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Research Article

Effect of *Azadirachta indica* (Neem) Aqueous Leaves Extract on Markers of Nephropathy in Streptozotocin-Induced Diabetic Wistar Rats

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ABSTRACT

Diabetes is a life-threatening health issue that is becoming more prevalent and poses a great health problem and a colossal responsibility for the global economy. Effective phytomedicine can serve as a good source for the management of diabetes. This study was carried out to evaluate the effect of *Azadirachta indica* aqueous leaf extract on markers of nephropathy in streptozotocin-induced diabetic Wistar rats. 60 mg/kg of STZ was administered intraperitoneally to Wistar rats which induced diabetes. Thirty male rats were randomly distributed into groups of 5 rats each. Normal and diabetic groups received distilled water; diabetic rats were orally treated with 100, 300 and 400 mg/kg *A. indica* leaves extract and 5 mg/kg glibenclamide for 4 weeks. Fasting blood glucose was monitored weekly. Administration of Streptozotocin (STZ) significantly ($p<0.05$) increased the concentrations of blood glucose, AGE, IL-6, VEGF and KIM-1. However, treatment with the extract reversed the effect of STZ on the above parameters. It is observed from the study that the extract has the potency to manage nephropathy. However, further research is encouraged to identify the most active compounds.

Keywords: *Azadirachta indica*; Diabetes; Glibenclamide; Phytomedicine; Streptozotocin

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INTRODUCTION

Diabetes mellitus is an endocrine ailment resulted from elevated blood glucose level that happens because of defect in insulin release, insulin sensitivity or all the two (Stafi *et al*., 2024). Early medical personnel were aware that the patient's urine contained sugar, thus the words "diabetes," implies passing through, and "mellitus," implies sweety (Vasudevan *et al*., 2011). If diabetes is not properly managed, it may result in serious health problems like cardiovascular problems (CVD), renal damage (nephropathy), nerve damage (neuropathy), visual loss or blindness (retinopathy), etc. that can be life-threatening or cause disability. However, if diabetes is well-managed, these serious complications can be delayed or avoided (IDF, 2021). It is estimated that 537 million people worldwide suffer from diabetes; this figure is expected to rise to 643 million by 2030 and 783 million by 2045, according to the International Diabetes Federation (IDF, 2021). It was anticipated that in 2021, diabetes-related causes of death claimed the lives of nearly 6.9 million people aged 20 to 79 (IDF, 2021). Diabetes already results in direct medical costs that are around $1 trillion USD, and this number will surpass the 2030 estimate (IDF, 2021). However, it has been forecasted that by 2045, the highest prevalence of diabetes (94%), will be found in low- and middle-income nations due to anticipated faster population growth (IDF, 2021). Diabetic nephropathy is a chronic problem of both type 1 and 2 diabetes (Vrhovac *et al*., 2008) which is the most frequent cause of chronic kidney failure in both developed and developing countries (Reutens *et al*., 2008). The underlying pathological changes comprise stiffening of basement membrane, atrophy, interstitial fibrosis and arteriosclerosis that initially results in glomerular

hyper filtration and subsequent progressive loss of renal function (Karunakaran, 2010). The conventional medicines for the treatment of diabetic nephropathy have side effects, noncompliant and expensive which increased the chance of developing renal failure. Due to that, the modern world is now turning to bioactive chemical components derived from plants which are cheap and readily available for the treatment and prevention of diseases (Innocent *et al*., 2021; Santosh *et al*., 2020; Stafi *et al*., 2024). These pants contain chemical compounds that provide the basis for the identification and creation of novel antihyperglycemic products (Umar *et al*., 2010). Through many methods, the utilization of these plants and phytoconstituents may enhance insulin secretion, postpone the onset of diabetic problems, and control metabolic anomalies (Switi *et al*., 2014). Consequently, a larger-scale, more pertinent and more carefully planned study is required to examine impact of plants on diabetic nephropathy. *Azardirachta indica* (figure 1), (divine tree) has been declared the tree of the 21st century by the United Nations, and the most versatile and useful medicinal plant ever found (Jose *et al*., 2020).

