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Hypoglycaemic, anti-hyperlipidemic, and anti-inflammatory activities of *Asparagus africanus* (Asparaceae) extract on high-fat diet/streptozotocin-induced diabetes in Wistar rats

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ABSTRACT

Objective: To investigate the toxicity and effect of the extract on some complications of diabetes in Wistar rats.

Methods: Type 2 diabetes was induced by a combination of a high-fat diet and streptozotocin (35 mg/kg, *i.p.*). Aqueous extract of *Asparagus africanus* (EAA) was prepared and administered (*p.o.*) for 28 d to groups of diabetic rats as well as to groups of normal rats for toxicity. Fasting blood glucose levels, inflammatory cytokines, and lipid profiles were assessed in diabetic rats. Body and organ weight as well as liver and kidney functions were examined to assess the sub-acute toxicity.

Results: EAA for 28 d did not affect the body weight, the weight of the liver, kidney, and heart as well as the serum level of aspartate aminotransferase, alanine aminotransferase, urea, uric acid and creatinine in normal rats. In diabetic rats, the administration of EAA significantly lowered hyperglycemia, reduced interleukin (IL)-6, IL-1β, and tumour necrosis factor-α levels, and increased the level of IL-10. EAA also lowered cholesterol, triglyceride, and low-density lipoprotein cholesterol levels and augmented high-density lipoprotein cholesterol in the serum. As a result of the anti-lipidemic effect, EAA reduced the atherogenic index, Castelli indices, and atherogenic coefficient in diabetic rats. EAA showed the presence of flavonoids, alkaloids, tannins, saponins, terpenes, and steroids.

Conclusions: The findings of this study demonstrated that EAA is safe. It has the potential to reduce the glucose level and the risk of inflammation and atherogenesis in diabetic patients.

KEYWORDS: Type 2 diabetes; Hypoglycaemia; Antihyperlipidemia; Antiinflammation; *Aspasragus africanus*; Aqueous extract; Cardiovascular indices; Phytochemicals

1. Introduction

Diabetes is an endocrine disease due to insufficient production or deficiency in insulin from the pancreas and is characterized by damage to several body systems, particularly blood vessels, eyes, kidneys, heart, and nerves[1-3]. Type Ⅱ diabetes being the most occurring form is characterized by peripheral insulin resistance

Significance

Diabetes is a global disease of significant public health implications and current medications or synthetic drugs are insufficient due to their adverse effects and their inaccessibility for many patients in resource-limited settings. *Asparagus africanus* extract was therefore investigated as an alternative medication for diabetes management. The findings support the traditional use of the plant to reduce the risk of inflammation and cardiovascular disorders in diabetes patients. The aqueous extract is also found safe.

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and impaired insulin secretion in the liver, adipose tissue, and muscles which causes the elevation in levels of free fatty acids and proinflammatory cytokines in plasma[4–7]. It accounts for more than 85% of diabetes[4] with a diabetic global death rate of 1.6 million in 2015 and 4 million in 2017[5]. Several studies suggest that diabetes is accompanied by oxidative stress[8,9] production of pro-inflammatory cytokines[3] and high lipidemia that promotes cardiovascular complications, peripheral vascular diseases, and inflammation[7]. The current conventional treatment of diabetes is essentially based on the administration of oral anti-diabetics like metformin[10] or the injection of insulin[2] which do not meet the needs of patients. Moreover, these therapies could cause serious side effects[11] and are only accessible to some category of the population. For this reason, most of the populations in developing countries use traditional medicine.

Asparagus africanus (*A. africanus*) (Asparaceae) is a small spiny deciduous tree, native to tropical America. It is traditionally used to treat fever, malaria, rheumatism, and hyperglycemia. A survey of the genus *Asparagus* revealed the presence of variable classes of compounds such as flavones, flavanones, flavone glycoside, alkaloids, sterols, tannins, terpenoids, glycerols, carotenoids, saponins, carbohydrates, free amino, and fatty acids[12,13]. However, there are no known proven scientific studies for the effectiveness of aqueous extract (aerial part) of *A. africanus* in control of hyperglycemia and hyperlipidemia, and modulation of immune response in diabetes to prevent disease complications. The present study was directed to assess the hypoglycemic, lipid-lowering, and anti-inflammatory response of aqueous extract of *A. africanus* in T2D Wistar rats.

2. Materials and methods

2.1. Plant material and extraction

A sample of the aerial part of the plant, *A. africanus* was harvested from Doukoula Kharhay in the Far North region of Cameroon in July 2022. A specimen of the plant was taken to the National Herbarium and authenticated in comparison with the material collected by Seville 277 (No: 14443 SRFCam). To prepare the extract, samples of the plant were collected and dried using an oven at 40℃. Dried plant was ground to powder using a blender. The aqueous extract was prepared by putting the fine powder in hot water (90℃) for 6 h, after which it was filtered using the Whatman 1 filter paper. The filtrate was then collected and dried at 40℃ in the oven. The dried matter, which constituted the extract, was collected and kept at 4℃.

