Anti-Diabetic Potential of Anacardium Occidentale Leaf Extract in Alloxan-Induced Diabetic Albino Rats

Abstract: This study investigates the anti-diabetic potential of the aqueous leaf extract of *Anacardium occidentale* in alloxan-induced diabetic male albino rats. Diabetes mellitus, characterized by chronic hyperglycemia due to insulin deficiency or resistance, poses significant health challenges, necessitating the exploration of alternative therapeutic agents. To induce diabetes, male albino rats received an intraperitoneal injection of alloxan at a dosage of 120 mg/kg body weight. Following confirmation of hyperglycemia, the diabetic rats were treated with aqueous leaf extract of *A. occidentale* at a dosage of 100 mg/kg for a duration of 28 days. The results demonstrated a statistically significant reduction in fasting blood glucose levels ($p < 0.05$) in the treated groups compared to the diabetic control group. Additionally, there were notable improvements in liver function markers, indicating the extract's potential hepatoprotective effects. The treatment also positively influenced lipid profiles, with reductions in total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels, while high-density lipoprotein (HDL) levels increased significantly. Furthermore, the extract enhanced antioxidant levels, as evidenced by increased concentrations of reduced glutathione and superoxide dismutase, suggesting a protective role against oxidative stress associated with diabetes. These findings support the hypothesis that *Anacardium occidentale* leaf extract possesses significant anti-diabetic properties, with potential mechanisms involving the modulation of glucose metabolism, improvement of lipid profiles, and reduction of oxidative stress. The results contribute to the growing body of evidence advocating for the utilization of plant-based remedies in the management of diabetes, emphasizing *A. occidentale* as a promising candidate for further investigation and potential therapeutic application in the treatment of diabetes and its associated complications. Future studies should aim to elucidate the underlying biochemical mechanisms and active compounds responsible for the observed effects, paving the way for the development of effective plant-based treatments for diabetes management.

Key words: Diabetes Mellitus, Alloxan induced, fasting glucose test,

Renal function, Anacardium occidentales.

1. Introduction

Diabetes mellitus (DM) is one of the oldest diseases known to humanity, with historical records tracing back over 3000 years in Egyptian manuscripts (Ahmed, 2002). It is characterized by

chronic hyperglycemia due to insulin deficiency or resistance, leading to serious complications, including microvascular damage (such as retinopathy, nephropathy, and neuropathy) and macrovascular issues like heart attacks, strokes, and kidney failure (Loukine et al., 2012). The rising global prevalence of DM, coupled with the limitations of conventional treatments, has stimulated interest in alternative therapeutic strategies, particularly those derived from medicinal plants. Traditional treatments, including sulfonylureas and metformin, often come with undesirable side effects and limited long-term efficacy (Moller, 2001), prompting the need for safer and more effective alternatives.

For centuries, plants have served as a vital source of medicinal agents. Traditional medicine, particularly in areas with limited access to modern healthcare, holds a wealth of knowledge regarding plants with therapeutic potential. Over 400 plant species have been documented to exhibit hypoglycemic effects, leading the World Health Organization (WHO) to encourage further research into plant-based diabetes treatments (WHO, 2002; Miura et al., 2002). The rich diversity of bioactive compounds in medicinal plants—such as alkaloids, flavonoids, saponins, and tannins—contributes to various pharmacological activities, including hypoglycemic, anti-inflammatory, and antioxidant effects (Fabricant & Farnsworth, 2001).

One such plant, *Anacardium occidentale* (cashew), has been extensively used in traditional medicine across various cultures. Indigenous to regions such as Brazil, Portugal, India, Southeast Asia, and Africa, this tropical evergreen plant is renowned for its leaves, stem, and bark, which have been utilized in folk medicine to treat a range of ailments, including diabetes, diarrhea, malaria, and yellow fever (Akinpelu, 2001). Phytochemical analyses of *A. occidentale* have identified several compounds, including flavonoids, glycosides, and saponins, that are believed to contribute to its therapeutic effects (Konan et al., 2007).