Figure 1: Leaves of *Azadirachta indica*

The research therefore investigated the effect of *Azadirachta indica* (neem) aqueous leaves extract on markers of nephropathy in streptozotocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Sample Collection and Identification

The plant sample (leaves) was collected from take-off Campus, Federal University Dutsin-Ma, Katsina State, Nigeria and was iidentified at the Herbarium of Botany Unit of the Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State. Voucher number: FUDMA/PSB/00002 was issued.

Sample Preparation

The leaves were sorted and air dried under shade at room temperature for two weeks, it was grounded to powder. Distilled water 1000 ml was added to each 150 g of the powdered sample and was soaked for 24 hours. It was filtered with muslin cloth followed by whatman filter paper number 1and the extract was collected and dried in oven at 60^{o}C which was used for the analyses.

Experimental Animals

A total of thirty (30) male Wistar rats with average weight 152.73 g were used and were purchased from the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. They were maintained and housed in aluminum cages in the Animal house of the Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma. They were allowed to acclimatize for two weeks. The animals were fed with standard diet and water *ad libitum* throughout the experiment.

GC-MS Analysis of the plant

The extract of *Azadirachta india* leaves was analyzed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 500, USA) equipped with a fused silica capillary column (30 x 0.25 mm i.d. x 0.25 µm film thickness) coupled with a Perkin Elmer Clarus 600C MS.

Induction of Diabetes

Diabetes was induced by a single intra-peritoneal injection of 60 mg/kg streptozotocin (Sigma St Louis, M.O., USA) dissolved in 0.9% normal saline to overnight fasted rats using insulin syringe. Diabetes mellitus was confirmed after the 7th day of streptozotocin treatment by the observation of fasting blood glucose (FBG)>300 mg/dl using glucometer (Accu-check) (Osibemhe *et al.,* 2018).

Experimental Design

After acclimatization period, the rats were divided randomly into six groups of 5 rats each:

Group 1: Normal rats

Group 2: STZ-induced diabetic treated with standard drug (glibenclamide)

Group 3: STZ-induced diabetic untreated

Group 4: STZ-induced diabetic treated with 100 mg/kg *A. indica* aqueous leaves extract

Group 5: STZ-induced diabetic treated with 300 mg/kg *A. indica* aqueous leaves extract

Group 6: STZ-induced diabetic treated with400 mg/kg *A. indica* aqueous leaves extract

The rats were maintained on their normal diets and water *ad libitum* for the period of 8 weeks.

Collection of Blood Sample

After the completion of the 4 week treatment, the rats were fasted overnight for eight hours, anaesthetized in using chloroform. Blood samples were collected from the animals through abdominal aorta into a sterile syringe and were put in heparinized containers.

Biochemical Analysis

Diagnostic kits that were utilized for the analyses of biochemical parameters include randox kits for

blood glucose test and Elisa kits for makers of nephropathy.

Data Analysis

Data obtained were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 21 (SPSS Inc., Chicago, Illinois, USA). The results were reported as Mean±SEM of the values and Duncan comparison was used to compare mean values. $P<0.05$ was considered significant.

RESULTS

Phytochemicals identified in the aqueous extract of *Azadirachta indica* Leaves by GC-MS

Table 1 represents the bioactive phytochemicals present in the aqueous extract of *A. indica* leaves identified by GC-MS analysis. On comparison of the mass spectra of the constituent with the National Institute of Standards and Technology (NIST) library, 33 phytochemicals were identified. The active principles with their retention time (RT), molecular formula and weight are presented in Table 1.

Weekly body weight changes of streptozotocin-induced diabetic rats administered *Azadirachta indica* aqueous leaves extract

Table 2 represents body weight changes of STZ-induced diabetic rats administered aqueous *A. indica* leaves extract. Treatment with *A. indica* leaves extract resulted to significant increase ($p<0.05$) in weight of the diabetic groups when compared with the untreated group. The significant increase in weight is said to be more in glibenclamide and 400 mg/kg leaves extract groups among the treated groups.

