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Research Article

Antidiabetic and Antioxidant Activities of Ethanolic Extract of *Anogeissus leiocarpus* in Alloxan-Induced Type 1 Diabetes Mellitus in Wistar Rats

Sabina Mbafan Num-Adom[1,2], Sani Adamu[1], Tagang Aluwong[3], Ngozi Ejum Ogbuagu[3], Ismaila Alhaji Umar[4] and King Akpofure Nelson Esievo[1]*

[1]*Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2]*Department of Veterinary Pathology, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria*

[3]*Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[4]*Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria*

***Corresponding Author:** King Akpofure Nelson Esievo, Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

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Abstract

Antidiabetic property of *Anogeissus leiocarpus* on hyperglycaemia-induced oxidative stress in spleen, kidney, heart, brain and liver. The study investigated antidiabetic and antioxidant effects of A. leiocarpus ethanolic extract in alloxan-induced diabetes mellitus and hyperglycaemia-induced oxidative stress in Wistar rats. Four groups of nine rats each; non-diabetic (ND), diabetic untreated (DU), diabetic insulin-treated (DI) and diabetic extract-treated (DE) were assayed for fasting blood glucose and oxidative stress biomarkers. Blood glucose significantly ($P < 0.001$) increased, as well as oxidative stress biomarkers, malondialdehyde (MDA) ($P < 0.05$; 0.002; 0.001) and significant ($P < 0.05$) variations in superoxide dismutase (SOD), catalase and glutathione (GSH) in DU. Ethanolic extract of A. leiocarpus significantly ($P < 0.001$) reversed the blood glucose and the oxidative stress biomarker to normal in DE as in DI. A. leiocarpus can serve as non-conventional treatment for diabetes-induced oxidative stress.

Keywords: *Antidiabetic; Antioxidant; Oxidative Stress; Anogeissus leiocarpus; Wistar Rats*

Introduction

Diabetes mellitus (DM) is a heterogeneous metabolic disease associated with disorders of carbohydrate, protein and lipid metabolism characterized by the presence of hyperglycaemia due to lack or impairment of insulin secretion, type 1 diabetes mellitus (TIDM), defective insulin action type 2 diabetes mellitus (T2DM) or both [1]. Hyperglycaemia, a common effect of uncontrolled diabetes led to serious complications, such as damage to the heart, blood vessels, eyes, kidneys and nerves, generally associated with T2DM [2]. DM was the most common noncommunicable disease afflicting over 425 million people worldwide [3,4] and might become the 7th leading cause of death in 2030 as projected by WHO, with worse projection for 2035 [4]. Therefore, DM became a challenging unresolved health problem for the 21st century [5]. An assessment of outpatient visits between 2007 and 2013 in the United States alone, showed 785 million people with diabetes [6,7] which further explains the worrisome nature of DM on the national budgets of countries worldwide. More worrisome is the risk factor induced by diabetes for the progression, prognosis and mortality of COVID-19, particularly, COVID-19 patients with pre-existing type 2 diabetes [8] and the global economic implications.

A consistent differentiating feature common to all cell types that are damaged by hyperglycaemia is an increased production of reactive oxygen species (ROS) and generation of ROS in diabetes was directly linked to chronic hyperglycaemia through the following

mechanisms: Increased polyol pathway flux in the peripheral nerve; increased formation of advanced glycation end products (AGEs), hyperglycaemia-induced activation of protein kinase C (PKC) isoforms and increased hexosamine pathway flux and consequent over modification of proteins by N-acetylglucosamine [9]. Increased production of ROS which resulted in oxidative stress in diabetic patients played an important role in development of microvascular and macrovascular disease [10]. Alloxan monohydrate-induced type 1 diabetes mellitus and initiated oxidative stress through inhibition of glucokinase, thereby inhibiting glucose-induced insulin secretion conferred its major pathophysiological effect [11]. The diagnostic criteria for diabetes were based on thresholds of glycaemia that were associated with microvascular disease, especially retinopathy [1]. The prevalence of complications, for example, diabetic retinopathy increased with the duration of diabetes. Additional risk factors included a lowered degree of glycemic control, the type of diabetes (for instance, type 1 or type 2); the presence of associated conditions such as hypertension, nephropathy, dyslipidaemia, and pregnancy were reported as additional risk factors [12].

Phytochemicals present in medicinal plants received remarkable attention because of their health benefits, such as antioxidant, anti-inflammatory, anti-genotoxic, antipyretic and anti-carcinogenic properties [13]. About 1,200 species of medicinal plants used in traditional medicine exhibited anti-diabetic properties [14- 17]. *Anogeissus leiocarpus*, due to its blood glucose lowering ability and enhanced erythropoiesis in Wistar rats [18], possessed antidiabetic properties and ameliorative effect on anaemia. Previous phytochemical studies of the plant revealed the presence of alkaloids, glycosides, phenol, tannins, saponins, flavanoids, ellagic acid and anthraquinones [18].

Although insulin therapy, pharmacotherapy, and diet therapy-controlled diabetes, increasing evidences indicated that enhanced pro-synthetic hypoglycaemic drugs have some disadvantages that included drug resistance, side effects, inability to control hyperlipidaemia and even toxicity and are often too expensive [19-21].

Very recently ethanolic extract of *A. leiocarpus* stem bark modulated both reduced erythrocyte surface and elevated free serum sialic acids which served as predictive, potent diagnostic biomarker and exhibited prognostic value in alloxan-induced type 1 diabetes mellitus in dogs [22].

The antidiabetic and antioxidant effects of *A. leiocarpus*, investigated on the hyperglycaemia and oxidative stress produced by alloxan-induced diabetes mellitus in Wistar rats are reported herein.

Materials and Methods

Ethical clearance

All procedures carried out on animals in this study were approved and performed in accordance with the ethical standards of the Ahmadu Bello University Committee on Animal Use and Care, Ahmadu Bello University Zaria (Ethical clearance No ABUCAC/2019/16).

Plant collection and preparation: The stem bark of *Anogeissus leiocarpus* was collected from Zaria environs, authenticated with a voucher sample number 167 and processed as detailed earlier [18,22].

Crude ethanolic extraction of stem bark of *A. leiocarpus*: Crude ethanolic extract of the plant was obtained as described earlier [18,22].

