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Original Article

Attenuating effects of *Azanza garckeana* fractions on glycemo-impaired-associated dyslipidemia, hepatopathy, and nephropathy

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Abstract: Objectives: The use of medicinal plants for diabetes treatment is increasing owing to their effectiveness and safety compared to synthetic drugs. Thus, the ameliorative effects of *Azanza garckeana* (F. Hoffm.) fractions in diabetes-induced dyslipidemia, hepatopathy, and nephropathy in rats were evaluated in this study. Methods: Rats with alloxan (120 mg/kg body weight (BW))-induced diabetes were randomized into different groups (n=5) and treated with the crude methanolic extract, and fractions (n-hexane, ethyl acetate, and aqueous fractions) of *A. garckeana* each at 100, 200, and 400 mg/kg BW. Glibenclamide (5 mg/kg BW) was used as a reference drug, and all treatments were administered orally daily for 6 weeks. Results: Our data revealed that treatment with the crude extract caused a dose-dependent hypoglycemic effect of 61.32±3.45%, 76.05±3.05%, and 78.59±5.90% at 100, 200, and 400 mg/kg BW, respectively and improved the BW of the animals. The extract also ameliorated the elevated cholesterol, triglyceride, low-density lipoprotein cholesterol, and increased serum levels of high-density lipoprotein cholesterol compared with untreated control animals. The extract also reversed serum biochemical alterations in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, total and direct bilirubin, urea, and uric acid that were observed in untreated diabetic rats. Interestingly, the *A. garckeana* fraction also exhibited significant protection against diabetes-induced dyslipidemia, hepatopathy, and nephropathy in rats, with the ethyl acetate fraction exhibiting a remarkable protective effect. The LC-MS characterisation of the active fraction identified the presence of various phenolic and flavonoid compounds that could be responsible for the bioactivity of the fraction. Conclusion: Collectively, this study suggests the potential application of *A. garckeana* for effective treatment of diabetic nephropathy, with the ethyl acetate fraction of this plant representing a reserve of potential candidates for developing new drugs.

Keywords: *Azanza garckeana*, dyslipidemia, hepatopathy, nephropathy, biochemical parameters

Introduction

Diabetes mellitus (DM) is a public health problem with a global estimate of 10.5% (536.6 million people) prevalence in 2021 and rising expectations with 12.2% (783.2 million) by 2045 [1, 2]. It is a heterogeneous disorder and multifactorial in its etiology, with genetic and environmental factors being the major risk factors that significantly contribute to its development and progression [3].

Mechanistically, DM is characterized by a complex disruption in insulin signaling, β-cell function, and regulation of energetic metabolism [4], which affects the biochemical reactions of carbohydrates, proteins and lipid, as well as other metabolisms [5, 6] in target tissues like liver, skeletal muscle, and adipose tissue [7]. Hence, diabetes is associated with multiple pathological conditions, including cardiovascular, neuropathy, renal and eye diseases [8, 9], leading to substantial socio-economic burden, low life quality, disability, and ultimately death [10, 11].

Although, several synthetic and conventional oral antidiabetic medications are commercially available [12, 13], the successes of these drugs are limited by undesirable adverse effects including gastrointestinal disturbances, hematological alterations, hypoglycemic coma, and impairment of liver and kidney function [12, 14, 15]. These have led to the increasing search for alternative therapies from natural products, particularly medicinal plants, which have served as the major source of natural medications [16, 17] for many centuries and still hold significant importance in the modern treatment of several human diseases [18-22]. The low cost, ready availability, accessibility, safety, and efficacy have placed plant-based therapies at an advantage over conventional chemotherapies [23].

Azanza garckeana (F. Hoffm.) Exell and Hillc., synonymously known as Rothm., *Shantzia garckeana* (F. Hoffm.), is commonly found in Nigeria, Botswana, Kenya, Malawi, South Africa, Mozambique, Sudan, and some other parts of Africa [24, 25]. The plant is commonly used in traditional medical systems as a herbal remedy for the treatment and management of several human diseases [25]. Previous studies identified and implicated several bioactive secondary metabolites including terpenes, alkaloids, flavonoids, saponins, ascorbic acid, carotenoids, glucosides, and phenols, in the diverse biological activities of *A. garckeana* [25, 26]. Pharmacologically, A. *garckeana* has been reported for anti-arthritic, wound healing, fecundity, antimicrobial, and reproductive potential [25, 27-29]. Our previous study also revealed that extracts from this plant contain some drug-like candidates that enhanced insulin secretion and glucose lowering effect in rats [30].

Considering the reputation of this plant in traditional medicine for the treatment of kidney disease, diabetes and its associated complications, we hereby hypothesize that the extract from this plant could exhibit protective effects against dyslipidemia, hepatopathy, and nephropathy, which are the common secondary complications associated with diabetes. Hence, this study aims to evaluate the hepatoprotective, nephroprotective, and antihyperlipidemic activities of both crude and solvent fractions *of A. garckeana pulp* extract in *an in vivo* model of experimental diabetics. Furthermore, the bioactive compounds were characterized using liquid chromatography-mass spectrometry (LC-MS).

Materials and methods

Reagents and chemicals

Organic solvents, including methanol, ethyl acetate, and n-hexane, were obtained from Sigma Chemical (St. Louis, MO, USA). All biochemical assay kits were Randox Liquizyme assay kits (Crumlin, UK).

Plant collection, extraction, and fractionation

The pulp of *A. garckeana* was collected at 10.00 am, 23 June 2021 from Tula village, Gombe State, Nigeria. Plant species authentication was conducted at the Biological Department, Federal University of Technology, Minna, Nigeria, and a voucher number was deposited. The pulp of the fruit was air-dried at 25°C for 14 days and then pulverized using a mechanical blender (Type VIII machine of Christy and Norris). A protocol of cold maceration was employed for plant extraction according to the method employed by Lawal et al. [30]. Briefly, 300 g of the pulverized sample was extracted with 1.5 L of methanol by soaking it

for 72 h at ambient temperature (37.8±2.0°C). Afterwards, the extract was filtered, concentrated in a rotary evaporator, and air-dried at ambient temperature until a constant weight was achieved. Fifty (50 g) of the *A. garckeana* methanolic extract was sequentially partitioned with n-hexane, ethyl acetate, and distilled water using a separating funnel [31]. The resulting solvent-partitioned fractions were concentrated in a rotary evaporator to give n-hexane, ethyl acetate, and aqueous fractions of *A. garckeana* with respective percentage yields of 24.2%, 16.48%, and 8.70%.

Acute toxicity and maximum tolerated dose (MTD) analysis

Azanza garckeana fractions (ethyl acetate, aqueous, and n-hexane) were subjected to MTD analysis in acute oral toxicity dosages according to Lorke's method [32]. Briefly, in the first phase, three sets of nine animals were divided into three groups of three animals and administered 10, 100, and 1000 mg/kg each of n-hexane, ethyl acetate, and aqueous fractions of *A. garckeana*. Animals were thereafter monitored for mortality and adverse effects over a period of 24 hours. In the second phase, three sets of three animals were administered higher doses of 1600, 2900, and 5000 mg/kg BW of the fractions. Animals were thereafter monitored for mortality and adverse effects over a period of 1 week.

The Lethal dose of 50 (LD_{50}) was calculated using the formula:

$$LD_{50} = \sqrt{D_0 \ X \ D_{100}}$$

D_0 = Highest mortality producing dose; D_{100} = Lowest mortality producing dose.

In vivo antidiabetic study

Ethical approval: This study was approved by the Animal Used and Research Committee of the Alex-Ekwueme Federal University Ndufu-Alike, Nigeria (No. AE-FUNAI-2020/0099), and all the procedures performed in the animal experiments were in accordance with the ethical standards of this institution.