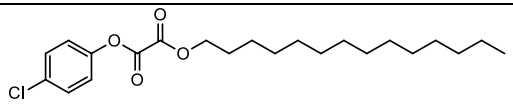
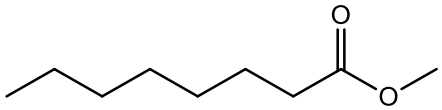
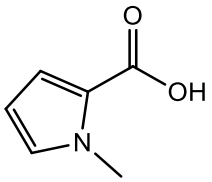
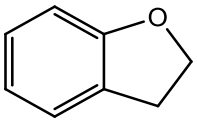
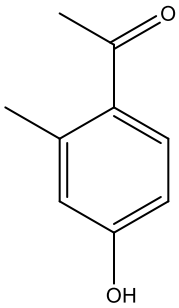
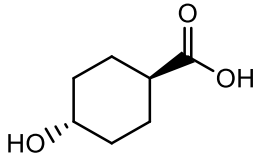
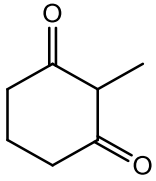
Changes in blood glucose concentrations of streptozotocin-induced diabetic rats administered aqueous *Azadirachta indica* leaves extract during the 4 weeks treatment

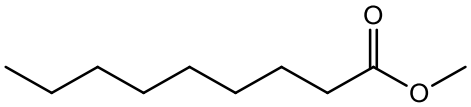
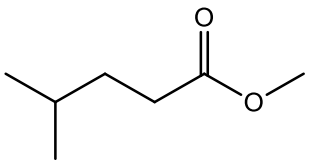
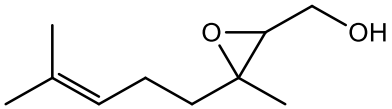
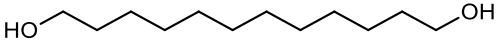
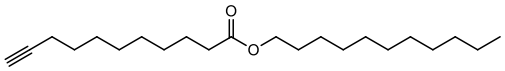
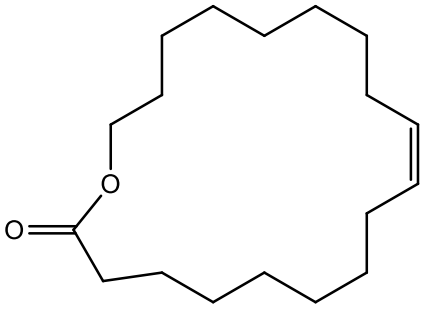
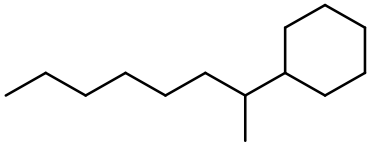
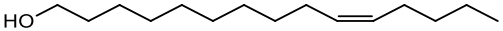
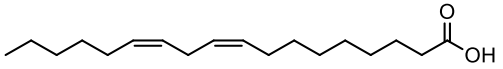
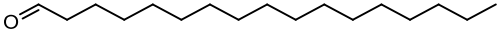
Table 3 shows the changes in blood glucose concentrations of STZ-induced diabetic rats administered *A. indica* aqueous leaves extract during the 4 weeks treatment. The result indicated significant increase ($p<0.05$) in blood glucose concentrations in the diabetic group when compared with the normal control. However, administration of *A. indica* aqueous leaves extract resulted to significant decrease ($p<0.05$) in blood glucose concentrations in the treated groups when compared with the untreated. Treatment with glibenclamide 5 mg/kg and 400 mg/kg aqueous leaves extract showed the most significant decrease among the treated groups.