Experimental animals: Thirty-six Wistar rats of both sexes were used for this study. They were housed in standard cages and kept at ambient temperature and humidity in the animal room of the Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria. They were fed on grower's marsh mixed with maize offal and groundnut cake in a ratio of 4:2:1 and given water *ad libitum.* They were allowed to acclimatize for two weeks before commencement of the experiments. The animals were fasted from feeds for 12h before the commencement of each experiment, but were allowed water *ad libitum.*

Experimental rat groups and experimental design: The animals were weighed and assigned at random, into 4 groups of nine rats each. The crude ethanolic extract was reconstituted by dissolving 1g of extract in 2.5 ml distilled water to obtain a concentration of 400 mg/ml. Insulin detemir (LEVEMIR®), which is a long-acting recombinant (rDNA origin) by Novo Nordisk A/S, Denmark, was administered at a dose rate of 0.5 IU/kg subcutaneously once daily as recommended by the manufacturers. All administrations and sample collections were carried out at 9:00 am daily. The dose of extract 1000 mg/kg bd. wt. was selected based on an earlier study [18].

Group 1 (ND): Diabetes was not induced and the animals were administered normal saline at a dose of 2 ml/kg body weight orally, daily and served as normal control.

Group 2 (DU): Diabetes was induced but the rats in this group were left untreated and served as negative control.

Group 3 (DI): Diabetes was induced and insulin at a dose of 0.5 IU/kg body weight was administered subcutaneously daily and served as positive control.

Group 4 (DE): Diabetes was induced and crude ethanolic extract of *Anogeissus leiocarpus* at a dose of 1000 mg/kg body weight was administered daily orally using a tiny plastic tube attached to a syringe.

Induction of diabetes mellitus with alloxan monohydrate in rats: After an acclimatization period of fourteen days, the rats assigned to the diabetic groups (groups 2 - 4) were fasted overnight and injected intraperitoneally with a single freshly prepared alloxan monohydrate, dissolved in sterile cold normal saline, at a dose of 150 mg/kg body weight, as applied by Aluwong., *et al* [23]. The rats were allowed free access to 5% glucose solution post-alloxan administration to prevent the fatal alloxan-induced hypoglycaemia [23]. Blood glucose concentrations of the rats were estimated after 72 hours post-alloxan administration and the rats with a blood glucose levels equal or greater than 14.0 mmol/L were considered diabetic for this study.

Measurement of blood glucose levels: Three rats from each group were used for the experiment every week for three weeks. The fasting blood glucose levels were measured at 9:00 am daily by cutting the tail-tip of the rats to obtain a drop of blood directly onto an Accu Chek® test strip inserted into the portable glucometer (Accu Chek® Active, Roche, Roche Diabetes Care Middle East, FZCO).

Evaluation of effects of administrations on oxidative stress: Following determination of blood glucose, each week, the three rats from each group, were sacrificed and the following organs, spleen, kidney, heart, brain, and liver were harvested for tissue antioxidant enzymes assay. Slices of the organs were homogenized in ice cold 10% buffered phosphate saline (PBS) to make 10% homogenates. The homogenates were centrifuged at 10,000 g for 15 minutes at 4°C and aliquotes separated and refrigerated until when required for assay. The malondialdehyde (MDA) concentrations were assayed using commercial kits. Concentrations of MDA in the spleen, kidney, heart, brain and liver were determined to evaluate lipid peroxidation in all the groups.

The antioxidant enzymes, superoxide dismutase (SOD), catalase and glutathione concentrations were analyzed using standard ELISA methods on PND 91.

Statistical analysis: The results obtained from this study were presented in charts and tables of means ± standard error of means (SEM). The data were subjected to ANOVA using the GraphPad Prism® statistical package with Tukey's post-hoc tests to analyze the difference between groups. Values of $P < 0.05$ were considered significant.

Results

Effects of administration of insulin and crude ethanolic extract of *A. leiocarpus* on fasting blood glucose levels of alloxan-induced diabetic rats

The effects of the administrations of insulin and crude extract of *A. leiocarpus* on fasting blood glucose in alloxan-induced diabetes mellitus in rats are demonstrated in figure 1. The fasting blood glucose in DU significantly ($P < 0.001$) increased and remained high throughout the 3-week study, when compared to the ND, DI and DE groups. By week three the fasting blood glucose values in DI group showed a reversal to hyperglycemic state after insulin withdrawal whereas the DE group, did not show a reversal to hyperglycaemia upon withdrawal of administration of the extract.

