± 0.25
Metformin	13.00 ± 1.44	0.56 ± 0.01	0.68 ± 0.04	5.47 ± 0.34
Extract	4.50 ± 2.02	0.47 ± 0.02	0.37 ± 0.01	5.10 ± 0.18
Treatment	11.00 ± 3.00	0.61 ± 0.03	0.76 ± 0.14	6.90 ± 0.51

Note: Data are weights of kidney, pancreas and liver, and are expressed as mean ± SEM. [a]p<0.05, compared with normal control group; [b]p<0.05, compared with diabetic control group

Table 2: Percentage change in body weight and relative organ weights of diabetic rats.

Group	% change in body weight	Relative kidney × 10^{-3}	Organ weight pancreas × 10^{3}	Liver × 10^{-2}
Normal control	12.76 ± 1.03	3.38 ± 0.04	3.17 ± 0.03	4.26 ± 1.49
Diabetic control	6.70 ± 0.36a	3.20 ± 0.04	2.51 ± 0.01	2.93 ± 0.15
Dmso	11.61 ± 0.88	3.23 ± 0.04	3.10 ± 0.01	3.39 ± 0.09
Metformin	7.08 ± 0.84	3.05 ± 0.02	3.71 ± 0.03	2.98 ± 0.04
Extract	2.95 ± 0.04b	3.08 ± 0.05	2.42 ± 0.02	3.34 ± 0.14
Treatment	4.99 ± 0.21b	2.77 ± 0.01	3.45 ± 0.25	3.13 ± 0.03

Note: Data are relative weights of kidney, pancreas and liver, and are expressed as mean ± SEM. [a]p < 0.05, compared with normal control group; [b]p < 0.05, compared with diabetic control group

Effect of aqueous extract of *A. occidentale* leaves on FBG of diabetic rats: As shown in Table 3, treatment of diabetic rats with aqueous extract of *A. Occidentale* leaves led to significant and time dependent reductions in their FBG levels (p<0.05) [14].

Table 3: Fasting blood glucose of diabetic rats.

Group	Fasting blood	Glucose (mg/dL)	
	Day 0	Day 1	Day 4
Normal control	81.00 ± 0.91	77.75 ± 2.84	84.00 ± 1.58
Diabetic control	147.25 ± 17.85	225.50 ± 8.22[a]	238.50 ±6.40[a]
DMSO	88.00 ± 1.22	91.50 ± 2.06	89.25 ± 2.29
Metformin	324.75 ± 44.39	157.75 ± 27.48[b]	89.75 ± 2.72[b]
Extract	76.75 ± 1.49	86.75 ± 1.44[b]	83.00 ± 0.91[b]

Treatment	211.00 ± 14.00	110.00 ± 2.00[b]	95.00 ± 1.00[b]

Note: Data are FBG levels, and are expressed as mean ± SEM. [a]p<0.05, compared with normal control group; [b]p<0.05, compared with diabetic control group

Effect of aqueous extract of *A. occidentale* leaves on lipid profile of diabetic rats: Treatment of diabetic rats with aqueous extract of *A. occidentale* leaves significantly reduced the circulating levels of TG, TC, VLDL-C and LDL-C, but it significantly increased HDL-C level (p<0.05). These results are shown in Table 4 [15,16].

Table 4: Lipid profile of diabetic rats.