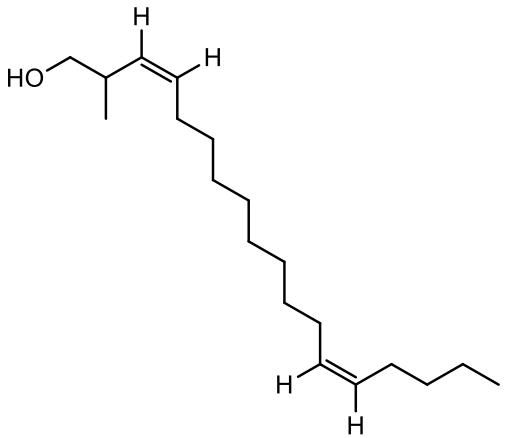
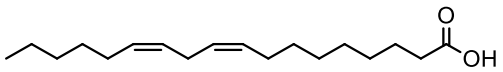
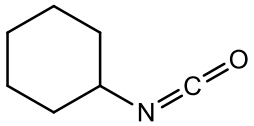
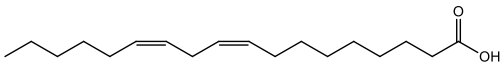
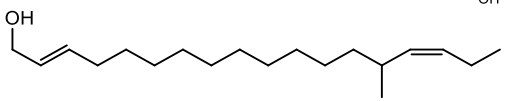
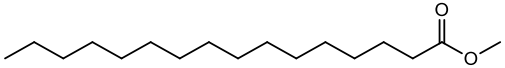
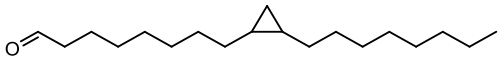
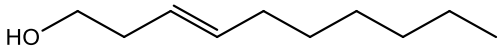
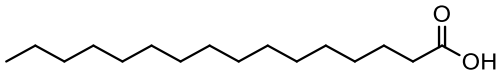
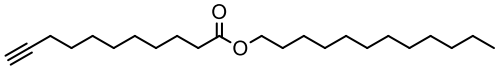
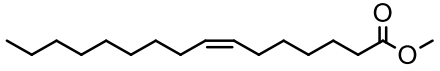
Concentrations of biomarkers of nephropathy in streptozotocin-induced diabetic rats administered *Azadirachta indica* aqueous leaves extract

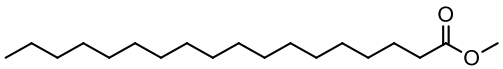
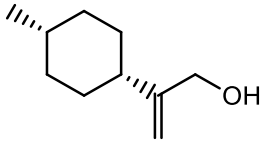
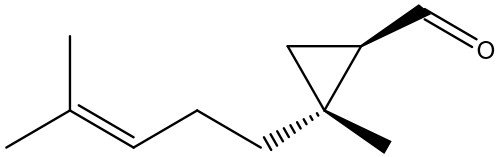
Table 4 shows AGE, IL-6, VEGF and KIM-1 levels of STZ-induced diabetic rats administered *A. indica* aqueous leaves extract. The result indicated significant increase ($p<0.05$) in AGE, IL-6, VEGF and KIM-1 levels in the diabetic untreated when compared with the normal control. However, administration of *A. indica* aqueous leaves extract resulted to significant decrease ($p<0.05$) in AGE, IL-6 VEGF and KIM-1 levels in the treated groups when compared with the diabetic untreated. Treatment with glibenclamide 5 mg/kg showed the most significant decrease in AGE, IL-6, VEGF and KIM-1 concentrations among the treated groups.

Table 1: Phytochemicals identified in the aqueous extract of *Azadirachta indica* leaves by GC-MS

S/N	RT	Area Pct	IUPAC	STRUCTURE	MF	MW
1	6.8072	0.6095	Oxalic acid, 4-chlorophenyl tetradecyl ester		$C_{22}H_{33}ClO_4$	396.95
2	8.004	0.5699	Octanoic acid, methyl ester		$C_9H_{18}O_2$	158.24
3	10.0184	1.9885	N-Methylpyrrole-2-carboxylic acid		$C_6H_7NO_2$	125.13
4	11.254	2.1455	Benzofuran, 2,3-dihydro-		C_8H_8O	120.15
5	13.3599	1.6702	4-Hydroxy-2-methylacetophenone		$C_9H_{10}O_2$	150.17
6	14.5945	8.9917	trans-4-Hydroxycyclohexanecarboxylic acid		$C_7H_{12}O_3$	144.17
7	15.026	0.316	1,3-Cyclohexanedione, 2-methyl-		$C_7H_{10}O_2$	126.15

8	18.558	2.0226	Nonanoic acid, methyl ester		$C_{10}H_{20}O_2$	172.26
9	23.4195	2.4191	Pentanoic acid, 4-methyl-, methyl ester		$C_7H_{14}O_2$	130.18
10	24.5972	2.3066	Oxiranemethanol, 3-methyl-3-(4-methyl-3-pentenyl)-		$C_{10}H_{18}O_2$	170.25
11	24.8314	0.3078	1,12-Dodecanediol		$C_{12}H_{26}O_2$	202.33
12	24.97	0.9254	Undec-10-ynoic acid, undecyl ester		$C_{22}H_{40}O_2$	336.55
13	25.0641	0.5312	(Z)-18-Octadec-9-enolide		$C_{18}H_{32}O_2$	280.45
14	25.1993	0.8875	Octane, 2-cyclohexyl-		$C_{14}H_{28}$	196.37
15	25.6894	0.8039	Z-10-Pentadecen-1-ol		$C_{15}H_{30}O$	226.40
16	26.1296	1.4567	2-Methyl-Z,Z-3,13-octadecadienol			
17	26.4751	2.147	9,12-Octadecadienoic acid (Z,Z)-		$C_{18}H_{32}O_2$	280.45
18	26.5694	0.6728	Heptadecanal		$C_{17}H_{34}O$	254.45