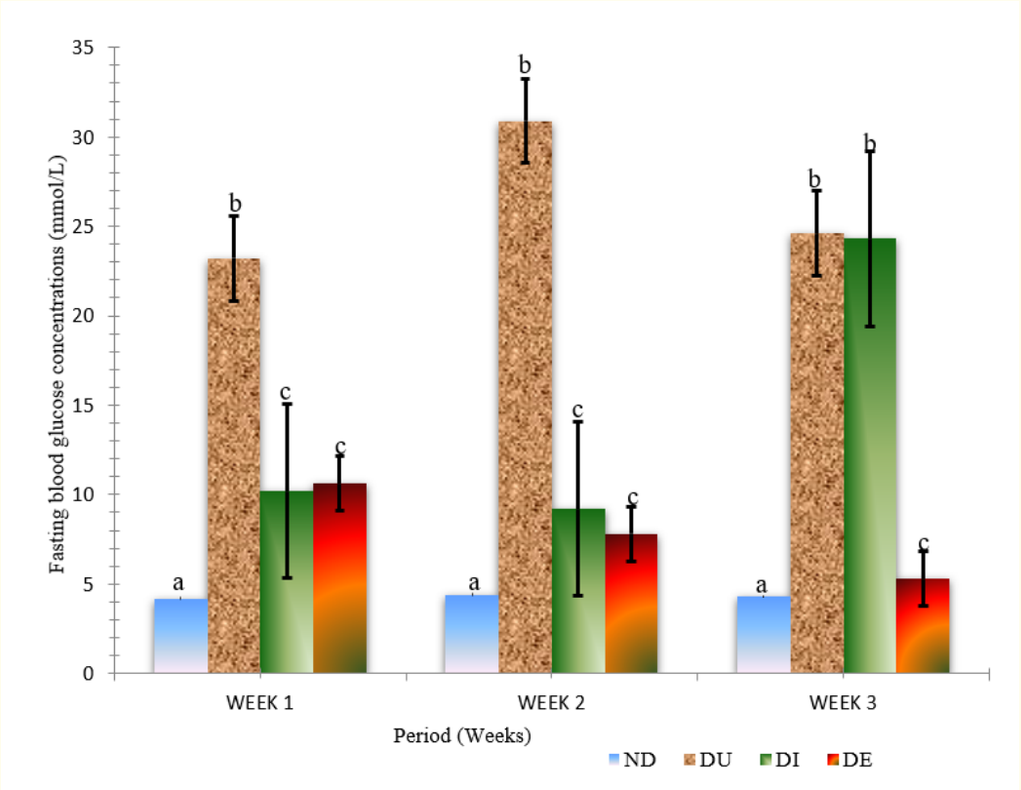

Figure 1: *Effects of administration of insulin and crude ethanolic extract of A. leiocarpus on fasting blood glucose levels of alloxan-induced diabetic rats.*
a = P < 0.05, b = P < 0.001, ND = Normal Non Diabetic Control, DU = Untreated Diabetic, DI = Insulin Treated Diabetic, DE = Extract Treated Diabetic.

Effects of administrations of insulin and crude ethanolic extract of *A. leiocarpus* on malondialdehyde concentrations in the spleen, kidney, heart, brain and liver of alloxan-induced diabetic rats

Spleen: The MDA concentrations were significantly ($P < 0.002$) higher after the first week and remained high throughout the period of study in DU group, when compared to ND. The MDA concentrations in DI and DE groups were significantly ($P < 0.001$) lower when compared to DU throughout the study period. By the third week, the concentrations in DE were not significantly ($P > 0.05$) lower when compared to DI (Table 1).

Kidney: The MDA concentrations were significantly ($P < 0.002$) higher after the first week and remained high throughout the period of study in DU group, when compared to ND. The MDA concentrations in DI and DE groups were significantly ($P < 0.002$) lower when compared to DU throughout the period of study. By the third week, the concentrations in DE were slightly higher, but not significant ($P > 0.05$) when compared to DI (Table 1).

Heart: The MDA concentrations were significantly ($P < 0.001$) higher after the first week and remained high throughout the period of study in DU group when compared to ND. The MDA concentrations in DI and DE groups were significantly ($P < 0.001$) lower when compared to DU throughout the study period. By the third week, the concentrations were lower in DI when compared to DE, but not significant ($P > 0.05$) (Table 1).

Brain: The MDA concentrations were significantly ($P < 0.002$) higher after the first week and remained high throughout the period of study in DU group (except at week two when the concentration was lowest) when compared to ND. The MDA concentrations in DI and

DE groups were significantly P < 0.002 and P < 0.001 respectively, lower when compared to DU throughout the study period. By the third week, the concentrations were significantly (P < 0.05) lower in the DE when compared to DI (Table 1).

Liver: The MDA concentrations were significantly (P < 0.05) higher after the first week and remained high throughout the period of study in DU group when compared to ND. The MDA concentrations in DI and DE groups were significantly P < 0.001 and P < 0.002, respectively, lower when compared to DU throughout the study period. By the third week, the concentrations were significantly (P < 0.05) lower in the DI when compared to DE (Table 1).

Duration	Organs	ND	DU	DI	DE
Week 1	Spleen	22.99 ± 0.34**	41.23 ± 4.31	35.08 ± 0.94**	21.96 ± 0.86**
	Kidney	27.86 ± 0.32**	75.58 ± 7.78	30.21 ± 7.67**	30.14 ± 2.08**
	Heart	14.46 ± 0.16***	27.33 ± 0.75	17.92 ± 0.21**	16.33 ± 1.02**
	Brain	33.63 ± 0.42**	67.65 ± 5.75	29.12 ± 2.12**	24.40 ± 7.88**
	Liver	48.86 ± 0.39*	69.42 ± 2.49	37.76 ± 6.03**	34.68 ± 1.78**
Week 2	Spleen	22.99.± 0.34***	40.19 ± 2.0	19.15 ± 1.67***	23.54 ± 1.41***
	Kidney	27.86 ± 0.32***	59.32 ± 4.04	25.02 ± 1.71***	23.58 ± 0.33***
	Heart	14.46 ± 0.16*	30.02 ± 3.66	15.41 ± 0.94*	12.96 ± 3.58**
	Brain	33.63 ± 0.42*	18.85 ± 6.29	25.07 ± 2.06*	27.10 ± 0.26*
	Liver	48.86 ± 0.39*	79.90 ± 7.66	48.22 ± 1.26**	28.29 ± 5.87***
Week 3	Spleen	22.99 ± 0.34**	47.34 ± 1.45	17.76 ± 4.47***	14.30 ± 2.95***
	Kidney	27.86 ± 0.32**	43.41 ± 2.22	24.16 ± 2.56**	25.74 ± 2.76**
	Heart	14.46 ± 0.16*	35.99 ± 2.32	15.94 ± 4.66**	18.87 ± 2.63*
	Brain	33.63 ± 0.42**	51.20 ± 5.29	32.99 ± 3.08**	22.26 ± 1.37***
	Liver	48.86 ± 0.39*	63.40 ± 5.20	16.60 ± 3.87***	24.24 ± 3.86**

Table 1: *Effects of administrations of insulin and crude ethanolic extract of A. leiocarpus on malondialdehyde concentrations ([MDA (µmol/mg protein)]) in organs of alloxan-induced diabetic rats.*
**= P < 0.05, **= P < 0.002, ***= P < 0.001, ND = Normal Non Diabetic Control, DU = Untreated Diabetic, DI = Insulin Treated Diabetic, DE = Extract Treated Diabetic, MDA = Malondialdehyde.*

Effects of administrations of insulin and crude ethanolic extract of *A. leiocarpus* on superoxide dismutase (SOD) in spleen, kidney, heart, brain and liver of alloxan-induced diabetic rats

Spleen: The activities of SOD in the spleen are shown in table 2. There was no significant (P > 0.05) difference in the activities of SOD between any of the groups, when compared to ND, from weeks 1 to 3 of the study.