As the global incidence of diabetes continues to rise, and with the limitations of existing therapeutic options, exploring alternative plant-based treatments becomes imperative. Previous studies have indicated that extracts from *A. occidentale* possess hypoglycemic, anti-inflammatory, and antioxidant properties, suggesting their potential in diabetes management. Research has demonstrated that its bioactive components can provide protection against streptozotocin-induced diabetes in animal models (Sokeng et al., 2001), and its leaves have been observed to induce vasorelaxation in isolated rat aorta (Runnie et al., 2004). Beyond diabetes, *A. occidentale* has applications in treating various conditions, including eczema, psoriasis, and venereal diseases (Arul & Thangavel, 2011).

This study aims to investigate the effects of *A. occidentale* leaf extract on lipid profiles and antioxidant levels in alloxan-induced diabetic albino rats. Enhancing lipid profiles and reducing oxidative stress are crucial in managing the complications associated with diabetes. The findings of this study may pave the way for the development of plant-based therapeutics that offer a safer and more affordable alternative to conventional diabetes treatments.

2. Materials and Methods for Manuscript Publication

2.1 Materials

2.1.1 Instruments/Apparatus

- **Electronic weighing balance** (Pioneer, OHAUS, USA)
- **Whatman Filter paper** (Whatman Lab Division, Springfield Mill, England)
- **Water bath** (Uniscope SM 101 Laboratory, Surgifriend Medicals, England)
- **GCMS** (Agilent Technologies 6890N/5975B)
- **Heating mantle** (Techmel & Techmel, USA)
- **Rotary Evaporator** (Buchi, Rotavapor R-200)
- **Spectrophotometer** (Jenway 6305, England)
- **Centrifuge** (Model 80-2 Microfield Instrument, England)
- **Digital glucometer** (Accu-Check Sensor Glucometer, Roche, Mexico City)
- **Centrifuge tubes** (Plastic)
- **Sample tubes** (Goodcare Blood Collection Tubes)
- **Dissecting kits** (Hawksley, England)
- **Latex surgical gloves** (Shieldtex SDN. BAD, Malaysia)
- **Measuring cylinder**
- **Desiccator**
- **Cotton wool**

2.1.2 Reagents/Chemicals

- **A.S.P Kit** (Agappe Laboratories Ltd, UK)
- **A.L.T Kit** (Agappe Laboratories Ltd, UK)
- **A.L.P Kit** (Agappe Laboratories Ltd, UK)
- **Formalin** (40%)
- All chemicals and reagents used were of analytical grade.

2.2 Methods

2.2.1 Sample Collection and Identification

Leaves of the study plant, *Anacardium occidentale*, were collected from Ogoni land, Rivers State. Identification of the plant was confirmed by a botanist at the Department of Plant Science and Biotechnology, Rivers State University (RSU), Port Harcourt, Nigeria. The leaves were washed, rinsed with distilled water, air-dried for four weeks, and powdered.

2.2.2 Preparation of Extract

Powdered plant material (500g) was macerated in water for 72 hours. The extract was filtered using a Whatman No. 1 filter paper and concentrated via a rotary evaporator (Buchi, Rotavapor R-200), stored at 4°C.

2.2.3 Animal Handling

Twenty-four male Wistar rats (90-120g) were obtained from Rivers State University and housed at the Pharmacology/Therapeutics Animal House, RSU. The animals acclimatized for two weeks prior to the study.

2.2.4 Research Design

The rats were divided into 4 groups (n=6):

- **Group 1**: Normal control (feed and water)
- **Group 2**: Diabetic control (alloxan only)
- **Group 3**: 120mg/kg alloxan + 100mg/kg *Anacardium occidentale*
- **Group 4**: 120mg/kg alloxan + 5mg/kg glibenclamide

2.2.5 Blood Sample Collection

After 28 days of treatment, the rats were sacrificed. Blood samples were collected via cardiac puncture, centrifuged at 5000 rpm for 10 minutes, and stored at 4°C. Kidneys and pancreas were preserved in 10% formaldehyde for histology.

2.2.6 Examination

Serum was used for biochemical assays such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lipid profile, and antioxidant assays.