					$C_{19}H_{36}O$	280.49
19	26.7654	5.2818	2-Methyl-Z,Z-3,13-octadecadienol			
20	26.89	3.7405	9,12-Octadecadienoic acid (Z,Z)-		$C_{18}H_{32}O_2$	280.45
21	27.0113	1.1426	Cyclohexane, isocyanato-		$C_7H_{11}NO$	125.17
22	27.0808	2.918	9,12-Octadecadienoic acid (Z,Z)-		$C_{18}H_{32}O_2$	280.45
23	27.3558	4.7351	12-Methyl-E,E-2,13-octadecadien-1-ol		$C_{19}H_{36}O$	280.49
24	27.8528	21.1849	Hexadecanoic acid, methyl ester		$C_{17}H_{34}O_2$	270.45
25	28.4497	5.7525	Cyclopropaneoctanal, 2-octyl-		$C_{19}H_{36}O$	280.49
26	28.6085	2.0928	3-Decen-1-ol, (E)-		$C_{10}H_{20}O$	156.27
27	28.8524	15.2498	n-Hexadecanoic acid		$C_{16}H_{32}O_2$	256.42
28	29.2585	2.796	Undec-10-ynoic acid, dodecyl ester		$C_{23}H_{42}O_2$	350.58
29	31.41	1.572	7-Hexadecenoic acid, methyl ester, (Z)-		$C_{17}H_{32}O_2$	268.43

30	31.8946	1.2851	Methyl stearate		$C_{19}H_{38}O_2$	298.50
31	32.3711	1.0976	cis-9,10-Epoxyoctadecan-1-ol		$C_{18}H_{36}O_2$	284.48
32	38.311	-0.4791	p-Menth-8(10)-en-9-ol, cis-		$C_{10}H_{18}O$	154.25
33	38.5511	0.8583	Cyclopropanecarboxaldehyde, 2-methyl-2-(4-methyl-3-pentenyl)-, trans-(.+-.)-		$C_{11}H_{18}O$	166.26

Key: RT: Retention time, IUPAC: International union of pure and applied chemistry, MF: Molecular Formula and MW: Molecular weight

Table 2: Weekly body weight (g) changes of streptozotocin-induced diabetic rats administered aqueous *Azadirachta indica* leaves extract

Treatment Group	1st Week	2nd Week	3rd Week	4th Week
1	224.86±1.96[a]	240.62±1.97[a]	261.02±1.64[a]	284.36±2.58[a]
2	198.82±1.56[c]	203.04±3.16[c]	216.70±1.45[d]	226.36±2.47[d]
3	179.04±1.98[d]	181.30±0.81[d]	181.72±1.16[e]	180.76±0.52[e]
4	184.86±2.79[b]	187.38±2.19[b]	193.82±3.01[b]	198.68±2.80[b]
5	184.26±0.79[b]	190.92±0.88[b]	200.50±1.30[c]	209.58±1.35[c]
6	203.16±0.59[c]	207.28±0.80[c]	215.48±1.18[d]	221.72±1.44[d]

Results are Mean±SEM of 5 determinations. Values in the same column with different superscript are statistically different; values in the same column with same superscript are statistically not different at ($p<0.05$). Group 1: normal control, Group 2: glibenclamide control, Group 3: diabetic untreated, Group 4: 100 mg/kg leaves extract, Group 5: 300 mg/kg leaves extract, Group 6: 400 mg/kg leaves extract.