Experimental design: Swiss albino male rats (126.98±6.90 g) were obtained from the animal facility of the Department of Biochemistry. Freshly prepared alloxan monohydrate (120 mg/kg body weight (BW)) was intraperitoneally administered to rats that were starved overnight as described by Etuk et al. [33]. After 72 hours of alloxan administration, blood was drawn from their tail veins with a test strip and inserted into the glucometer (Roche Diagnostic, Mannheim, Germany). A diabetic state was confirmed by the glucose level >200 mg/kg BW [33]. Rats were randomized into 14 different treatment groups. Groups 1-3 were treated with 100, 200, and 400 mg/kg BW methanol extract of *A. garckeana.* Groups 4-12 were treated with 100, 200, and 400 mg/kg BW of n-hexane, ethyl acetate, and aqueous fractions of *A. garckeana* respectively. Groups 13 and 14 served as diabetic control (5 ml/kg BW normal saline), and standard control (5 mg/kg BW glibenclamide (GLB)) [34, 35] respectively. All treatments were orally administered daily for 6 weeks [36] with the aid of an esophageal cannula. The blood glucose level and BW were examined weekly.

Sample collection and processing: After the last dose of treatment, animals in all groups were anesthetized using pentobarbital sodium and sacrificed via dislocation of the cervical spine, blood was collected and centrifuged. Serum was then collected as described in previous studies [37, 38]. The resulting sera were aspirated and kept frozen at -20°C until used for biochemical analysis [39]. Furthermore, the rats were quickly dissected, and the organs, including kidney and liver were isolated and weighed. The remaining liver tissue was fixed with 10% formalin for histopathological analysis.

Organ viewing and organ weight ratios: The isolated liver and kidney were weighed and carefully examined for any gross physical pathology. The organs weight ratios were computed using the standard formula shown below [40].

$$\text{Organ body weight ratio} = \frac{\text{Weight of organ}}{\text{Total body weight of the rats}}$$

Histopathological analysis: The liver and kidney tissues were prepared for microscopic evaluation of the organ histology by the conventional paraffin-embedded protocol [41]. The liver and kidney sections (0.4-0.5 μ thickness) were further stained with hematoxylin and eosin (H&E)

to visualize the overall morphology. Images were captured at ×40 using a Light Microscope equipped with digital camera software. The photomicrographs of the treatment groups were compared with the normal control and the untreated diabetic group for any histo-architectural changes. The organ tissues were ranked for lesion severity based on a semi-quantitative scoring of 0-3 depending on the degree and extent of the alteration [42].

Analysis of serum biochemical parameters of hepatopathy/nephropathy

Serum biochemical parameters were analyzed using clinical diagnostic kits following the manufacturer's protocol. The level of serum total protein was estimated based on the principle that cupric ions interact with protein peptide bonds in an alkaline medium leading to the formation of a colored complex that demonstrates maximum absorption at 546 nm [43]. The activity of alanine transaminase (ALT) was analyzed based on the principle of the catalytic action of ALT on alanine and α-oxoglutarate to form pyruvate and glutamate [44], while aspartate transaminase (AST) activity was measured by monitoring the levels of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine [45]. The serum albumin (ALB) level was estimated based on its quantitative binding to 3,3',5,5'-tetrabromo-cresol sulphonephthalein (bromocresol green, BCG) [46] to form an albumin-BCG complex with absorption at 578 nm. The level of serum bilirubin was assayed based on the reaction between diazotized sulphanilic acid and bilirubin in an alkaline medium to form a colored complex with maximal absorption at 578 nm [47]. Urea levels were estimated spectrophotometrically based on Berthelot's reaction in the presence of urease [48]. The creatinine concentration was assayed based on the formation of a colored complex when creatinine reacted with picric acid [49]. Serum lipid profiles including triacylglyceride (TGL) [50], total cholesterol [51], high-density lipoprotein (HDL)-cholesterol concentration, and low-density lipoprotein (LDL)-cholesterol [52] were analyzed using standard protocols.

Liquid chromatography mass spectrometry (LCMS) analysis of ethyl acetate fraction of Azanza garckena pulp

The Liquid chromatography mass spectrometry (LCMS) analysis of the ethyl acetate fraction of *A. garckena* pulp was carried out with the highly sensitive and ultrafast Shimadzu LC-MS - 8040 ultrafast mass spectrometry. The column consists of Analysis Column - Shim-pack FC-ODS (2.0 mmI.D. × 150 mmL., 3 μm), Mobile Phase A - 5 mmol/L ammonium acetate - Water, Mobile Phase B - 5 mmol/L ammonium acetate - Methanol, Gradient Program 15%B (0 min) - 40%B (1-3.5 min) - 50%B (6 min) - 55%B (8 min) - 95%B (17.5-30 min) - 15%B (30.01-40 min), Flow Rate - 0.2 mL/min and Column Temperature 40°C. The spectrum generated within the retention time frame of 0.00-50.00 was monitored using the Shimadzu Lab solution software for LCMS. The result obtained was exported in CDF format and used for the successive steps required for compound identification with mzmine software (version 2.53) [53].

Statistical analysis

All analyses were conducted in replicates and analyzed using Graph-pad version 8.0, and the results are presented as the mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) with post-hoc and Duncan's multiple range tests were used to compare significant differences between treatment groups. Statistical differences were considered significant at $P<0.05$.

Results

Acute oral toxicity profile of the solvent fractions of A. garckeana pulp in rats

Safety evaluation is an important overriding criterion for the selection and use of medicinal plants for therapeutic applications. *A. garckeana* is a commonly consumed medicinal plant without report of adverse effects. We hypothesize that the solvent fractions of this plant may exhibit a high safety profile. To test this hypothesis, we explored the acute toxicity model in rats and found that the n-hexane, ethyl acetate, and aqueous fractions of *A. garckeana* pulp demonstrated LD_{50} values of >5 g/kg BW, with no death recorded throughout the dosing and observation periods. In addition, the groups of animals dosed with the fractions at concentrations of 10-1600 mg/kg BW were devoid of any adverse or behavioral changes. At a higher dose of 5000 mg/kg BW of the hexane and aqueous fractions, the rats demonstrated mild

Table 1. Acute oral toxicity profiles of the solvent fractions of *Azanza garckeana* pulp in rats

Dosage (mg/kg BW)		n-Hexane	Ethyl-acetate	Aqueous
10	0/3	---	---	---
100	0/3	---	---	---
1000	0/3	---	---	---
1600	0/3	---	Restlessness	
2900	0/3	Redness of the eyes	Rubbing of the mouth on the wall of the cage; restlessness, hyperactivity	Redness of the eyes
5000	0/3	Hyperactivity, restlessness, lasting for 30 min	Hyperactivity, restlessness, hair straightening, profuse breathing lasting for 24 h	Hyperactivity, restlessness, lasting for 30 min
Mortality		0/3	0/3	0/3

BW, body weight.

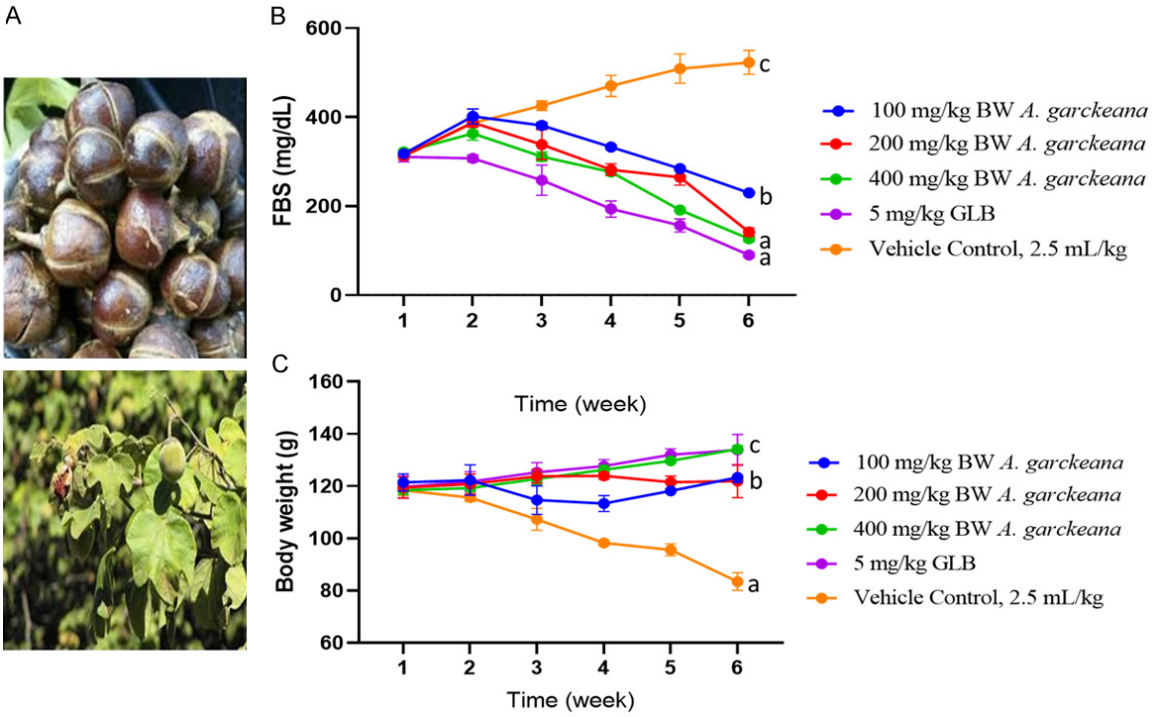

Figure 1. *Azanza garckeana* pulp extract demonstrated hypoglycemic effect in alloxan-induced diabetic rats. (A) Images of the leaf and pulp of *A. garckeana,* (B) Fasting blood sugar concentrations vs. time, and (C) Body weight changes vs. time in alloxan-induced diabetic rats treated with CMEAZ. Values are the mean ± SEM. Different superscript letters indicate a significant difference (P<0.05) between the treatment groups (n=5).