2.2.7 Data Analysis

Data were analyzed using GraphPad Prism. Statistical significance was determined using ANOVA.

2.3 Method of Assay and Procedure

2.3.1 Phytochemical Analysis of Aqueous Extract

A 1 µl sample of the aqueous extract was analyzed using GCMS (Agilent 6890N/5975B). Identification of active compounds was performed by comparing spectral peaks with the NIST 2014 database.

2.3.2 Determination of Changes in Body Weight

Body weight was measured at the start of the study, weekly, and on the 28th day using an electronic balance. The percentage change in body weight was calculated.

2.3.3 Determination of Fasting Plasma Glucose

Fasting plasma glucose was measured using the glucose oxidase method. Blood samples (0.2 ml) were drawn from the tail vein every 7 days for 28 days, and glucose levels were measured with an Accu-Check Sensor glucometer.

2.3.4 Alanine Aminotransferase (ALT) Assay

ALT activity was measured using Reitman and Frankel's method (1957). Absorbance was measured at 405 nm and ALT activity calculated.

2.3.5 Alkaline Phosphatase (ALP) Assay

ALP activity was measured using a standard method (Bergmeyer et al., 1974). Absorbance was read at 426 nm.

2.3.6 Aspartate Aminotransferase (AST) Assay

AST activity was determined using Reitman and Frankel's method. Absorbance was measured at 405 nm and AST activity calculated.

2.3.7 Determination of Total Cholesterol

Cholesterol concentration was determined using the Allain et al. (1976) method and a Biosystem cholesterol kit.

2.3.8 Determination of Triacylglycerides (TG)

TG concentration was measured using the Fletcher (1968) method. Absorbance was recorded at 500 nm.

2.3.9 Determination of HDL-Cholesterol

HDL-cholesterol was measured using Grove's method (1979). Precipitated LDL and VLDL were removed, and the supernatant was used for analysis.

2.3.10 Determination of LDL-Cholesterol

LDL concentration was calculated using Friedewald's formula (Friedewald et al., 1972).

2.3.11 Renal Function Assay (Urea)

Urea levels were determined using an enzymatic assay based on the urease-GLDH system (Talke & Schubert, 1965).