Table 3: Changes in blood glucose concentrations (mg/dl) of streptozotocin-induced diabetic rats administered aqueous *A. indica* leaves extract during the 4 weeks

Treatment Group	1st Week	2nd Week	3rd Week	4th Week
1	80.00±0.70[a]	76.80±1.06[a]	85.80±0.86[a]	81.00±0.70[a]
2	398.20±1.68[b]	383.80±0.86[e]	260.80±1.15[b]	94.60±1.43[b]
3	460.60±1.20[d]	450.60±1.16[f]	405.60±2.13[f]	401.20±0.86[e]
4	400.40±0.81[b]	360.40±1.20[d]	300.40±1.77[e]	273.40±1.93[d]
5	397.90±1.20[b]	327.60±1.02[c]	287.40±1.16[d]	186.20±2.22[c]
6	418.20±1.06[c]	301.60±1.77[b]	267.00±0.70[c]	92.60±1.69[b]

Results are Mean±SEM of 5 determinations. Values in the same column with different superscript are statistically different; values in the same column with same superscript are statistically not different at ($p<0.05$). Group 1: normal control, Group 2: glibenclamide control, Group 3: diabetic untreated, Group 4: 100 mg/kg leaves extract, Group 5: 300 mg/kg leaves extract, Group 6: 400 mg/kg leaves extract.

Table 4: Concentrations of biomarkers of nephropathy in streptozotocin-induced diabetic rats administered *Azadirachta indica* aqueous leaves extract

Group	AGE (pg/ml)	IL-6 (pg/ml)	VEGF (pg/ml)	KIM-1(pg/ml)
1	43.02±0.03^{a}	46.45±1.91^{a}	282.07±0.14^{a}	180.22±0.22^{a}
2	47.72±0.23^{b}	59.19±0.32^{b}	398.91±0.09^{b}	479.56±0.33^{b}
3	148.79±0.03^{f}	120.28±0.20^{e}	1105.52±0.27^{f}	1529.33±0.50^{e}
4	104.98±0.34^{e}	98.24±0.08^{d}	973.08±0.10^{e}	1240.93±0.13^{d}
5	81.93±4.07^{d}	76.84±0.15^{c}	727.80±0.27^{d}	711.80±3.63^{c}
6	54.87±0.32^{c}	77.65±1.08^{c}	401.45±0.41^{c}	712.57±7.52^{c}

Results are Mean±SEM of 5 determinations. Values in the same column with different same superscript are statistically different and values in the same column with same superscript are not statistically different at ($p<0.05$). Key: AGE: Advanced glycation end products, IL-6: Interleukin 6, VEGF: Vascular endothelial growth factor and KIM-1: Kidney injury molecule-1. Group 1: normal control, Group 2: glibenclamide control, Group 3: diabetic untreated, Group 4: 100 mg/kg leaves extract, Group 5: 300 mg/kg leaves extract, Group 6: 400 mg/kg leaves extract.

DISCUSSION

GC-MS Analysis

From the 33 compounds detected in leaves extract, 9 were believed to be associated with diabetes and its complications. These compounds are; Octanoic acid, methyl ester, N-Methylpyrrole-2-carboxylic acid, Benzofuran, 2, 3-dihydro-, Nonanoic acid, methyl ester, 2-Methyl-Z,Z-3,13-octadecadienol, 9,12-Octadecadienoic acid (Z, Z)-, 12-Methyl-E,E-2,13-octadecadien-1-ol, Hexadecanoic acid, methyl ester and n-Hexadecanoic acid. A study conducted by Wilson *et al*. (2006) reported that Octanoic acid increased weight and the concentration of albumin in diabetic rats. In a study reported by Azhagu and Madhavan (2021) indicated N-Methylpyrrole-2-carboxylic acid as antidiabetic agent. More so, 9, 12-Octadecadienoic acid (Z, Z)- was also identified by Ityo *et al*. (2023), Mariel *et al*. (2020) to have significantly reduced glucose concentration in diabetic rats. In another studies by Shi *et al*. (2022) and Xu *et al*. (2022) reported Pyrazine, tetramethyl- being antidiabtic agent. Similarly, Deepika *et al*. (2022) and Satish *et al*. (2011) reported the compound Benzofuran as antioxidant agent. Nonanoic acid, methyl ester, 2-Methyl-Z, Z-3,13-octadecadienol, 9,12-Octadecadienoic acid (Z, Z)-, Hexadecanoic acid, methyl ester and n-Hexadecanoic acid were also believed to be antioxidant compounds as reported by (Ityo *et al*., 2020; Mariel *et al*., 2020). The effects of these compounds can be attributed to the increased production of insulin by the pancreas that activate glucose transporters to convey glucose to the cells for effective utilization, or could be the ability of the extract to regenerate beta cells of the pancreas to produce the needed insulin required to signal the glucose transporters to convey glucose in to the cells (Rekha *et al*., 2022) which eventually prevent the degradation of structural proteins and muscle wasting. The antidiabetic activity of these agents could also be inhibition of the enzyme α - glucosidase (Rania *et al*., 2023) or through reduced glucagon secretion and possible helped in taking of physiologically important elements such as Cu^{2+} and Mg^{2+} for beta cells functions (N'doua *et al*., 2015). It could also be inhibition of the enzyme aldose reductase which catalyzes the conversion of glucose to sorbitol, which contributes to complications of diabetes (Rania *et al*., 2023). It could also be by increased insulin secretion through antioxidant activity (Rania *et al*., 2023). Furthermore, it could also be attributed to the possible enhancement of protein synthesis in the liver resulting in an increase in insulin secretion, and increase hepatic absorption of glucogenic amino acids (Arika *et al*., 2016).