hyper activeness and restlessness, but these effects lasted for only about 30 min. Additionally, with 5000 mg/kg BW of the ethyl acetate fraction, the rats were hyperactive and restless and were breathing profusely. This persisted with decreased severity for at least 24 h (**Table 1**). Collectively, the results suggested that-hexane, ethyl acetate, and aqueous fractions of *A. garckeana* pulp exhibited a high safety profile and could be explored for oral remedy at doses of ≤1600 mg/kg BW.

Azanza garckeana pulp demonstrated a hypoglycemic effect in alloxan-induced diabetic rats

Based on the traditional use of *A. garckeana* pulp for the treatment of diabetes in traditional medicine, we hypothesize that the extract of

Table 2. Percentage hypoglycemic effect of the crude methanol extract of *Azanza garckeana* pulp (CMEAZ) in alloxan-induced diabetic rats

	Conc. (mg/kg BW)	Initial FBS	Final FBS	Hypoglycemic (%)	Weight gain (%)
Azanza garckeana	100	317.90±4.16^{a}	229.34±1.63^{c}	61.32±3.45^{a}	1.42±0.05^{a}
	200	313.03±2.48^{a}	141.98±5.46^{b}	76.05±3.05^{b}	2.08±0.15^{a}
	400	321.80±3.32^{a}	126.93±3.94^{b}	78.59±5.90^{b}	11.97±1.35^{c}
Glibenclamide	5	310.86±6.77^{a}	90.33±2.97^{a}	84.76±4.93^{c}	10.60±0.95^{c}
Control	5 mL/kg BW	311.90±4.59^{a}	592.97±18.85^{d}	-	-41.88±5.24^{a}

Data are presented as the mean ± SEM (n=5). Values with different superscript alphabets (a, b, c, d) are comparable (P>0.05) to other group values with either of the alphabets within a row. BW, body weight; Conc., concentration; FBS, fasting blood sugar.

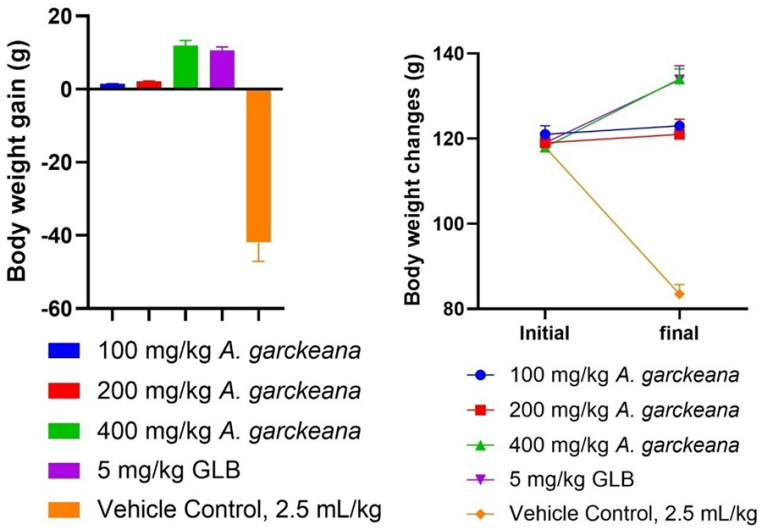

Figure 2. Weight gain of alloxan-induced diabetic rats treated with the crude methanol extract of *Azanza garckeana* pulp (CMEAZ). Values are the mean ± SEM (n=5).

this plant would exhibit hypoglycemic effects. Consequently, we evaluated it *in vivo* hypoglycemic effects in alloxan-induced diabetic rats, and our results revealed that untreated diabetic rats exhibited progressive increases in fasting blood glucose levels and BW loss (**Figure 1**). Treatment with the methanol extract of *A. garckeana* caused a dose-dependent hypoglycemic effect with respective glucose reductions of 61.32±3.45%, 76.05±3.05%, and 78.59±5.90% at 100, 200, and 400 mg/kg BW (**Table 2**). In addition, treatment with the *A. garckeana* crude extract caused a dose-dependent improvement in BW in alloxan-induced diabetic rats compared with the untreated diabetic rats (**Figure 2**). Altogether, the data generated revealed that *A. garckeana* pulp exhibited a hypoglycemic effect and could prevent body weight loss associated with diabetes conditions.

Crude methanolic extract of A. garckeana pulp reversed the serum biochemical alterations in alloxan-induced diabetic rats

Diabetes is associated with hyperlipidemia and tissue impairment. We hypothesize that diabetic untreated rats would exhibit dysregulated biochemical markers of liver and kidney dysfunction and that *A. garckeana* could offer some protective effects. Interestingly, we found that the untreated diabetic rats exhibited dyslipidemia characterized by significant (P<0.05) elevation of serum levels of cholesterol, triglycerides, and LDL-cholesterol with decreased serum levels of HDL-cholesterol (**Table 3**). Furthermore, significant decreases in serum total protein, albumin, and potassium, and increased serum activities/concentrations of AST, ALT, ALP, creatinine, total and direct bilirubin, urea, and uric acid were observed in untreated diabetic rats. Interestingly, treatment with the crude extract of *A. garckeana* pulp significantly reversed these biochemical alterations in dose-independent manner. However, no significant differences (P>0.05) were observed in serum bicarbonate or chloride concentrations in untreated diabetic rats compared to rats treated with the crude extract of *A. garckeana* pulp. In conclusion, our results support the hypothesis that *A. garckeana* extracts can protect against bio-

Table 3. Effect of the crude methanolic extract of *Azanza garckeana* pulp on biochemical parameters in alloxan-induced diabetic rats