Kidney: The activities of SOD of the kidney are shown in table 2. There was no significant (P > 0.05) difference in the activities of SOD between DU and ND. However, by week 3, there was an significant (P < 0.05) decrease in SOD activity in DI and DE, when compared to DU.

Heart: The activities of SOD of the heart are presented in table 2. There was no significant (P > 0.05) difference in the activities of SOD between ND and DI groups, when compared to DU. Conversely; there was a significant (P < 0.05) decrease in activity of SOD in the DE when compared to ND, DI and DU, by week 3.

Brain: The activities of SOD of the brain are presented in table 2. The SOD activities were significantly (P < 0.05) lower in the DU when compared to ND, DE and DI at weeks 1 and 3 of the study.

Liver: The activities of SOD of the liver are presented in table 2. There were significant (P < 0.002) increases in activity of SOD in the ND and DE when compared to DU and DI, by the 3rd week of the study.

Duration	Organs	ND	DU	DI	DE
Week 1	Spleen	6.92 ± 0.41	5.79 ± 0.44	6.33 ± 0.36	6.58 ± 1.30
	Kidney	7.22 ± 0.88**	6.68 ± 0.09	6.57 ± 0.11	5.88 ± 0.42*
	Heart	7.61 ± 0.16	6.14 ± 0.26**	6.60 ± 0.07	6.61 ± 0.16
	Brain	7.22 ± 0.06	5.64 ± 0.32	6.19 ± 0.11	6.88 ± 0.38
	Liver	7.65 ± 0.21*	5.87 ± 0.31	6.85 ± 0.24	6.65 ± 0.48
Week 2	Spleen	6.92 ± 0.41	5.85 ± 0.87	5.16 ± 0.80	6.02 ± 0.26
	Kidney	7.22 ± 0.88	6.79 ± 0.34	4.43 ± 0.92**	5.38 ± 0.13
	Heart	7.61 ± 0.16	6.51 ± 0.03	6.47 ± 0.31	6.03 ± 0.32*
	Brain	7.22 ± 0.06	5.80 ± 0.44	6.01 ± 0.44	6.85 ± 0.31
	Liver	7.65 ± 0.21*	6.51 ± 0.38	6.86 ± 0.15	7.07 ± 0.23
Week 3	Spleen	7.22 ± 0.88	7.26 ± 0.02	5.68 ± 0.14*	5.28 ± 0.14*
	Kidney	7.86 ± 0.32**	43.41 ± 2.22	24.16 ± 2.56**	25.74 ± 2.76**
	Heart	7.61 ± 0.16	6.89 ± 0.14	6.29 ± 0.61	5.74 ± 0.04*
	Brain	7.22 ± 0.06	5.55 ± 0.33	6.46 ± 0.22	7.46 ± 0.28*
	Liver	7.65 ± 0.21	5.05 ± 0.45	6.67 ± 0.43	7.90 ± 0.35**

Table 2: *Effects of administrations of insulin and crude ethanolic extract of A. leiocarpus superoxide dismutase activity ([SOD (IU/mg protein)]) in organs of alloxan-induced diabetic rats.*
**= P < 0.05, **= P < 0.002, ND = Normal Non Diabetic Control, DU = Untreated Diabetic, DI = Insulin Treated Diabetic, DE = Extract Treated Diabetic, SOD = Superoxide Dismutase.*

Effects of administrations of insulin and crude ethanolic extract of *A. leiocarpus* on catalase in spleen, kidney, heart, brain and liver of alloxan-induced diabetic rats

Spleen: The activities of catalase in the spleen are shown in table 3. The catalase activity in DU increased significantly ($P < 0.05$) when compared to ND, there were significant decreases in the catalase activities of DI ($P < 0.002$) and DE ($P < 0.05$) when compared to DU throughout the study (Table 3).

Kidney: Catalase activities were significantly lower in ND ($P < 0.05$), DI ($P < 0.001$) and DE ($P < 0.002$) when compared to DU all through the 3 weeks study (Table 3).

Heart: The catalase activity in DU increased when compared to ND and DI. There was a significant ($P < 0.05$) decrease in the catalase activity of DE when compared to DU, throughout the 3 week study (Table 3).

Brain: There was a significant ($P < 0.05$) decrease in catalase activity in the DE when compared to the DU, DI (not quite at week 1) and ND all through the 3 weeks study period (Table 3). Catalase activity was not significant ($P > 0.05$) in ND and DI when compared to DU, by week 3 (Table 3).

Liver: Catalase activity was significantly ($P < 0.05$) lower in the DI and DE groups, when compared to the DU and ND groups, by week 3 of the study (Table 3).

Duration	Organs	ND	DU	DI	DE
Week 1	Spleen	9.26 ± 0.18	15.93 ± 1.48	8.26 ± 0.18	7.44 ± 0.11**
	Kidney	9.36 ± 1.14	15.28 ± 2.45	6.03 ± 0.08*	9.62 ± 0.22
	Heart	9.05 ± 0.34	17.91 ± 2.65*	7.05 ± 0.34*	12.01 ± 2.24
	Brain	15.28 ± 0.97	15.24 ± 1.27	7.05 ± 1.35*	8.68 ± 0.82
	Liver	13.45 ± 0.72*	17.28 ± 1.51	11.45 ± 0.72*	12.83 ± 0.53
Week 2	Spleen	9.26 ± 0.18	14.88 ± 1.85	4.99 ± 0.14	7.24 ± 0.40**
	Kidney	9.36 ± 0.53	15.28 ± 2.45	6.03 ± 1.07*	8.62 ± 0.22
	Heart	9.05 ± 0.34***	20.10 ± 0.46	16.73 ± 1.77**	15.68 ± 0.48**
	Brain	15.28 ± 0.48	16.97 ± 1.47	14.26 ± 0.95	10.36 ± 1.56*
	Liver	13.45 ± 0.72	16.92 ± 0.99*	10.73 ± 3.46	7.03 ± 1.67*
Week 3	Spleen	9.26 ± 0.18	14.24 ± 2.05	4.84 ± 1.72	7.25 ± 0.88*
	Kidney	10.03 ± 0.15	17.16 ± 2.24	4.88 ± 0.95**	8.19 ± 0.59
	Heart	9.05 ± 0.34	14.48 ± 2.34	8.28 ± 1.45	6.63 ± 0.99*
	Brain	15.28 ± 0.48	16.36 ± 2.20	14.29 ± 0.27	10.50 ± 1.09*
	Liver	13.45 ± 0.72	13.42 ± 0.31	7.44 ± 2.89*	7.04 ± 0.96*