Effect on Body weight

The results revealed that aqueous *A. indica* leaves extract has the power to increase the body weight of STZ-induced diabetic rats. This result is in line with the findings by Ezeigwe *et al*. (2019) who revealed significant increase in the body weight of STZ-induced diabetic rats treated with *A. indica* leaves extract. It is also in accordance with a study by Abubakar *et al*. (2023) who reported significant increase in body weight of STZ-induced diabetic rats treated with *A. indica* leaves extract. The finding also conforms to the results of Athraa (2022) who reported significant increase in the body weight of STZ-induced diabetic mice after treatment with *A. indica* leaves extract. McCalla *et al*. (2016) reported significant ($p<0.05$) increase in the weight of STZ-induced diabetic Sprague-Dawley rats treated with *A. indica* leaves extract. Similar study reported by Fattah *et al*. (2020) revealed that *A. indica* leaves extract supplementation in diabetic rats showed a significant increase in body weight which could possibly be due to improved glucose metabolism. This effect can be attributed to the increased production of insulin by the pancreas that activate glucose transporters to convey glucose to the cells for effective utilization, or could be the ability of the

extract to regenerate beta cells of the pancreas to produce the needed insulin required to signal the glucose transporters to convey glucose in to the cells (Rekha *et al*., 2022) which eventually prevent the degradation of structural proteins and muscle wasting.

Effect on Fasting Glucose

The significant decrease in blood glucose conforms to the results of Athraa (2022) who reported significant decrease in blood glucose level after treatment with *A. indica* leaves extract in STZ-induced diabetic mice. This report agrees with the results of Tiwari *et al*. (2014), and Arika *et al*. (2016). Gutierrez *et al*. (2011) reported that the leaves extract of *A. indica* significantly (p< 0.05) decreased the blood glucose levels even at 60, 90 and 120 minutes in STZ-induced diabetic Wistar rats. Rania *et al.* (2023) reported hypoglycemic property of *A. indica* leaves extract in alloxan-induced diabetic rats which corresponds to the findings from this study. In a corresponding study, Satyanarayana *et al.* (2015) reported that oral administration of *A. indica* leaves extract in high-fat-induced diabetic rats normalized the altered levels of blood glucose and is very effective in the management of type 2 diabetes mellitus. It was reported that the *A. indica* is very effective in maintenance of blood glucose concentration and also good in preventing and delaying the onset of diabetes (Rania *et al*., 2023). The result also agrees with the findings by Ezeigwe and Francis, (2020) who revealed that leaves extract of *A. indica* significantly decreased blood glucose levels in STZ-induced diabetic rats. The effect of extract on diabetic rats may be attributed to the possible enhancement of protein synthesis in the liver resulting in an increase in insulin secretion, and increase hepatic absorption of glucogenic amino acids (Arika *et al*., 2016). It could also be due to decrease in proteolysis by activating the amino acids transamination enzymes (Owolabi *et al*., 2011). The antidiabetic activity of the extract also be by stimulating insulin secretion, augmenting peroxisome proliferator-activated receptors (PPARs), inhibiting α-amylase or α-glucosidase, inhibiting the secretion of incretin, glucagon-like peptide-1 (GLP1), inhibiting advanced glycation end product (AGE) formation, free radical scavenging and antioxidant activity, elevating translocation of glucose transporter type 4 (GLUT-4) and preventing development of insulin resistance (Zainab *et al*., 2018).