Biochemical parameter	100 mg/kg BW *A. garckeana*	200 mg/kg BW *A. garckeana*	400 mg/kg BW *A. garckeana*	5 mg/kg BW GLB	Negative control
Cholesterol (mmol/L)	167.21±3.40^{b}	173.92±2.83^{b}	144.85±3.90^{a}	149.54±3.52^{a}	195.72±4.38^{c}
Triglycerides (mmol/L)	129.84±2.78^{c}	112.67±3.28^{b}	98.83±2.53^{a}	124.84±4.16^{c}	158.64±3.78^{d}
HDL-C (mmol/L)	57.14±2.89^{c}	67.21±2.37^{d}	50.88±2.64^{b}	59.54±2.52^{c}	31.93±2.07^{a}
LDL-C (mmol/L)	39.23±2.30^{ab}	48.41±1.90^{b}	32.19±2.75^{a}	65.22±3.57^{c}	109.34±3.80^{d}
Total protein (g/L)	28.46±1.59^{bc}	23.12±3.01^{b}	34.55±4.30^{c}	23.78±1.66^{b}	12.23±1.49^{a}
Albumin (g/L)	15.63±1.01^{b}	18.34±2.88^{bc}	23.28±2.29^{c}	16.98±0.79^{b}	7.19±0.88^{a}
ALT (U/L)	27.46±1.97^{b}	20.45±1.67^{a}	15.84±0.65^{a}	37.84±1.99^{c}	42.46±2.38^{c}
AST (U/mL)	34.57±1.84^{b}	23.84±2.49^{a}	28.45±2.74^{ab}	33.74±1.84^{b}	48.85±2.38^{c}
ALP (U/L)	89.56±3.29^{b}	73.64±4.24^{a}	62.13±4.01^{a}	86.23±3.97^{b}	113.54±3.60^{c}
Creatinine (mg/dL)	4.12±0.43^{a}	6.32±0.56^{b}	3.75±0.48^{a}	5.43±0.55^{ab}	8.79±0.74^{c}
Ind_bilirubin (mg/dL)	0.65±0.05^{b}	0.54±0.05^{b}	0.37±0.02^{a}	0.64±0.03^{b}	0.95±0.04^{c}
Total bilirubin (mg/dL)	0.98±0.06^{bc}	1.06±0.06^{c}	0.87±0.04^{ab}	0.74±0.02^{a}	1.32±0.07^{d}
Uric acid (mg/dL)	5.46±0.51^{a}	5.12±0.57^{a}	4.93±0.44^{a}	6.41±0.40^{a}	8.65±0.63^{b}
Urea (mg/dL)	45.23±2.76^{b}	40.11±3.65^{ab}	32.96±3.14^{a}	32.92±2.75^{a}	45.21±2.64^{b}
Sodium (mEq/L)	125.94±3.59^{ab}	118.56±2.79^{a}	129.55±3.54^{bc}	126.94±3.39^{ab}	139.67±2.87^{c}
Potassium (mEq/L)	12.81±0.70^{b}	16.94±1.06^{c}	10.88±1.11^{b}	10.28±0.68^{b}	6.88±0.67^{a}
Chloride (mEq/L)	60.12±3.29^{b}	49.74±2.75^{a}	56.11±2.38^{ab}	69.27±2.57^{c}	58.34±2.31^{ab}
Bicarbonate (mEq/L)	25.89±1.85^{b}	22.55±2.38^{ab}	28.32±2.75^{b}	16.93±1.08^{a}	28.45±1.71^{b}

Data are presented as the mean ± SEM (n=5). Values with different superscript letters (a, b, c, d) for a given parameter within a row are significantly different from each other (Duncan's multiple range tests, *post hoc* test at P<0.05). Values with 2 superscript alphabets (ab, bc) are comparable (P>0.05) to other group values with either of the alphabets within a row. GLB, glibenclamide; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

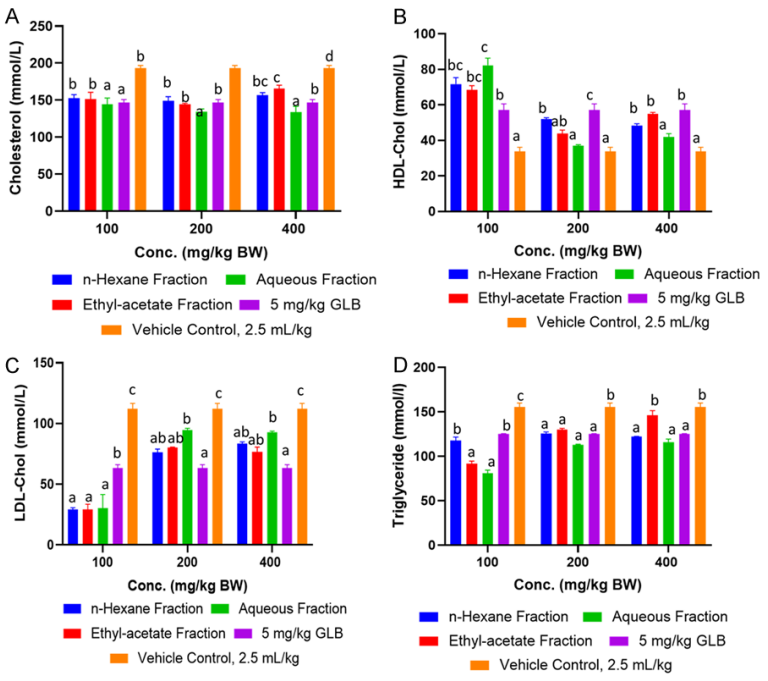

Figure 3. *Azanza garckeana* pulp fraction abrogated diabetes-induced dyslipidemia in alloxan-induced diabetic rats. Effects of n-hexane, ethyl acetate, and aqueous fractions of *A. garckeana* on the levels of (A) Serum cholesterol, (B) High-density lipoprotein (HDL)-cholesterol, (C) Low-density lipoprotein (LDL)-cholesterol, and (D) Triglycerides in alloxan-induced diabetic rats. Values are the mean ± standard error of the mean (SEM) (n=5). Different superscript letters in different histograms indicate a significant difference between groups (P<0.05).

chemical alterations in alloxan induced diabetic rats.

Azanza garckeana pulp fraction abrogated diabetes-induced dyslipidemia in alloxan-induced diabetic rats

Following the observed therapeutic effect of *Azanza garckeana* crude extract, we moved further to fractionate and evaluate the anti-hyperlipidemic potential of the fractions. We hypothesize that different fractions may elicit different levels of hypolipidemic effects in a dose-dependent or independent manner. Our results revealed significant (P<0.05) increases in serum concentrations of total cholesterol, triglycerides, and LDL-cholesterol and a decreased level of HDL-cholesterol in untreated diabetic rats compared to the other experimental groups (**Figure 3**). Interestingly, the n-hexane, ethyl acetate, and

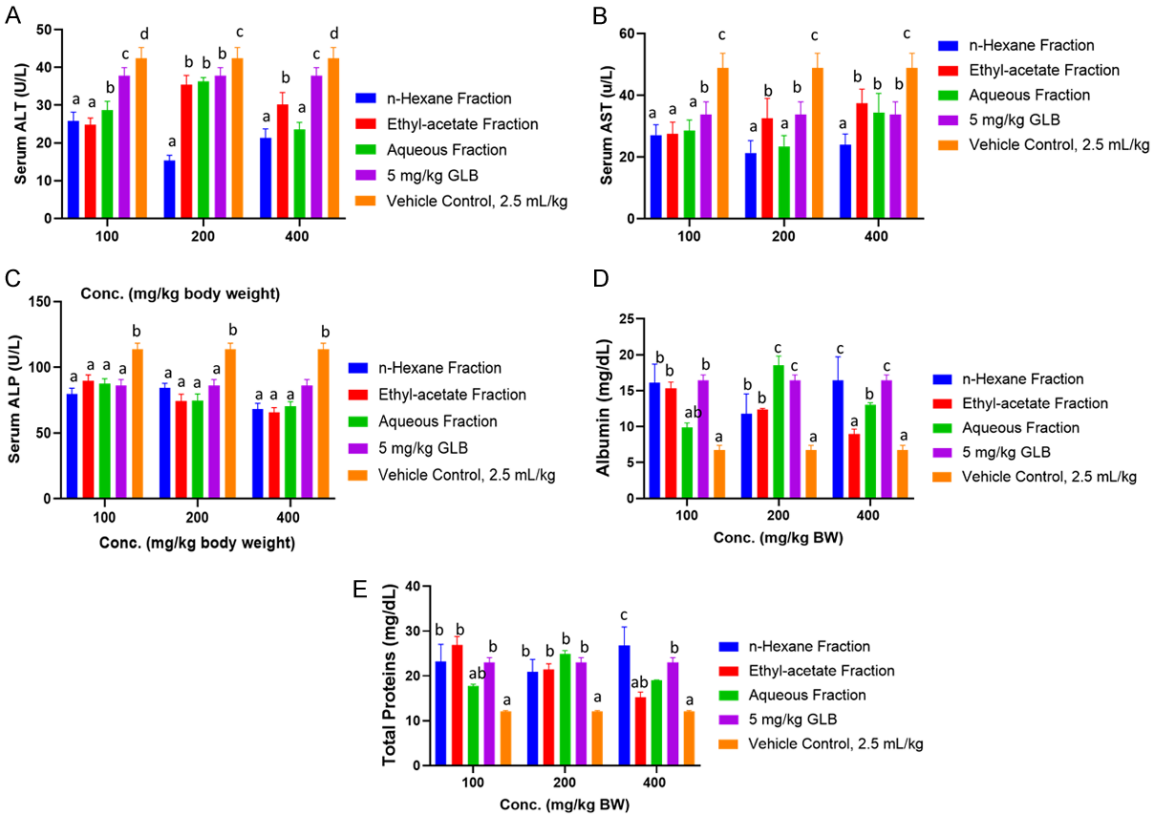

Figure 4. *Azanza garckeana* pulp fraction preserved the functional integrity of the liver in alloxan-induced diabetic rats. Effects of n-hexane, ethyl acetate, and aqueous fractions of *A. garckeana* on serum activity/levels of (A) Alanine transaminase, (B) Aspartate transaminase, (C) Alkaline phosphatase, (D) Albumin, and (E) Total proteins in alloxan-induced diabetic rats. Values are the mean ± standard error of the mean (SEM) (n=5). Different superscript letters in different histograms indicate a significant difference between groups (P<0.05).