2.2. Maintenance of animals and ethical statement

Adult albino rats (Wistar strain) 3 months old with an average weight of 250 g were used for this research. Rats were acquired from LANAVET (The National Veterinary Laboratory) of Garoua, Cameroon and they were housed in cages under conditions of normal room temperature and 12∶12-hour cycles of light and darkness. They had free access to a standard rat pellet diet and tap water *ad libitum*. All animals were acclimatized to the working environment for at least 1 week before the beginning of the experiment. All animal experiments, procedures, and techniques used in this study were conducted in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals. The study was approved by the Departmental Scientific Committee, Department of Biochemistry, the University of Bamenda (Approval No:003/22/DSC/BCH).

2.3. Sub–acute toxicity studies

2.3.1. Administration of extract

Animals were randomly allocated to 3 groups of 6 animals each for both male and female. The normal control group animals received normal saline and the other two groups received 500 mg/kg and 1000 mg/kg of the extract dissolved in saline solution. The administration of the extract was done daily by gavaging for 28 d. On day 29, animals were sacrificed after injection of diazepam and ketamine (50 mg/kg and 10 mg/kg, respectively), and organs namely: the liver, kidney, and heart were collected and weighed using an electronic balance.

2.3.2. Body and organ weights

Measurement of body weight was done weekly during the experimentation period using a weighing balance. The weights of the kidneys, liver, and heart of the rats were weighed using the balance, and the relative organ weight was also calculated.

Weight gain (%) = [(Body weight on specific week − Initial body weight)/Initial body weight] ×100

Relative organ weight = (Organ weight/Body weight) × 100

2.3.3. Plasma parameters

After sacrifice, blood samples were taken by cardiac puncture to measure plasma biochemical parameters. The following parameters: creatinine, urea, uric acid, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were evaluated.

To estimate the creatinine levels, 100 µL of the plasma sample was added to 1000 µL of alkaline picrate (SGM ITALIA: Via Olivadi, 2000126 Roma) in a test tube and mixed in a water bath for 30 s at 37℃. The absorbance was read at 510 nm immediately (OD1) and after 2 min (OD2). Alongside, 100 µL of distilled water was mixed in place of plasma sample for the blank and 100 µL of standard solution was used for the standard.

Creatinine concentration = $[(OD2 - OD1)/(OD2_{standard} - OD1_{standard})]$ ×Standard concentration

To determine the plasma urea concentration, the commercial kit

(SGM ITALIA: Via Olivadi, 2000126 Roma) was used. In this regard, 10 μL of plasma or standard was mixed with 1 000 μL of solutions R1 and R2 and incubated in a water bath at 37℃ for 30 s, the absorbance was read at 340 nm (OD1 and $OD1_{Standard}$ for sample and standard respectively) and after 2 min (OD2 and $OD2_{Standard}$ for sample and standard respectively). The concentration of urea was calculated as follows:

Urea concentration = $[(OD2 - OD1)/(OD2_{Standard} - OD1_{Standard})]$ ×Standard concentration

The uric acid concentration was analyzed using the Cypress diagnostic kit. In this concern, 25 μL of plasma and standard were mixed with 1 mL of working reagent in different cuvettes and kept at room temperature for 10 min. The absorbance was read against blank at 510 nm. The concentration was calculated as follows:

Uric acid = $[(OD_{sample} - OD_{blank})/(OD_{standard} - OD_{blank})] \times 6 \times$ Standard concentration

To determine activities of AST and ALT, 0.2 mL of plasma was mixed with 2 mL of solutions R1 and R2 (SGM ITALIA: Via Olivadi, 2000126 Roma) at 37℃. The absorbance read against reagent blank after 1 min and 2 min using a photometric spectrometer at 340 nm. The activity of the AST and ALT was calculated as follows:

AST/ALT = 1 746 × ΔAbs 340 nm/min.

2.4. Assessment of the effect on diabetic rats

2.4.1. Diabetes induction

Type 2 diabetes was induced using a high-fat diet associated with streptozotocin (STZ) (Sigma-Aldrich Inc., St. Louis, MO, USA). STZ was administered at a low dose of 35 mg/kg, *i.p.*, after animals were fed for 8 weeks with a high-fat diet. The rats were allowed to drink 5% fructose solution overnight to overcome drug-induced hypoglycemia. Using a glucose meter (ACCU-ANSWER), fasting blood glucose (FBG) was monitored 2 days after injection of STZ to confirm induction of diabetes and 9 days after to confirm the stability of diabetic condition. Rats with FBG above 200 mg/dL were considered diabetic[14,15] and were selected for further study.