Table 3: *Effects of administrations of insulin and crude ethanolic extract of A. leiocarpus catalase activity ([CAT (IU/mg protein)]) in organs of alloxan-induced diabetic rats.*
**= P < 0.05, **= P < 0.002, ***= P < 0.001, ND = Normal Non Diabetic Control, DU = Untreated Diabetic, DI = Insulin Treated Diabetic, DE = Extract Treated Diabetic, CAT = Catalase.*

Effects of administrations of insulin and crude ethanolic extract of *A. leiocarpus* on glutathione concentration in spleen, kidney, heart, brain and liver of alloxan-induced diabetic rats

Spleen: GSH concentration significantly (P < 0.001) increased in DU when compared to ND, DI and DE, all through the 3 weeks study period (Table 4).

Kidney: GSH concentrations significantly (P < 0.001) decreased in ND, DI and DE, when compared to DU, all through the 3-week study period (Table 4).

Heart: GSH concentrations significantly (P < 0.05) decreased in ND, DI and DE when compared to DU.

Brain: GSH concentrations were significantly (P < 0.002) lower in DU and DI when compared with ND and DE, by week 3 of the study period (Table 4).

Liver: GSH in the liver showed no difference in concentration among all the groups, except by third week, when significant (P < 0.05) decreases occurred in DI and DE when compared with ND and DU (Table 4).

Duration	Organs	ND	DU	DI	DE
Week 1	Spleen	16.18 ± 0.80*	24.77 ± 2.42	14.52 ± 0.35**	11.60 ± 1.28***
	Kidney	15.93 ± 0.67***	37.43 ± 2.22	15.30 ± 0.53***	13.06 ± 1.16***
	Heart	26.60 ± 0.86**	47.02 ± 1.96	14.41 ± 0.96***	13.88 ± 0.69***
	Brain	23.25 ± 0.59	16.31 ± 2.58	18.24 ± 2.02	19.84 ± 2.54
	Liver	17.81 ± 0.24	14.20 ± 0.82	16.32 ± 1.50	16.29 ± 2.10
Week 2	Spleen	16.18 ± 0.80	38.88 ± 1.52***	15.34 ± 3.17***	12.79 ± 1.56***
	Kidney	15.93 ± 0.67***	34.68 ± 3.13	13.75 ± 1.91***	12.41 ± 1.22***
	Heart	9.05 ± 0.34***	20.10 ± 0.46	16.73 ± 1.77**	15.68 ± 0.48**
	Brain	23.25 ± 0.59**	16.26 ± 0.62	20.24 ± 1.03*	23.17 ± 1.35**
	Liver	17.81 ± 0.24	17.24 ± 4.15	17.01 ± 1.03	19.93 ± 1.22
Week 3	Spleen	16.18 ± 0.80	41.63 ± 2.41***	14.80 ± 0.34***	14.85 ± 0.81***
	Kidney	15.93 ± 0.67***	40.68 ± 9.39	14.96 ± 0.59***	13.98 ± 1.17***
	Heart	26.60 ± 0.86*	31.76 ± 3.29	14.04 ± 0.38*	16.29 ± 4.13*
	Brain	23.25 ± 0.59**	17.50 ± 1.08	19.34 ± 0.22	23.04 ± 1.39**
	Liver	13.45 ± 0.72	13.42 ± 0.31	7.44 ± 2.89*	7.04 ± 0.96*

Table 4: *Effects of administrations of insulin and crude ethanolic extract of A. leiocarpus glutathione activity ([GSH (umol/mg protein)]) in organs of alloxan-induced diabetic rats.*
**= P < 0.05, **= P < 0.002, ***= P < 0.001, ND = Normal Non Diabetic Control, DU = Untreated Diabetic, DI = Insulin Treated Diabetic, DE = Extract Treated Diabetic, GSH = Glutathione.*

Discussion

The administration of alloxan monohydrate at a dose of 150 mg/kg successfully induced type 1 diabetes mellitus in Wistar rats in this study as previously reported in experimental animals [22,23]. Alloxan selectively inhibited glucose-induced insulin secretion through its ability to specifically inhibit glucokinase, the glucose sensor of beta cells and produced insulin dependent diabetes mellitus (IDDM) by inducing selective necrosis of pancreatic beta cells [11]. In dog's IDDM, sialic acid expression is 18% lower than normal [22] Pancreatic beta cell toxicity and resultant diabetogenicity of alloxan was due to redox recycling and the generation of toxic reactive oxygen species (ROS) in combination with hydrophilicity and the glucose similarity of the molecular shape of alloxan [11]. It was shown that effective prevention of redox cycling and generation of ROS prevented pancreatic beta cell death and counteracted the development of alloxan diabetes *in vivo* [24].

The present study showed that crude ethanolic extract of stem bark of *A. leiocarpus* reduced blood glucose levels in the diabetic Wistar rats as earlier reported in non-diabetic Wistar rats [18]. In addition, ethanolic extract of stem bark of *A. leiocarpus* significantly reduced blood glucose in alloxan-induced type 1 diabetes mellitus of dogs [22] and the ability to reduce blood glucose was attributed to the phytochemical components found in this plant, namely, alkaloids, glycosides, flavonoids, saponins, dietary fibres, polysaccharides, glycolipids, peptidoglycans, amino acids and others, also obtained from various plant sources, reported to have potent hypoglycaemic agents [18,25]. It is being suggested that use of *A. leiocarpus* phytoconstituents may delay the development of diabetic complications and may regulate the metabolic abnormalities as earlier observed [26]. Numerous mechanisms of actions were proposed for the ameliorative effects of the extracts of these medicinal plants on diabetes mellitus such as their effects on the pancreatic ß-cells (synthesis, release, cell regeneration/revitalization) or the increase in the protective/inhibitory effect against insulinase and the increase of the insulin sensitivity or the insulin-like activity of the plant extracts, improved glucose homeostasis, increased peripheral utilization of glucose, increased synthesis of hepatic glycogen and/or decreased glycogenolysis, inhibited intestinal glucose absorption, reduced glycaemic index of carbohydrates and reduced effect of glutathione [27-29].