aqueous fractions of *A. garckeana* significantly attenuated diabetes-induced dyslipidemia in doseindependent manner. The hypolipidemic effects of the n-hexane, ethyl acetate, and aqueous fractions were comparable to the activities demonstrated by the standard drug, glibenclamide (5 mg/kg BW). However, rats treated with the aqueous fraction exhibited the lowest serum cholesterol levels compared to the other treatment groups (**Figure 3**). Altogether, our results demonstrated that *A. garckeana* pulp fractions abrogated diabetes-induced dyslipidemia and thus could be useful in managing diabetic complications.

Azanza garckeana pulp fraction preserved the functional integrity of the liver in alloxan-induced diabetic rats

We subsequently evaluated the potential of *A. garckeana* fractions in restoring the functional integrity of the liver, with the aim of identifying the most active fraction. The results obtained revealed significant (P<0.05) increases in the levels of ALT (**Figure 4A**), AST (**Figure 4B**), and ALP (**Figure 4C**) in the serum of untreated diabetic rats compared to the other experimental groups. In contrast, untreated diabetic rats exhibited low serum albumin (**Figure 4D**) and total protein (**Figure 4E**) concentrations. However, rats treated with 100, 200, and 400 mg/kg BW of n-hexane, ethyl acetate, and aqueous fractions exhibited significantly attenuated levels of albumin and total protein with decreased activity of serum enzymes compared to untreated diabetic rats. Activities demonstrated by the fractions were dose-independent and were comparable to the levels of biochemical indices observed in rats treated with 5 mg/kg BW GLB. Altogether, these findings suggest that *A. garckeana* fractions could protect against diabetes-induced liver impairment.

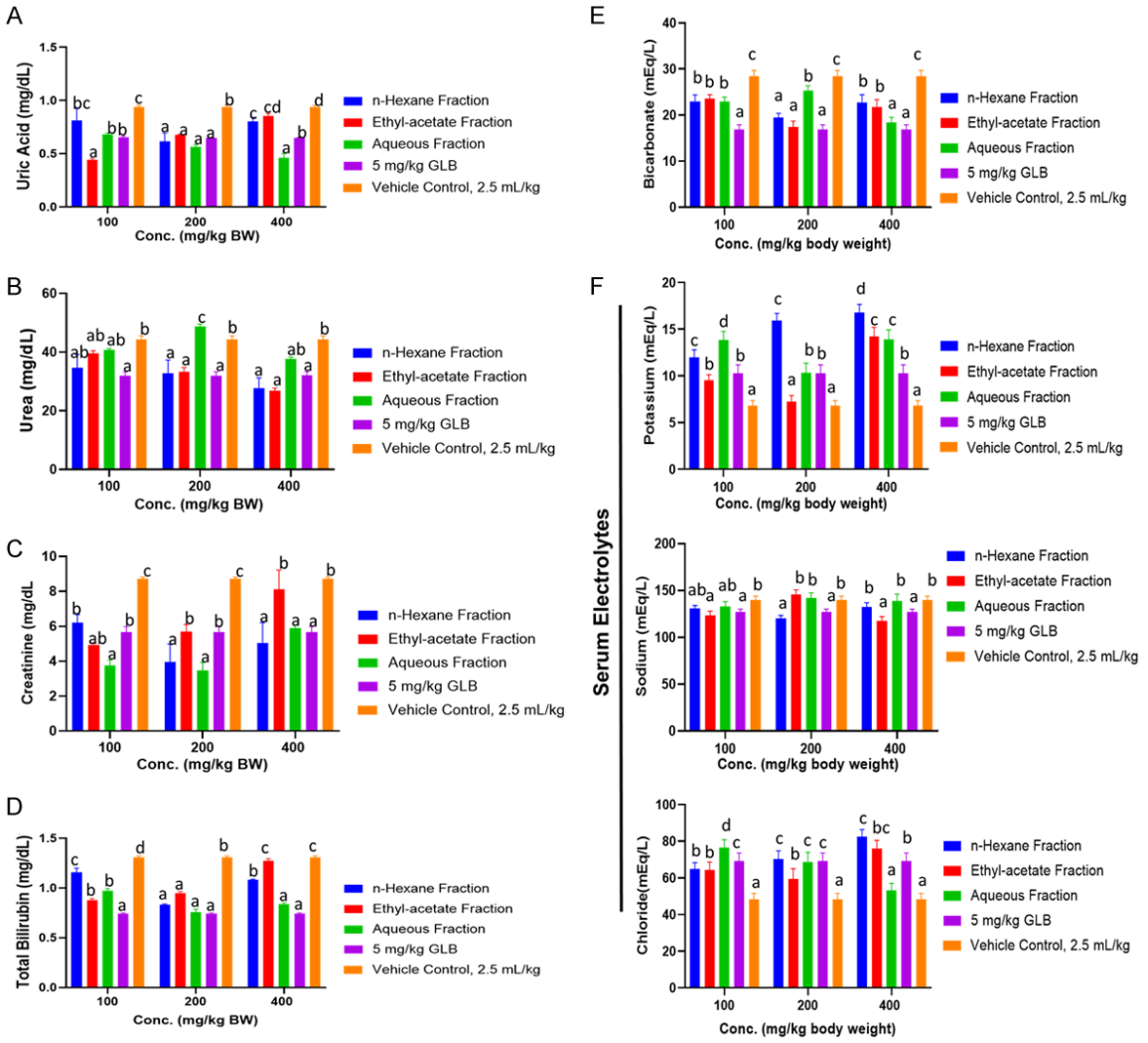

Figure 5. *Azanza garckeana* pulp fraction preserved the functional integrity of the kidneys in alloxan-induced diabetic rats. Effects of n-hexane, ethyl acetate, and aqueous fractions of *A. garckeana* on (A) Uric acid, (B) Urea, (C) Creatinine, (D) Total bilirubin, (E) Bicarbonate, and (F) Electrolyte concentrations in the serum of alloxan-induced diabetic rats. Values are the mean ± SEM (n=5). Different superscript letters in different histograms indicate a significant difference between groups (P<0.05).

Azanza garckeana pulp fraction preserved the functional integrity of the kidneys in alloxan-induced diabetic rats

Similarly, we evaluated the potential of the fractions in restoring the functional integrity of the liver. We found that the concentrations of urea, uric acid, creatinine, and bilirubin were significantly elevated in untreated diabetic rats. Similarly, there were significant (P<0.05) increases in the concentrations of serum sodium and bicarbonate, while the concentrations of chloride and potassium decreased in untreated diabetic rats (**Figure 5**). Interestingly, our results demonstrated that the diabetes-induced alterations in the levels of the biochemical indices of functional integrity were significantly (P<0.05) attenuated by treatment with n-hexane, ethyl acetate, or aqueous fractions and those treated with 5 mg/kg BW GLB. The restoration of functional indices by the fractions was dose-dependent (P<0.05) and was more pronounced with the ethyl acetate and aqueous fractions. In conclusion, ethyl acetate fraction of *Azanza garckeana* exhibited a more remarkable protective effect against diabetes-induced hepatorenal impairment and thus could be considered as a reserve template for new antidiabetic drugs.