3 RESULT

3.1 PHYTOCHEMICAL ANALYSIS OF AQUEOUS LEAF EXTRACT OF *Anacardium Occidentale*

Figure 1 GCMS result of aqueous leaf extract of *Anacardium Occidentale*

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File       :C:\msdchem\1\data\AMARA\CASHEW LEAF EXTRACT.D
Operator   : EBIC
Acquired   : 24 Mar 2023  14:52     using AcqMethod Scan_.M
Instrument :    GCMSD
Sample Name: Cashew Leaf
Misc Info  :
Vial Number: 6
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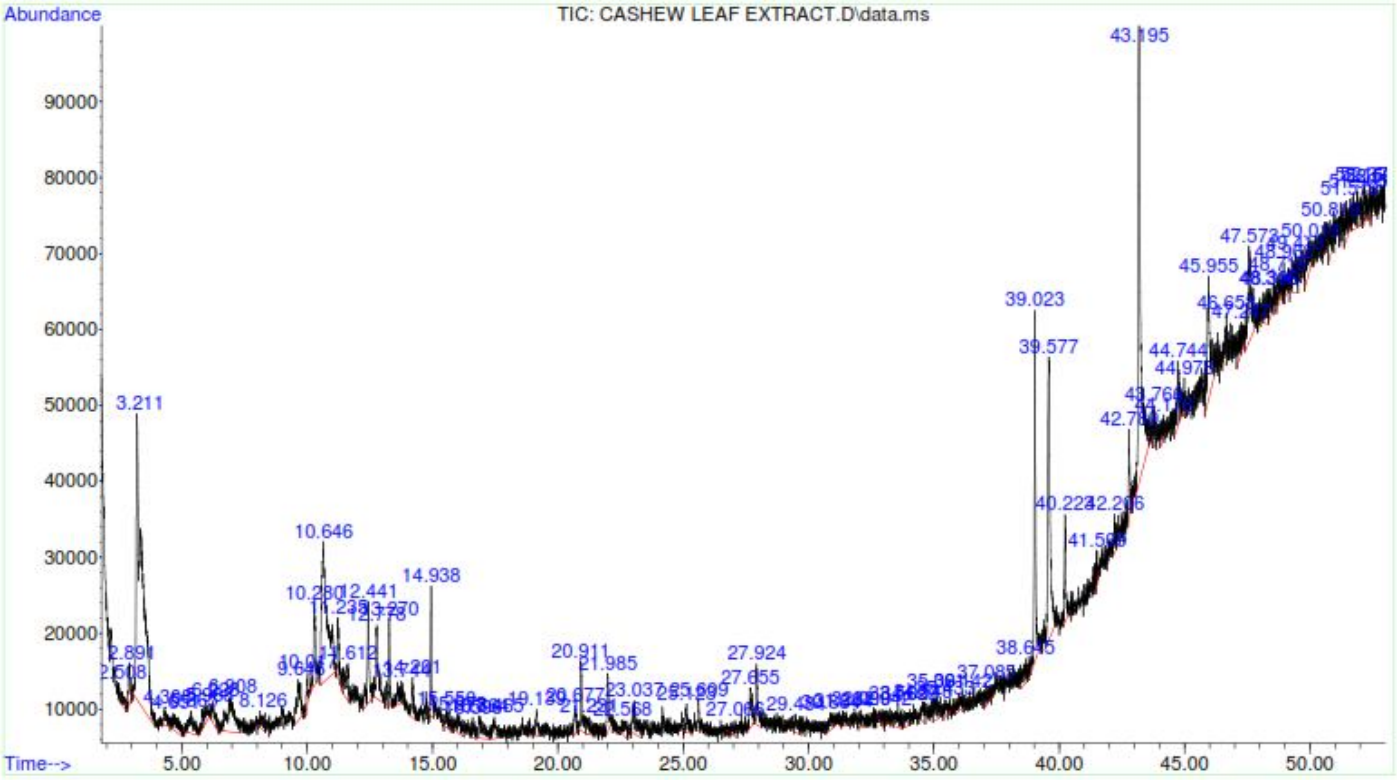
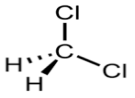
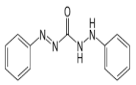
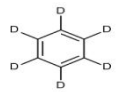
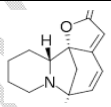
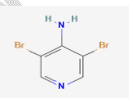
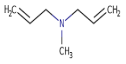
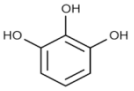
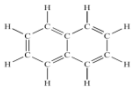
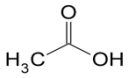
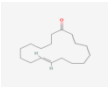
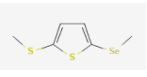


Figure 1 shows the result of the GC-MS analysis of the aqueousleaf extract of *anacardium occidentale*. The GC-MS spectrum confirmed the presence of various components with different retention times. The list of constituents is given in Table 1. The major components and their molecular formular, molecular weight, biological activity and structural formular are also shown.

TABLE 1: Bioactive compounds present in aqueous leaf extract of *anacardium occidentale*

RT (MIN)	NAME OF COMPOUND	MOELCULAR FORMULAR	MOLECULAR WEIGHT	PEAK AREA %	STRUCTURE
2.508	Methylene chloride	CH2Cl2	84.933	0.41	

2.891	Diphenylcarbazone	C13H12N4O	240.26	0.48	
3.211	.gamma.-Terpinene	C10H16	136.234	13.62	
4.651	Benzene-D6	C6D	84.148	0.69	
6.908	Securinine	C13H15NO2	217.26	2.08	
9.646	4-amino-3,5-dibromopyridine	C5H4Br2N2	251.91	1.251	
10.28	Methyldiallylamine	C7H13N	111.18	1.463	
10.65	1,2,3-Benzenetriol	C6H6O3	126.11	6.537	
12.44	Naphthalene	C10H8	128.17	1.78	
12.78	Pyrazole-3-carboxylic acid,	C4H4N2O2	112.09	1.665	
14.94	9-Octadecene	C18H36	252.47	1.339	
20.91	Behenic alcohol	C22H46O	326.6	1.073	
21.99	9-Cycloheptadecen-1-one,	C17H30O	250.419	0.81	
27.07	Phenol, 2,4-dibromo-	C6H4Br2O	251.905	0.35	
30.88	Thiophene, 2-(methylselenyl)-5-(propylthio)	C6H8S2Se	223.2	0.67	