In the present study, increased lipid peroxidation occurred in the spleen, kidney, heart, brain and liver of DU rats, evidenced by the significant increases in malondialdehyde (MDA) concentrations in these organs, as earlier reported by others [23,30-33] and found indicative of oxidative stress on the heart of alloxan-induced diabetic rats, which gives credence to the report that oxidative stress played a pivotal role in the development of diabetes-induced cardiovascular disease [34] and both microvascular and macrovascular complications of diabetes mellitus, reported much earlier [10], associated with chronic or intermittent hyperglycaemia [35-37]. Hyperglycaemia, caused more oxidative damage in the blood and tissue of diabetic patients [38] and led to diabetic nephropathy [30,39-41]. The brain is especially vulnerable to oxidative damage, ascribed to the brain's high oxygen consumption rate, abundant lipid content and relative paucity of antioxidant enzymes as compared to other tissues [42]. The increased MDA in the kidneys of DU supports increased lipid peroxidation and oxidative damage which altered the transbilayer fluidity gradient of plasma membranes and damaged the membrane components, the membrane-bound enzymes and receptor activity and led to inflammation and cell necrosis [43,44]. MDA concentrations in organs indicated the extent of lipid peroxidation in such organs thus MDA served as a biomarker of lipid peroxidation [45]. The ethanolic extract of *A. leiocarpus* ($P < 0.001$) like insulin ($P < 0.002$) significantly reduced MDA in these organs, suggesting that *A. leiocarpus* antioxidant properties effectively prevented lipid peroxidation in alloxan-induced type 1 diabetes mellitus in Wistar rats.

In the present study, the alloxan-induced type 1 diabetes mellitus in DU showed oxidative damage in these organs, spleen, kidney and heart, evidenced by their elevated catalase activities and GSH concentrations. The significantly high activities of catalase and glutathione concentrations reported in the spleen of untreated diabetic rats [46,47] gives support to the present study. In addition, significant increases in SOD, catalase activities and glutathione concentrations in the kidneys of untreated diabetic rats in the present study, were similarly observed by others [30,48,49]. In contrast, in the heart, brain and liver, the SOD levels were significantly reduced in the diabetic untreated rats, when compared to the normal control [23] while Guido., *et al*. [50] reported unchanged SOD levels in the heart after 8 weeks of diabetes induction which appear similar with the findings in the present study at the end of 3 weeks. The catalase activities and glutathione concentrations on the other hand were significantly increased in the heart and liver of DU rats in the present study which agrees with earlier reports [50-53]. The observed reduction in some of the antioxidant enzyme activities including SOD in DU in the present study, if maintained may render tissues susceptible to oxidative stress leading to diabetic complications, as earlier reported by Lipinski [54], except for kidney at week 3. These variations from unchanged and between decreased and increased values of these oxidative stress biomarkers in the present study can partly be explained from reports that animal cells responded to oxidative stress by

either repairing the damage or by decreasing the state of pro-oxidation through enzymatic, such as SOD and catalase and non-enzymatic, such as GSH, antioxidant defence system (ADS) which scavenged free radicals and reactive oxygen species (ROS) [55-58]. In the present study, the significant increases of SOD and catalase activities and GSH concentrations in DU organs under investigation at any time up to the 3rd week of the study is suggestive that the first line of ADS against free radicals and ROS has not been utilized to handle the lipid peroxidation while significant decreases of SOD, catalase and GSH in any organ in DU during the 3-week study is suggestive that the first line of ADS against free radicals and ROS has been depleted. Free radicals formation in diabetes from increased lipid peroxidation led to increased insulin resistance due to oxidative stress [59]. The possible sources of oxidative stress in brain injury included lipid peroxidation and decreased tissue concentrations of low molecular weight antioxidants such as reduced glutathione (GSH) [42,60] as observed in DU in the present study. Both oxidative stress and inflammatory responses acted as damaging agents in aggravating the pathological condition of DM [61,62]. Earlier reports showed that a decrease in SOD and CAT activities within a hyperglycaemic state led to an increase in ROS, which eventually contributed to oxidation-induced liver damage [51-53] as observed for SOD in DU rats, in the present study, except for the kidney at week 3. The liver played a pivotal role in the homeostasis of the GSH enzyme family and reduced concentrations of GSH in the livers of in diabetic rat as observed in this study particularly at week 1, was earlier associated with depletion of decreased glutathione *S*-transferases (GSTs) glutathione peroxidase (GPX) and glutathione reductase activities and with the accumulation of oxidative stress products, such as advanced glycation end-products (AGEs), protein oxidation products (POPs) and lipid peroxidation [39,63]. Therefore, prevention of oxidative imbalance in the liver cells might stop progression into hepatic complications associated with diabetes mellitus [52].

Antioxidant therapy was an effective intervention for minimizing or even preventing the complications associated with diabetes mellitus [64]. *Anogeissus leiocarpus* contained phytochemicals, which in addition to having potent hypoglycaemic properties [18], potent plasma sialic acid reducing properties [22] also possessed potent antioxidants properties [17], from its polysaccharides, terpenoids, flavonoids, sterols, alkaloids. saponins, tannins, anthraquinones and glycosides contents [18,22,65].

In the present study, the ameliorative and modulatory effects of ethanolic extracts of *A. leiocarpus* on MDA concentration, SOD, Catalase activities and GSH concentration showed good attenuation of the oxidative damage on the spleen, kidney, heart, brain and liver as it modulated the activities of the antioxidant enzymes and lipid peroxidation in these organs in alloxan-induced type 1 diabetes mellitus in Wistar rats comparable to insulin-treated diabetic rats and gives credence to the observation of others [17].