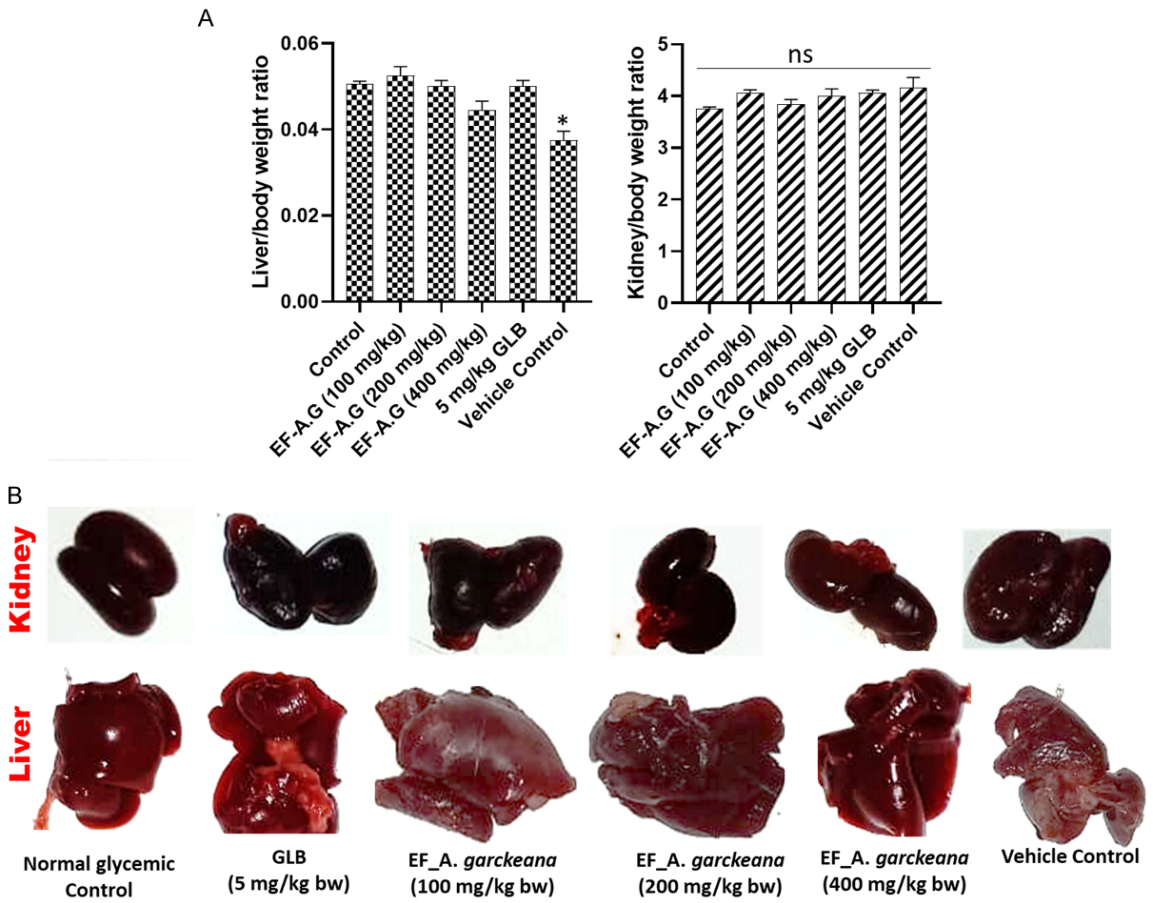

Figure 6. Pictorial view and organs weight ratio. A. Organs-body weight ratio in alloxan induced diabetic rats treated with the ethyl acetate fraction of *A. garckeana*. EF_*A. garckeana.* B. Macroscopic pictures of liver and kidney of alloxan induced diabetic rats treated with the ethyl acetate fraction of *A. garckeana*. EF_*A. garckeana*; ethyl acetate fraction of *Azanza garckeana* pulp. GLB, Glibenclamide.

Macroscopic assessment and organs weight ratio

To support our findings from biochemical analysis, post treated liver and kidney samples from animals treated with the ethyl acetate fraction were subjected to macroscopic assessment of the liver and kidney to further ascertain the fraction's restorative potential. Our results revealed that the liver-body weight ratio was significantly ($P<0.05$) lowered in the diabetic untreated rats when compared with the control and treatment groups. However, there was no observed significant ($P>0.05$) change in the liver-body weight ratio between the experimental groups (**Figure 6A**). In comparison to the normal liver topology with a regular and smooth surface, the livers in the diabetic untreated group were rough with a white fibrous appearance. Treatment with the ethyl acetate fraction of *Azanza garckeana* at 400 mg/kg BW remarkably enhanced the recovery of alloxan-induced liver structure damage (**Figure 6B**).

Azanza garckeana fraction preserved the histological architecture of the liver and kidney in alloxan-induced diabetic rats

Following the macroscopic observation, we hypothesize that the liver and kidney of diabetic rats are histological distorted, and that the ethyl acetate fraction of *A. garkeana* may protect against histological alterations. Thus, we conducted histological assessment of the tissue samples. In agreement with our hypothesis, we found necrosis of hepatocytes and enlarged hepatocyte sinusoids in the liver section of diabetic untreated rats, while the normal control rats show hepatic tissue with preserved architecture, composed of cords of normal hepatocytes, normal portal tract and central vein. There are no features of acute or chronic

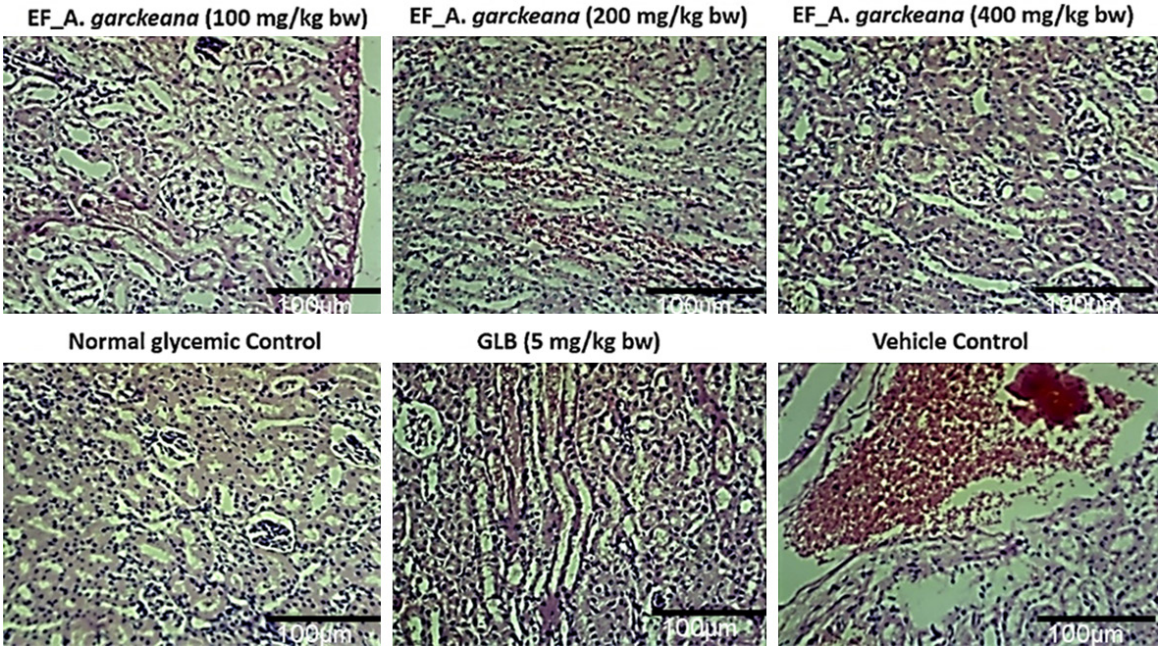

Figure 7. Representative slides of the liver histology of alloxan-induced diabetic rats treated with the ethyl acetate fraction of *Azanza garckeana.* EF_*A. garckeana*; ethyl acetate fraction of *Azanza garckeana* pulp. GLB, Glibenclamide (×40).

damage. Rats treated with 400 mg/kg BW of ethyl acetate fraction of *A. garckeana* and those treated with GLB (5 mg/kg BW) showed similar liver histology to the normal control groups (**Figure 7**). Similarly, the kidney section of normal control rats shows renal tissue with preserved architecture, composed of normal glomeruli, tubules, and interstitium. There are no features of acute or chronic damage. However, the kidney section of the diabetic untreated rats shows renal tissue with distorted glomeruli and capsular space. There was also necrosis of the nephrocytes. Interestingly, treatment with the ethyl acetate fraction of *A. garckeana* showed increased improvement in renal histology with increased treatment doses. Rats treated with 400 mg/kg BW show preserved architecture composed of normal glomeruli, tubules, and interstitium, and no features of acute or chronic damage (**Figure 8**). Collectively, results of the present study show that the ethyl acetate fraction *of Azanza garckeana* preserved the histological architecture of the liver and kidney in alloxan-induced diabetic rats.