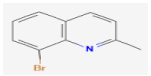
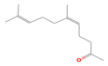
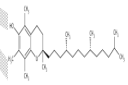
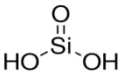
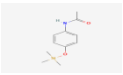
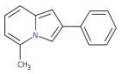
34.02	Mercury, chloromethyl-	CH3ClHg	251.08	0.74	
35.43	8-Bromo-2-carbamoylquinoline	C10H8BrN	222.08	0.35	
39.02	5,9-Undecadien-2-one, 6,10-Dimethyl	C13H22O	194.313	3.908	
39.58	dl-.alpha.-Tocopherol	C29H50O2	430.7	5.6	
40.22	Silicic acid	H4SiO4	192.23	1.231	
44.74	Tetrasiloxane, decamethyl	C10H0O3Si	351.522	1.399	
47.57	Methyltris(trimethyl siloxy)silane	C10H24O6Si	301.46	2.536	
49.41	Acetamide, N-[4-(trimethylsilyl) phenyl]	C11H17NOSi0	207.34	1.217	
50.02	5-Methyl-2-phenylindolizine	C15H13N	207.104	1.078	
51.91	Cyclotrisiloxane, hexamethyl-	C6H18O3Si	222.46	1.017	
52.56	Tetrasiloxane, decamethyl-	C10H30OSi4	310.68	0.38	

Table 1 shows that the GC-MS analysis of aqueous leaf extract of *anacardium occidentale* confirmed the presence of lots of phytochemically active biocompounds whose strength contribute to the medicinal bioactivity of the plant.

3.2 CHANGES IN BODY WEIGHT

TABLE 2: Effect of aqueous leaf extract of *anacardium occidentale* on body weight (B.WT) of alloxan induced diabetic male rats after 28 days

GROUPS	BODY WEIGHT (g)			
	WEEK1	WEEK2	WEEK 3	WEEK4
GROUP 1	108.0±0.34	136.2±0.83	141.2±0.63	128.0±0.46
GROUP 2	76.6±0.63	53.2±0.54	50.8±0.74	72.0±0.13
GROUP 3	122.9±1.45	126.8±0.28	130.1±0.46	118.9±0.41
GROUP 4	85.4±0.44	111.6±0.72	104.6±0.94	96.8±0.33

Result presented as mean ±SD

n=5

Where

Group 1 - Normal (control) feed and water

Group 2 - Diabetic control (only alloxan)

Group 3 - Alloxan + Anacardium Occidentale

Group 4 - Alloxan + Glibenclamide

Table 2 showed that there was a significant decrease ($P<0.05$) in the body weight of animals of the diabetic control group in the 4 weeks of experiment when compared to the normal control and other groups. However, there was a significant increase ($P<0.05$) in body weight of *Anacardium occidentale* treated group in week 3 of the experiment when compared to diabetic group and glibenclamide treated rats in group 4.

3.3 FASTING PLASMA GLUCOSE CONCENTRATION

TABLE 3: Effect of aqueous leaf extract of *anacardium occidentale* on Fasting Plasma Glucose (Glu) inmg/dl of alloxan induced diabetic male rats after 28 days

GROUPS	GLUCOSE (GLU) (mg/dl)			
	WEEK1	WEEK2	WEEK 3	WEEK4
GROUP 1	113.0±0.34	99.6±0.21	79.4±0.40	104.2±0.31
GROUP 2	321.4±0.45	573.6±1.23	282.8±0.50	254.2±0.09
GROUP 3	299.0±0.49	182.2±0.41	164.2±0.64	193.6±0.91
GROUP 4	309.4±0.54	72.2±0.08	201.4±0.11	198.4±0.12

Result presented as mean ±SD

n=5

Where Group 1 - Normal (control) feed and water

Group 2 - Diabetic control (only alloxan)

Group 3 - Alloxan + Anacardium Occidentale

Group 4 - Alloxan + Glibenclamide

Table 3 revealed that there was a significant increase ($P<0.05$) in plasma fasting glucose concentration in the diabetic control group throughout the 4 weeks of experiment. The highest increase was recorded in the week 2. The result also showed that there was a significant decrease ($P<0.05$) in *Anarcadium occidentale* treated group when compared with the diabetic control and Glibenclamide group in week 3, although Glibenclamide treated group at week 2 showed a greater reduction in glucose concentration when compared with the control and other groups.