Group	TG (mg/dL)	TC (mg/dL)	VLDL-C (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal control	46.84 ± 2.14	61.04 ± 3.15	9.37 ± 0.43	35.62 ± 3.62	16.05 ± 1.08
Diabetic control	96.84 ± 6.5	125.86± 13.6	19.37 ± 1.31	25.84 ± 3.62	80.65 ± 5.17
Dmso	50.90 ± 5.16	75.84 ± 19.06	10.18 ± 1.03	20.32 ± 1.93	45.34 ± 2.51
Metformin	86.93 ± 4.58	72.41 ± 14.90	17.39 ± 0.98	31.31 ± 2.21	23.71 ± 1.42
Extract	21.18 ± 2.61	57.18 ± 5.49	4.24 ± 0.52	47.39 ± 7.40	5.55 ± 0.71
Treatment	72.41 ± 3.94	87.83 ± 4.39	14.48 ± 0.94	61.37 ± 7.77	11.98 ± 1.02

Note: Data are lipid profile parameters, and are expressed as mean ± SEM. [a]p < 0.05, compared with normal control; [b]p < 0.05, compared with diabetic control

Effect of aqueous extract of *A. occidentale* leaves on activities of liver enzymes: Aqueous leaf extract of *A. occidentale* significantly reduced the activities of ALT and ALP in serum of diabetic rats (p<0.05) (Table 5).

Table 5: Effect of aqueous extract of *A. occidentale* leaves on activities of liver enzymes.

Group	Enzyme activity (IU/L)	
	Alt	Alp
Normal control	0.06 ± 0.87	9.00 ± 0.19
Diabetic control	70.07 ± 3.35	64.37 ± 7.04
Dmso	23.97 ± 0.79	12.50 ± 3.70
Metformin	23.63 ± 0.54	29.37 ± 2.78
Extract	22.64 ± 0.95	12.06 ± 0.46
Treatment	34.35 ± 1.41	13.44 ± 2.41

Note: Data are activities of ALT and ALP, and are expressed as mean ± SEM. [a]p < 0.05, compared with normal control; [b]p < 0.05, compared with diabetic control

Discussion

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [17,18].

Several pathogenic processes are involved in the development of DM. These range from autoimmune destruction of the pancreatic beta cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in DM is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action.

Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia [19]. As a tropical plant *A. occidentale* is indigenous to Brazil, Portugal, India, Southeast Asia and Africa. Extracts of *Anacardium occidentale* (cashew tree) are used in folk medicine for the treatment of DM, diarrhea, skin diseases, arthritis, fever, aches and pains. This study investigated the effect of aqueous leaf extract of *A. occidentale* on blood glucose level and lipid profile of diabetic rats [20].

Alloxan ((5,5-dihydroxyl pyrimidine-2,4,6-trione), a carcinogen and cytotoxic glucose analog, is one of the common diabetogenic agents often used to assess the antidiabetic potential of both pure compounds and plant extracts in studies involving DM. Among the known diabetogenic agents which include dithizone, monosodium glutamate, gold thioglucose, high fructose load, high glucose load and anti insulin serum; alloxan and Streptozotocin (STZ) are the most widely used in diabetes studies. Alloxan is administered as single or multiple doses, *via* different routes (intraperitoneal, intravenous and subcutaneous); with single intraperitoneal administration apparently the most employed mode [21]. In DM, the levels of free fatty acids are usually elevated. The circulating free fatty acids have deleterious effect on endothelial functions through various pathways and mechanisms which include free radical production, protein kinase c activation and increase in severity of dyslipidemia. Oxidative stress, caused by an increase in free radical production, is strongly linked to insulin resistance and progression of DM. With an increase in oxidative stress, coupled with increase in free fatty acids and blood glucose level, insulin secretion and activity are adversely affected. The liver function markers (ALT and ALP) were also assessed in this study to ascertain the effect of drug treatment in diabetic rats.

CONCLUSION

The results obtained in this study suggest that aqueous extract of *Anacardium occidentale* leaves mitigates diabetogenic action of alloxan in Wistar rats. It has been reported that alloxan has a non specific action on the destruction of vasculature of other organs, besides pancreas. The accumulation of exogenous insulin in the blood after the destruction of pancreatic cells may be due to impaired renal and hepatic functions. The results of this study showed that aqueous extract of *A. occidentale* leaves significantly mitigated alloxan induced metabolic derangement in rats, and are in agreement with those of previous reports.

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