LC-MS characterisation

We characterised the most active fraction using LC-MS analysis with the aim of identifying the bioactive metabolites that could be attributed to the remarkable activities of the ethyl acetate fraction *Azanza garckeana*. The LC-MS characterisation of this fraction generated a total of twenty-two peaks (**Figure 9**) revealing the presence of 3,4-dihydroxyl-l-phenylalanine (RT 4.62, 14.598%, m/z; 198.1), 5-heptadecylresorcinol (RT 30.567, 13.463%, m/z; 6.49), Thiamine (RT 4.567, 7.76%, m/z; 266.15), stigmasterol (RT 35.5, 6.348%, m/z; 413.3) as the major bioactive compounds in the fraction. Other compounds including nonyl flavanone, 2-Bromoacetaldehyde, ethyl Piperazine-1-Carboxylate, Mansonone N, 4-Aminophenol, 1-methylpiperidin-2-one were less abundantly present (**Table 4**).

Discussion

The rapid increase in the rate, burden, and complications of diabetes suggest the urgent need for new, effective strategies for treating and managing DM [54]. Medicinal plants are rich sources of therapeutic agents for treating several human illnesses [20, 21, 55]. Traditionally, plants have served as major sources of antidiabetic medications for many centuries and still hold significant importance in the modern treatment of diabetes [16]. The accessibility, safety, and efficacy give plant-

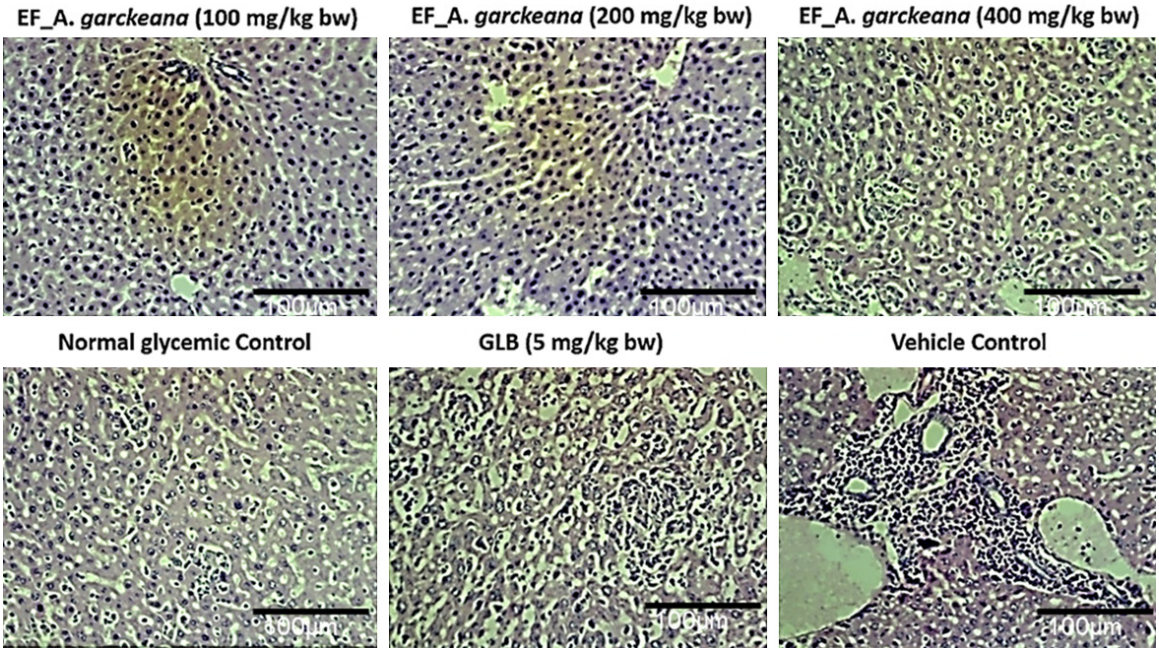

Figure 8. Representative slides of the kidney histology of alloxan-induced diabetic rats treated with the ethyl acetate fraction of *Azanza garckeana*. EF_*A. garckeana*; ethyl acetate fraction of *Azanza garckeana* pulp. GLB, Glibenclamide (×40).

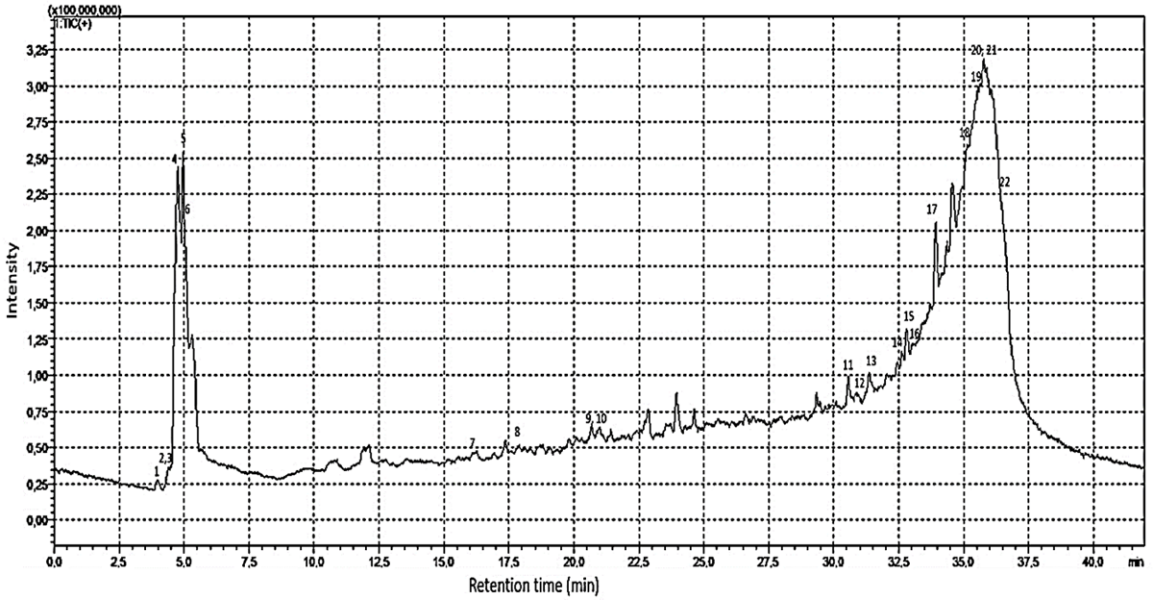

Figure 9. LCMS Chromatogram of phytoconstituents of ethyl acetate fraction of *Azanza garckeana*.

based therapy advantages over conventional chemotherapy [23, 56, 57]. Herein, we demonstrated the ameliorative effects of different fractions of *A. garckeana* pulp on diabetes-induced alterations of serum biochemical parameters.

Results obtained from the acute toxicity study showed that the crude extract and ethyl acetate and n-hexane fractions of *A. garckeana* pulp demonstrated a high degree of safety since the animals tolerated up to 5000 mg/kg BW of the extracts with no mortality recorded.