Effect of the Extracts Advanced Glycation End Products (AGEs)

The decreased AGE level agrees with a report by Maria de Jesus (2014) who reported significant decrease in the level of AGE in STZ-induced diabetic rats administered *A. indica* leaves extract. The result also is consistent to a study by Gutierrez *et al*. (2011) who reported that the chloroform leaves extract of *A. indica* exhibited significant inhibitory effect on AGEs formation that is similar to amino guanidine, an AGEs inhibitor that was tested in clinical trials for the treatment of diabetic complications. The significant decrease could either be due to inhibition of the formation of methylglyoxal-derived advanced glycation end-products in a bovine serum–albumin–methylglyoxal system, or could act by blocking conversion of dicarbonyl intermediates to advanced glycation end-products or by reaction with carbonyl groups from the reducing sugars, Amadori adducts, and dicarbonyl intermediates, therefore, blocking their conversion to advanced glycation end-products. Akilona *et al*. (2011) reported that AGE formation can be delayed by the antioxidants.

Effect on Interleukin-6 (IL-6)

The significant decrease in IL-6 level is in line with the findings by Bharali *et al*. (2022) reported significant decrease in serum IL-6 level in STZ-induced diabetic Wistar rats treated *A. indica* leaves extract for 30 days in dose dependent manner. The result also agrees with a study by Abubakar *et al*. (2023) who reported that oral administration of *A indica* for 28 days of treatment significantly (p<0.05) decreased the IL-6 compared to diabetic control rats. It also conforms with a finding by Usharani *et al*. (2020) who revealed that oral administration of *A indica* for 4 and 12 weeks treatment significantly (p<0.05) decreased the IL-6 compared to diabetic untreated rats. The significant decrease in IL-6 concentration could be associated with the constituents in the extract that decrease nuclear factor kappa (NF-kβ) p56 units, interleukin-1β and nitric oxide (Aba and Asuzu, 2018).

Effect on Vascular Endothelial Growth Factor (VEGF)

The significant decrease in VEGF level is in line with the finding of Thungrung *et al*. (2020) who revealed significant decrease in VEGF level in STZ-induced diabetic rats treated with *Moringa oleifera* extract. In contrast, Panaskar *et al*. (2013) reported increase in VEGF levels after treatment with *A. indica* leaves extract in STZ-induced diabetic rats. The significant decrease in VEGF concentration could possibly be through inhibition of aldose reductase which ameliorates VEGF expression by the compounds present in the extracts (Sung *et al*., 2010). It could also be due enhanced angiogenesis mediated through the inhibition of hyperglycemia, oxidative stress and down-and-up regulation of inflammatory mediators and growth factor expression. Natural antioxidants were found to reduce angiogenesis by interfering with the formation of VEGF receptor

complex which may have physiological significance in the management of diabetic nephropathy (Al-Maliki *et al*., 2015).

Effect on Kidney Injury Molecule-1(KIM-1)

The significant decrease in KIM-1 level is in line with the work of Putra *et al.* (2023) who reported significant decrease KIM-1 after treatment with *Coroton hookeri* methanol leaves extract at 200 mg/kg in STZ-induced Sprague Dawley diabetic rats within the period of 14 days. It also conforms to a study by Al-Hassan *et al*. (2024) who revealed significant decrease in KIM-1 levels after treatment with phloretamide in STZ-induced diabetic rats. The significant decrease in KIM-1 concentration could be through improvement in the action of antioxidants present in the extracts that decrease oxidative stress parameters, normalize lipoproteins and improve recovery in hepatic insulin and leptin sensitivity (Aba and Asuzu, 2018).

CONCLUSION

The study observed that aqueous *A. indica* leaves extract is potent in the management of diabetes and its complication. This showed that *A. indica* is a store of phytoconstituents that can serve as a good source of diabetic drug.

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CONFLICT OF INTEREST

Authors declared no conflict interests regarding the publication of this article.

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