3.4 LIVER FUNCTION ASSAY

TABLE 4: Effect of aqueous leaf extract of *anacardium occidentale* on liver function parameter (ALT, ALP and AST) in u/l of alloxan induced diabetic male rats after 28 days

GROUPS	LIVER FUNCTION PARAMETERS (U/L)		
	ALT (U/L) (Alanine Aminotransferase)	ALP (U/L) (Alkaline Phosphatase)	AST (U/L) (Aspartate Aminotransferase)
GROUP 1	21.30 ± 0.32	84±0.84	50.02±0.92
GROUP 2	28.90 ± 0.41 #	118±0.78	102.40±0.36
GROUP 3	12.40 ± 0.20 ***	108±0.62	52.31±0.67

GROUP 4	14.34±0.24***	105±0.65	40.93±0.55

Result presented as mean ±SD, n=5

* shows highly symbolic

shows statistically significant

Where Group 1 - Normal (control) feed and water

Group 2 - Diabetic control (only alloxan)

Group 3 - Alloxan + Anacardium Occidentale

Group 4 - Alloxan + Glibenclamide

Table 4 showed that in ALT assay, there was a significant decrease ($p < 0.05$) in *Anacardium occidentale* treated group when compared with the control and other groups. Furthermore, the standard drug (Glibenclamide) showed a significant decrease ($p < 0.05$) in ALT activity when compared with the control. However, the plant extract significantly decreased more when compared to Glibenclamide treated group. On the other hand, the AST assay showed that there was a significant increase ($p < 0.05$) of AST activity in the diabetic control group when compared to the normal control and other groups. However, there was a significant decrease ($p < 0.05$) in the *A. Occidentale* treated group when compared with the diabetic group. Although Glibenclamide group showed a more significant decrease ($P<0.05$) in AST activity when compared with *A. Occidentale* treated group. Similarly, the ALP assay showed significant decrease ($p < 0.05$) in the *A. Occidentale* treated group when compared with other groups while the diabetic group showed a significant increase ($p < 0.05$) in activity when compared with normal control and other groups.

3.6 RENAL FUNCTION ASSAY

TABLE 5: Effect of aqueous leaf extract of *anacardium occidentale* on renal function parameters of alloxan induced diabetic rats after 28 days

GROUPS	**RENAL FUNCTION PARAMETERS (mg/dl)**			
	UREA	**CREATININE**	**SODIUM**	**POTASSIUM**
GROUP 1	15.85±0.43	1.25±0.34	5.50±0.14	8.40±0.04
GROUP 2	28.45±0.54	3.30±0.65	8.54±0.23	12.43±0.93
GROUP 3	17.91±0.65	1.90±0.38	4.43±0.24	5.23±0.13

GROUP 4	16.87±0.24	2.52±0.26	3.51±0.63	4.81±0.75

Result presented as mean ±SD, n=5

* shows highly symbolic

shows statistically significant

Where Group 1 - Normal (control) feed and water

Group 2 - Diabetic control (only alloxan)

Group 3 - Alloxan + Anacardium Occidentale

Group 4 - Alloxan + Glibenclamide

Table 3.6 revealed that the serum urea concentration of the diabetic control significantly increased ($p < 0.05$) when compared with the control and other groups. The *Anarcadium occidentale* treated group also showed slight increase in activity when compared with the normal control and glibenclamide group. Similarly, there was a significant increase ($p < 0.05$) in the creatinine concentration of diabetic control group, when compared with other groups. And while the *Anarcadium occidentale* treated group also showed slight increase in serum creatinine concentration when compared with the normal control, there was a decrease when compared with Glibenclamide treated group. Again, there was a significant increase ($p < 0.05$) in sodium and potassium level in the diabetic control group when compared with normal control and other groups. However, a decrease in sodium and potassium was noticed in *A. occidentale* treated group when compared with the control and diabetic control. Glibenclamide treated group showed more reduced sodium and potassium level in the blood of experimental animal when compared with *Anarcadium occidentale* treated group and the other groups.