Table 4. LCMS-based identification of compounds from the ethyl acetate fraction of *Azanza garckeana* pulp

Peak#	Ret. Time	Area	Area %	Height	Height %	A/H	$[M + H]^+$ m/z	MS/MS fragment ions	Name	Molecular formula
1	4.25	5072606.5	0.50411	1036005	0.75618	4.89631	122.95	65, 122	2-Bromoacetaldehyde	C_2H_3BrO
2	4.567	41916256	4.16559	10638061	7.76472	3.94022	266.15	118, 180, 198, 235, 266	Thiamine	$C_{12}H_{17}N_4OS$
3	4.62	245600055	24.4075	20000000	14.598	12.28	198.1	65, 118, 163, 180, 198, 266, 295	3,4-Dihydroxy-L-phenylalanine	$C_9H_{11}NO_4$
4	4.75	22758762	2.26174	2748482	2.00612	8.28048	159.1	45, 69, 85, 118	Ethyl Piperazine-1-Carboxylate	$C_7H_{14}N_2O_2$
5	4.95	23782395	2.36347	6439785	4.7004	3.69304	279.2	126, 198, 235, 278	Mansonone N	$C_{16}H_{22}O_4$
6	5.017	16065698	1.59659	4445506	3.24478	3.61392	110.1	65, 109	4-Aminophenol	C_6H_7NO
7	16.083	11136487	1.10673	1231053	0.89855	9.04631	114.1	74, 114	1-methylpiperidin-2-one	$C_6H_{11}NO$
8	17.833	15150952	1.50569	2181538	1.59231	6.94508	194.1	74, 91, 194	(3-Arylcarbonyl)-alanine	$C_{10}H_{11}NO_3$
9	20.717	14528468	1.44382	2873622	2.09746	5.0558	415.15	74, 95, 345, 415	BETA-SITOSTEROL	$C_{29}H_{50}O$
10	20.983	13505380	1.34215	2773664	2.0245	4.86915	415.15	345, 415	Stigmastanone	$C_{29}H_{50}O$
11	30.567	119810017	11.9066	18449470	13.4663	6.49395	349.35	74, 88, 349	5-Heptadecylresorcinol	$C_{23}H_{40}O_2$
12	31.35	25234293	2.50776	4295539	3.13531	5.87453	351.4	351	Nonyl Flavanone	$C_{24}H_{30}O_2$
13	32.633	34945019	3.4728	4262203	3.11098	8.19882	313.4	65, 88, 102, 164	Dodecyl octanoate	$C_{20}H_{40}O_2$
14	32.8	18742623	1.86262	2953660	2.15588	6.34556	338.3	96, 164, 235, 337	Lobeline	$C_{22}H_{27}NO_2$
15	32.95	9898871.5	0.98374	1967565	1.43613	5.03103	397.4	164, 312, 397	Hexacosanoic acid	$C_{26}H_{52}O_2$
16	33.117	7136272	0.7092	1173069	0.85622	6.08342	365.25	164, 192, 249, 279, 337, 381	Tetrahydrocortisone	$C_{21}H_{32}O_5$
17	33.95	43609292	4.33384	6206293	4.52997	7.02662	280.2	74, 205, 237, 279	4-hydroxy-3-methoxy-N-octylbenzamide	$C_{16}H_{25}NO_3$
18	35.283	4596901	0.45684	982199	0.71691	4.68021	310.25	164, 290, 310	Catechin hydrate	$C_{15}H_{16}O_7$
19	35.5	113895493	11.3188	8698242	6.34885	13.0941	413.3	74, 83, 164, 239, 325, 413	Stigmasterol	$C_{29}H_{48}O$
20	35.767	34526121	3.43117	5892243	4.30075	5.85959	391.299	164, 391	3alpha,12alpha-Dihydroxy-5beta-Chol-8--En-24-Oic Acid	$C_{24}H_{38}O_4$
21	35.9	5001915	0.49708	825402	0.60246	6.05997	531	531	Lupeol benzoate	$C_{37}H_{54}O_2$
22	36.65	14736443	1.46449	1559039	1.13794	9.45226	385.35	164, 275, 341, 384	Cholest-4-en-3-one	$C_{27}H_{44}O$

According to Hodge and Sterner [58], substances that demonstrate an LD_{50} of 5000 mg/kg BW in rats should be tagged as harmless substances. Thus, the high safety of this plant upon oral exposure justifies the widespread use of this plant for treating various ailments by traditional healers in northern Nigeria.

Interestingly, the dose-dependent hypoglycemic effect of the extract is in line with the study by Yusuf *et al*. [25] which reported that several pharmacological activities of *A. garckeana,* including anti-inflammatory, antimicrobial, antioxidant, anti-arthritic, and analgesic actions, were dose-dependent. The improvement in the BW of diabetic rats following treatment with the extracts suggested effective glucose utilization and ameliorative effects of the plant extract on diabetes-induced catabolism of fats and proteins, consequently leading to sparing of muscle reduction [59].

Biochemical parameters are associated with the health status and are of strong diagnostic relevance in clinical evaluations of the state of health [60]. The transaminases, ALT and AST, are widely used to assess liver impairment [61]. These transaminases are produced in the liver and released in regulated amounts into the serum, with higher levels of these transaminases thus indicating liver impairment [61, 62]. Consequently, the significantly high levels of these enzymes in the serum of untreated diabetic rats were an indication of compromised liver integrity [63]. These alterations will affect the metabolism of amino acids and consequently impair ATP production. ALP activity, on the other hand, is related to the integrity of the endoplasmic reticulum and hepatocytes [64]; thus, the significant increase ($P<0.05$) in ALP activity in the serum of untreated diabetic rats suggested compromised plasma membrane and endoplasmic reticulum.

Measurements of total protein, bilirubin, and albumin may reflect the metabolic status and may be used to diagnose and evaluate the extent of kidney and liver impairment and many other pathological conditions [65]. Results of the present study revealed that untreated diabetic rats demonstrated alterations in urea, uric acid, creatinine, and bilirubin. The decreased serum albumin and total proteins in diabetic rats could be attributed to increased protein catabolism; this would compromise the hydration status of rats, which is detrimental to cellular homeostasis and subsequently affect the health of the animals [66]. Studies showed that albumin and bilirubin concentrations are altered during metabolic disorders and liver impairment [67]. Thus, the significant alterations in the levels of albumin and total protein indicate liver dysfunction and provide logistic support for liver damage commonly observed in patients with untreated DM.

The higher restoration of functional indices by the ethyl acetate fraction when compared with the n-hexane fraction could be attributed to the differences in the polarity of the solvents. It has been reported that the phytochemical and biological activity of plant extract differs with the solvent fraction used in the extraction process [68].

There are a number of reports on nephrotic impairment associated with experimental and clinical diabetes [69, 70]. With kidney impairment, the elimination of urea and creatinine is altered, which results in their accumulation in the blood and consequently in several pathological conditions that affect the health of the animal [71]. Consequently, untreated diabetic rats demonstrated marked nephrotic impairment as revealed by elevated serum urea, uric acid, creatinine, and bilirubin concentrations. Since urea is a byproduct of protein metabolism, the significant increase in urea concentration in untreated diabetic rats could be attributed to the increased protein catabolism that was observed in diabetic rats.

Although diabetes is well known to be associated with organ assault, particularly the liver and kidneys, accumulating evidence indicated that medicinal plants with hypoglycemic effects may offer protection against diabetes-induced secondary complications [72]. Fortunately, our results revealed that the crude extract as well as the n-hexane, ethyl acetate, and aqueous fractions of *A. garckeana* exhibited significant protection against diabetes-induced dyslipidemia, hepatopathy, and nephropathy, with the ethyl-acetate fraction exhibiting remarkable protective effects.

Diuresis is a common feature associated with diabetes, which may be the reason for the structural changes observed with glomerulus [73]. Coherently with the results of biochemical

parameters, our histological analysis also revealed that treatment of alloxan induced diabetic rats with the ethyl acetate fraction of *A. garckeana* significantly attenuated the liver and kidney histological abnormalities observed in the diabetic untreated rats. These histopathological findings strongly suggested the cytoprotective and hepatoprotective effects of the extract in the amelioration of liver and kidney injury in diabetic rats.

The LCMS characterization revealed the presence of phytoconstituents of pharmacological effect which contributed to the observed antidiabetic and associated complications activity of the fraction. Reports have shown that stigmasterol enhances GLUT 4 translocation, thiamine normalizes cholesterol and triacylglycerol levels, while nonyl flavanones and other flavonoids generally regulate glucose metabolism and improve liver enzyme activity in diabetes complications. Based on the study, our results revealed that the ethyl-acetate fraction of *A. garckeana* exhibited the most remarkable protection against diabetes-induced dyslipidemia, hepatopathy, and nephropathy due to the bioactive constituents identified.

Conclusions

Our research findings provide preclinical evidence of the ameliorative effects of *Azanza garckeana* fractions in diabetes-induced dyslipidemia, hepatopathy, and nephropathy in rats. Results of the present study therefore strongly suggest the potential application of *A. garckeana* for the effective treatment of diabetes, and the ethyl acetate fraction of this plant represents a reserve of potential candidates for developing new drugs. Further studies to establish the mechanism of the reaction are suggested.

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Disclosure of conflict of interest

None.

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