Conclusion

Anogeissus leiocarpus ethanolic extract modulated the biomarkers of oxidative stress caused by alloxan-induced type 1 diabetes in various organs of Wistar rats. This may be useful in preventing progression into complications associated with diabetes mellitus. *A. leiocarpus* can serve as a non-conventional treatment for diabetes-induced oxidative stress where insulin is unaffordable.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Bibliography

1. Punthakee Z., *et al*. "Definition, classification and diagnosis of diabetes, pre-diabetes and metabolic syndrome diabetes". *Canadian Journal of Diabetes* 42 (2018): 10-15.

2. Fowler MJ. "Microvascular and macrovascular complications of diabetes". *Clinical Diabetes* 26.2 (2008): 77-82.

3. World Health Organization. "Global report on diabetes. 1. Diabetes Mellitus - epidemiology. 2. Diabetes Mellitus - prevention and control. 3. Diabetes, Gestational. 4. Chronic Disease. 5. Public Health. I". World Health Organization. France (2016).

4. Guariguata L., *et al*. "Global estimates of diabetes prevalence for 2013 and projections for 2035". *Diabetes Research and Clinical Practice* 103 (2014): 137-149.

5. Amjad S., *et al*. "A novel strategy of nanotized herbal drugs and their delivery in the treatment of diabetes: Present status and future prospects". *Journal of Herbal Medicine* 17 (2019): 100279.

6. Armstrong DG., *et al*. "Diabetic foot ulcers and their recurrence". *New England Journal of Medicine* 376.24 (2017): 2367-2375.

7. Skrepnek GH., *et al*. "Health care service and outcomes among an estimated 6.7 million ambulatory care diabetic foot cases in the US". *Diabetes Care* 40.7 (2017): 936-942.

8. Zhu, L., *et al*. "Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes". *Cell Metabolism* 31 (2020): 1068-1077.

9. Kawahito S., *et al*. "Problems associated with glucose toxicity: Role of hyperglycaemia-induced oxidative stress". *World Journal of Gastroenterology* 15.33 (2009): 4137-4142.

10. Giacco F and Brownlee M. "Oxidative stress and diabetic complications". *Circulatory Research* 107 (2010): 1058-1070.

11. Lenzen S. "Alloxan and streptozotocin diabetes". *Advance Research Institutes of Diabetes Animals* 6.4 (2010): 113-122.

12. Zhang X., *et al*. "A1C level and future risk of diabetes: A systematic review". *Diabetes Care* 33 (2010): 1665-1673.

13. Kang P., *et al*. "Antiinflammatory effects of anethole in lipopolysaccharide-induced acute lung injury in mice". *Life Science* 93 (2013): 955-961.

14. Babu PA., *et al*. "A database of 389 medicinal plants for diabetes". *Bioinformation* 1.4 (2006): 130-131.

15. N'guessan K., *et al*. "Ethnobotanical study of plants used to treat diabetes, in traditional medicine, by Abbey and Krobou People of Agboville (Côte-d'Ivoire)". *American Journal of Scientific Research* 4 (2009): 45-58.

16. Rugerio-Escalona C., *et al*. "Diabetes and Metabolism Disorders Medicinal Plants: A Glance at the Past and a Look to the Future 2018: Anti-hyperglycemic Activity of Hamelia patens Jacq. Extracts". *Evidence-based Complementary and Alternative Medicine* (2018).

17. Zaid H., *et al*. "Diabetes and metabolism disorders medicinal plants: A glance at the past and a look to the future 2018". *Evidence-based Complementary and Alternative Medicine* (2018): 5843298.

18. Num SM., *et al*. "Some observations in Wistar rats administered ethanol extracts of the stem barks of Anogeissus leiocarpus". *Asian Journal of Pharmacology and Toxicology* 2.3 (2014): 4-10.

19. Dey L., *et al*. "Alternative therapies for type 2 diabetes". *Alternative Medicine Review* 7 (2002): 45-58.

20. Kooti W., *et al*. "The role of medicinal plants in the treatment of diabetes: A systematic review". *Electronic Physician* 8.1 (2016): 1832-1842.

21. Raj R and Tripathi SS. "Medications of diabetes mellitus and antidiabetic medicinal plants: A review". *International Journal of Indian Herbs and Drugs* 1 (2016): 19-28.

22. Esievo KAN., *et al*. "Elevated serum sialic acids, a potent biomarker of alloxan-induced type 1 diabetes in dogs by ethanolic extract of Anogeissus leiocarpus". *Journal of Diabetes and Metabolic Disorders* (2021): 1-8.

23. Aluwong T., *et al*. "Amelioration of hyperglycaemia, oxidative stress and dyslipidaemia in alloxan-induced diabetic Wistar rats treated with probiotic and vitamin C". *Nutrients* 8.151 (2016): 1-15.

24. Elsner M., *et al*. "Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin". *Diabetolgia* 12 (2007): 1528-1533.

25. Gaikwad SB., *et al*. "Phytochemicals for diabetes management". *Pharmaceutical Crops* 5.1 (2014): 11-28.

26. Mukherjee PK., *et al*. "Leads from Indian medicinal plants with hypoglycemic potentials". *Journal of Ethnopharmacology* 106 (2006): 1-28.

27. Bnouham M., *et al*. "Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000)". *International Journal of Diabetes and Metabolism* 14 (2006): 1-25.

28. Govindappa M. "A review on role of plant(s) extracts and it's phytochemicals for the management of diabetes". *Journal of Diabetes and Metabolism* 6 (2015): 565.

29. Bacanli M., *et al*. "Effects of phytochemicals against diabetes". *Advances in Food and Nutrition Research* (2019): 1-28.

30. Kanadhasan R and Venkataraman S. "In vitro capacity and in vivo antioxidant potency of sedimental extract of Tinospora cordifolia in streptozotocin induced type 2 diabetes". *Avicenna Journal of Phytomedicine* 3.1 (2013): 7-24.

31. Kumawat M., *et al*. "Antioxidant enzymes and lipid peroxidation in Type 2 diabetes mellitus patients with and without nephropathy". *North American Journal of Medical Sciences* 5.3 (2013): 213-219.

32. Tiwari B., *et al*. "Markers of Oxidative stress during diabetes mellitus". *Journal of Biomarkers* (2013): 378790.

33. De Souza Bastos A., *et al*. "Diabetes and increased lipid peroxidation are associated with systemic inflammation even in well-controlled patients". *Journal of Diabetes and its Complications* 30.8 (2016): 1593-1599.