4. Discussion

The present study investigated the anti-diabetic potential of the aqueous leaf extract of *Anacardium occidentale* in alloxan-induced diabetic male albino rats. The findings revealed that the extract significantly reduced fasting blood glucose levels, improved liver function markers, and positively influenced lipid profiles and antioxidant levels. The reduction in blood glucose levels observed in the treated groups supports the hypothesis that *A. occidentale* possesses bioactive compounds that modulate glucose metabolism, making it a potential natural remedy for diabetes management.

The induction of diabetes using alloxan was effective, as indicated by the marked increase in fasting plasma glucose concentrations in the diabetic control group. This is consistent with

previous studies, where alloxan is known to induce diabetes by selectively destroying insulin-producing beta cells in the pancreas, leading to hyperglycemia (Wokocha et al., 2024 Vessal et al., 2003). The extract-treated group demonstrated a significant decrease in plasma glucose levels from week two to week four, suggesting a time-dependent therapeutic effect of the extract. This is in agreement with earlier reports on the hypoglycemic effects of plant extracts in diabetic animal models (Khan et al., 2015).

Furthermore, the assessment of liver function parameters revealed that the extract significantly decreased the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in the treated groups compared to the diabetic control group. Elevated levels of these enzymes typically indicate liver damage or dysfunction, often associated with diabetes (Raza et al., 2015). The observed hepatoprotective effect of *A. occidentale* may be attributed to its phytochemical constituents, which possess antioxidant properties that mitigate oxidative stress and inflammation in diabetic conditions (Gholap et al., 2017).

The lipid profile results further corroborate the efficacy of *A. occidentale* in managing diabetes. The diabetic control group exhibited elevated total cholesterol (TC) and low-density lipoprotein (LDL) levels, which are common lipid abnormalities in diabetic patients and contribute to cardiovascular risks (Kumar et al., 2013). The treated groups, particularly the glibenclamide group, showed significant reductions in TC and LDL levels, while the *A. occidentale* group exhibited improvements in high-density lipoprotein (HDL) cholesterol levels. These findings suggest that the extract not only helps in controlling blood glucose levels but also ameliorates dyslipidemia associated with diabetes.

Additionally, the phytochemical analysis using GC-MS identified several bioactive compounds present in the aqueous extract of *A. occidentale*. The presence of compounds such as *gamma-Terpinene*, *dl-alpha-Tocopherol*, and others noted for their medicinal properties aligns with the observed therapeutic effects in this study. This supports the traditional use of *A. occidentale* in managing diabetes and suggests potential avenues for further research into the specific mechanisms of action of these compounds.

5. Conclusion

In conclusion, the results of this study suggest that the aqueous leaf extract of *Anacardium occidentale* exhibits significant anti-diabetic effects in alloxan-induced diabetic albino rats. The extract effectively reduced fasting blood glucose levels, improved liver function parameters, and positively affected the lipid profile of the diabetic rats. Given the presence of various phytochemicals in the extract, *A. occidentale* may serve as a promising natural remedy for diabetes management. Future studies should focus on elucidating the precise mechanisms by which these bioactive compounds exert their effects and exploring the clinical relevance of these findings in human subjects.