34. Kayama Y., *et al*. "Diabetic Cardiovascular Disease Induced by Oxidative Stress". *International Journal of Molecular Science* 16.10 (2015): 25234-25263.

35. Kesavulu MM., *et al*. "Lipid peroxides and antioxidant enzyme levels in type 2 diabetics with microvascular complications". *Diabetes and Metabolism* 26.3 (2000): 387- 392.

36. Asmat U., *et al*. "Diabetes mellitus and oxidative stress: A concise Review". *Saudi Pharmaceutical Journal* 24 (2016): 547-553.

37. Volpe CMO., *et al*. "Cellular death, reactive oxygen species (ROS) and diabetic complications". *Cell Death and Disease* 9.119 (2018): 1-9.

38. Sireesha K and Rao SP. "Oxidative stress and diabetes: An overview". *Asian Journal of Pharmacology Clinical Research* 8.1 (2015): 15-19.

39. Ahmed D., *et al*. "Antidiabetic, antioxidant, antihyperlipidemic effect of extract of Euryale ferox salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin induced diabetic rats". *Springer Plus* 4 (2015): 315.

40. Chikezie PC., *et al*. "Oxidative stress in diabetes mellitus". *Integrative Obesity and Diabetes* 1.3 (2015): 71-79.

41. Ebokaiwe AP., *et al*. "Alteration in sperm characteristics, endocrine balance and redox status in rats rendered diabetic by streptozotocin treatment: attenuating role of Loranthus micranthus". *Redox Report* 23.1 (2018): 195-205.

42. Muriach M., *et al*. "Diabetes and the brain: oxidative stress, inflammation, and autophagy". *Oxidative Medicine and Cellular Longevity* (2014).

43. Sebeková K., *et al*. "Markedly elevated levels of plasma advanced glycation end products in patients with liver cirrhosis: Amelioration by liver transplantation". *Journal of Hepatolology* 36 (2002): 66-71.

44. Mahmoud AM., *et al*. "Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats". *Journal of Diabetes Complications* 26 (2012): 483-490.

45. Aluwong T., *et al*. "Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens". *Antioxidants* 2 (2013): 326-339.

46. Yilmaz HR., *et al*. "Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver". *Journal of Biochemistry and Molecular Toxicology* 18 (2004): 234-238.

47. Okutan H., *et al*. "Effects of caffeic acid phenethyl ester on lipid peroxidation and antioxidant enzymes in diabetic rat heart". *Clinical Biochemistry* 38 (2005): 191-196.

48. Genet S., *et al*. "Alterations in antioxidant enzymes and oxidative damage in experimental diabetic rat tissues: Effect of vanadate and fenugreek (Trigonella foenum graecum)". *Molecular Cell Biochemistry* 236 (2002): 7-12.

49. Koya D., *et al*. "Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats". *Journal of American Society of Nephrology* 14.3 (2003): S250-S253.

50. Guido MC., *et al*. "The effects of diabetes induction on the rat heart: differences in oxidative stress, inflammatory cells, and fibrosis between subendocardial and interstitial myocardial areas". *Hindawi Oxidative Medicine and Cellular Longevity* (2017): 5343972.

51. Parveen K., *et al*. "Protective effects of Pycnogenol on hyperglycemia-induced oxidative damage in the liver of type 2 diabetic rats". *Chemical and Biological Interactions* 186 (2010): 219-227.

52. Lucchesi AN., *et al*. "Diabetes mellitus triggers oxidative stress in the liver of alloxan-treated rats: a mechanism for diabetic chronic liver disease". *Acta Cirurgica Brasileira* 28.7 (2013): 502-508.

53. Mohammed J., *et al*. "Mechanisms of diabetes-induced liver damage: The role of oxidative stress and inflammation". *Sultan Qaboos University Medical Journal* 16.2 (2016): 32-41.

54. Lipinski B. "Pathophysiology of oxidative stress in diabetes mellitus". *Journal of Diabetes and its Complications* 15.4 (2001): 203-210.

55. Kirkman HN and Gaetani GF. "Mammalian catalase: a venerable enzyme with new mysteries". *Trends in Biochemical Sciences* 32.1 (2007): 44-50.

56. Boots AW., *et al*. "Health effects of quercetin: from antioxidant to nutraceutical". *European Journal of Pharmacology 585*.2-3 (2008): 325-337.

57. Otitoju O., *et al*. "Oxidative stress and superoxide dismutase activity in brain of rats fed with diet containing permethrin". *Biokemistri* 20.2 (2008): 93-98.

58. Abdel-Daim MM., *et al*. "Alleviation of drugs and chemicals toxicity: biomedical value of antioxidants". *Oxidative Medicine and Cellular Longevity* (2018).

59. Maritim AC., *et al*. "Diabetes, oxidative stress, and antioxidants: a review". *Journal of Biochemistry and Molecular Toxicology* 17.1 (2003): 24-38.

60. Singhal SS., *et al*. "Antioxidant role of glutathione S-transferases: 4-Hydroxynonenal, a key molecule in stress-mediated signaling". *Toxicology and Applied Pharmacology* 289.3 (2015): 361-370.

61. Guven A., *et al*. "Effects of melatonin on streptozotocin-induced diabetic liver injury in rats". *Acta Histochemical* 108 (2006): 85-93.

62. Romagnoli M., *et al*. "Xanthine oxidase-induced oxidative stress causes activation of NF-kappaB and inflammation in the liver of type I diabetic rats". *Free Radical Biology and Medicine* 49 (2010): 171-177.

63. Yagmur E., *et al*. "Elevation of Nepsilon-(carboxymethyl)lysine-modified advanced glycation end products in chronic liver disease is an indicator of liver cirrhosis". *Clinical Biochemistry* 39 (2006): 39-45.

64. Aydın S., *et al*. "Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control". *Clinical Biochemistry* 34 (2001): 65-70.

65. Chigayo K., *et al*. "Phytochemical and antioxidant properties of different solvent extracts of Kirkia wilmsii tubers". *Asian Pacific Journal of Tropical Biomedicine* 6.12 (2016): 1037-1043.

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