References

1. Ahmed, A. (2002). Historical records of diabetes mellitus. *Journal of Medical History, 46*(3), 322-335.

2. Akinpelu, D. A. (2001). Ethnomedicinal properties of *Anacardium occidentale*. *Nigerian Journal of Natural Products and Medicine, 5*(1), 12-14.
3. Arul, A. M., & Thangavel, K. (2011). Medicinal uses of *Anacardium occidentale* and its potential in treating skin disorders. *International Journal of Pharmacology, 7*(6), 759-764. doi:10.3923/ijp.2011.759.764.
4. Ezekwe, A.S., Nwadike, C.N., Wokocha, P.G. Orlando, B.G (2021) Phytochemistry and Antioxidant Activity of Irvingiagabonensis (Bush mango) Seed Sample.Asian Journal of Biochemistry, Genetics and Molecular Biology 7(4): 25-34, 2021; Article no.AJBGMB.63380 ISSN: 2582-3698
5. Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives, 109*(1), 69-75. doi:10.1289/ehp.01109s169.
6. Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry, 18*(6), 499-502.
7. Gholap, S. K., Dhananjay, N. N., Raju, G. K., & Bansal, S. (2017). A review on medicinal properties of *Anacardium occidentale*. *Asian Pacific Journal of Tropical Biomedicine, 7*(3), 293-302.
8. Grove, D. (1979). The measurement of high-density lipoprotein cholesterol in plasma. *Annals of Clinical Biochemistry, 16*(1), 34-36.
9. Khan, M. N., Ullah, M. F., Khan, M. S., & Ali, M. (2015). Herbal plants: A potential source of hypoglycemic agents. *International Journal of Pharmaceutical Sciences and Research, 6*(1), 54-64.
10. Konan, N. A., et al. (2007). Phytochemical analysis of *Anacardium occidentale* leaves: Potential health benefits. *African Journal of Pharmacy and Pharmacology, 1*(3), 037-041. doi:10.5897/AJPP2007.000-1018.
11. Kumar, A., Shukla, V., & Singh, A. K. (2013). Hyperlipidemia in diabetes mellitus. *Indian Journal of Clinical Biochemistry, 28*(4), 368-374.
12. Loukine, L., et al. (2012). Complications of diabetes mellitus: The importance of prevention. *Diabetes Care, 35*(6), 1240-1248. doi:10.2337/dc11-1495.
13. Miura, T., et al. (2002). Antidiabetic properties of plant-derived substances. *Journal of Ethnopharmacology, 81*(1), 11-16. doi:10.1016/S0378-8741(02)00240-2.
14. Moller, D. E. (2001). New drug targets for type 2 diabetes and the metabolic syndrome. *Nature, 414*(6865), 821-827. doi:10.1038/414821a.
15. Raza, H., Shakoor, S., & Khan, M. A. (2015). The role of antioxidants in diabetic liver disease. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 9*(4), 310-313.
16. Runnie, I., et al. (2004). The vasorelaxation effect of *Anacardium occidentale* leaf extract on isolated rat aorta. *Journal of Ethnopharmacology, 93*(1), 115-120. doi:10.1016/j.jep.2004.03.018.
17. Sokeng, S. D., et al. (2001). Pharmacological evaluation of *Anacardium occidentale* (cashew) for hypoglycemic activity. *Phytotherapy Research, 15*(4), 320-324. doi:10.1002/ptr.1301.
18. Vessal, M., Mohammadpour, A. A., &Vessal, M. (2003). Antidiabetic effects of *Ferulaassa-foetida* in alloxan diabetic rats. *Journal of Ethnopharmacology, 84*(1), 159-164.

19. World Health Organization (WHO). (2002). *Traditional medicine strategy 2002-2005*. Geneva: WHO. Retrieved from WHO website.
20. Wokocha, P.G., Ezekwe, A.S., Achor, M.T., Fubara, B.N. (2024). Synergistic Biochemical Effects of Combined Aqueous Extracts of Vernonia Amygdalina Leaf and IrvingiaGabonensis Seed in Alloxan Induced Diabetic Rats, International Journal of Research Publication and Reviews, Vol 5, no 9, pp 1333-1339
21. Wokocha, P.G., Ezekwe, A.S., Achor, M.T., Fubara B. N. (2024). Determining the Effects of Aqueous Seed Extract of IrvingiaGabonensis on Selected Biochemical and Parameters in Alloxan-Induced Diabetic Rats, International Journal of Research Publication and Reviews, Vol 5, no 9, pp 2657-2665.