2.4.2. Treatment and measurement of blood glucose

Upon the induction of diabetes, selected animals were randomly allocated to groups of 6 animals each. The normal control group made up of non-diabetic animals received normal saline at 2 mL/kg; the negative control made up of diabetic animals was given 2 mL/kg of saline solution (vehicle); the positive control made of diabetic rats received metformin (200 mg/kg) in saline solution; three test groups made up of diabetic animals received extract dissolved in saline solution (250 mg/kg, 500 mg/kg and 1 000 mg/kg). The treatment was done daily by gavage for 4 weeks[15] during which blood glucose was determined weekly using a glucose meter (ACCU-ANSWER) following the manufacturer's guide.

2.4.3. Analysis of serum parameters

In this study, after 4 weeks of treatment animals were anesthetized by injection of diazepam and ketamine (50 mg/kg and 10 mg/kg, respectively). Blood samples were taken by cardiac puncture into EDTA tubes (anticoagulant). Afterward, serum was prepared by centrifuging blood samples at 1 800 *g* for 10 min at room temperature. Serum samples were stored at −20℃ until analysis for cytokines and lipid parameters.

We measured concentration of tumour necrosis factor (TNF)-α, interleukin (IL)-10, IL-1β, and IL-6 using ELISA reagents (Quantiquine®; France). The assay was carried out following manufacturer's guide. For each cytokine, a microplate (NUNC) was coated overnight with the specific antibody. The following day, 10 μL of samples or control were added to the well, sealed, and incubated for 2 h at room temperature. Plates were washed by filling each well with 400 μL of wash buffer and decanted by inverting the plates and blotting them against clean paper towels. Thereafter, 200 μL of peroxidase-conjugated antibody (detection antibody) was added and incubated at room temperature for 2 h. Microplates were washed again as previously described and 200 μL of substrate (chromogen-substrate solution of *O*-phenylenediamine dihydrochloride) was added to each well and incubated at room temperature for 20 min. The reaction was stopped by adding 50 μL of stop solution and the optical density was read at 450 nm after 30 min.

Triglyceride (TG) was quantified using the TG kit following the manufacturer's guide (Cypress diagnostics kits). For the analysis, 1 mL of reagent was put into test tubes followed by 10 μL of sample and standard in their respective test tubes. The mixtures were then incubated for 10 min at 37℃ in a water bath. Absorbance was then measured against the blank and recorded. The concentration of TG was determined as follows:

TG (mg/dL) = $[(A_{sample} - A_{blank})/(A_{standard} - A_{blank})] \times 200 \times$ Standard concentration

The total cholesterol (TC) was determined using the commercial reagent (Cypress Diagnostics). As described by the manufacturer, 1 mL of the reagent was put into test tubes followed by 10 μL of sample and standard in their respective test tubes. The mixtures were then incubated for 10 min at 37℃ in a water bath. Absorbance was then measured against the blank and recorded and concentration was calculated as follows:

TC (mg/dL) = $[(A_{sample} - A_{blank})/(A_{standard} - A_{blank})] \times$ Standard concentration

The quantity of low-density lipoprotein cholesterol (LDL-c) was estimated from that of cholesterol using the formula:

LDL-c (mg/dL) = 0.545 ×TC concentration[16]

High-density lipoprotein cholesterol (HDL-c) was quantified using

the commercial reagent (Cypress Diagnostics). Serum (10 µL) was mixed with 750 µL of R1 (detergent). The mixture was stirred and incubated for 5 min at 37℃ in a water bath and the absorbance (Abs 1) was read at 600 nm against the blank for both samples and calibrator. Then, R2 (Enzyme) was added, mixed, and incubated at 37℃ for 5 min in a water bath. The absorbance (Abs 2) was read against the blank.

HDL-c (mg/dL) = $[(\Delta Ab_{sample} - \Delta blank)/(\Delta Ab_{calibrator} - \Delta blank)] \times$ Calibrator concentration

ΔAbs represents the difference between Abs1 and Abs2 (Abs 2 – Abs 1).

Various cardiovascular indices were determined using the lipid parameters. Atherogenic index (AI) was calculated as follows: AI = Log (TG/HDL-c). Castelli risk index (CRI) was calculated using the formula CRI = TC/HDL-c, and atherogenic coefficient (AC) was also calculated using the formula: AC = TC-HDL-c/HDL-c[17].

2.5. Qualitative screening of phytochemicals

The phytochemical constituents of the aqueous extract of *A. africanus* were screened using standard phytochemical reagents. This test was a qualitative analysis based on precipitation and/or color reactions. The tests assessed the presence of tannins, saponins, flavonoids, alkaloids, glucosides, anthraquinones, terpenoids, carbohydrates, steroids, proteins, coumarins, and reducing sugar[2,18–20]. The presence of alkaloids was tested using Mayer's reagent (Potasiomercuric iodide solution). Steroids were determined using Salkowski Test and Libermann-Burchard reagent. For terpenoids, the Salkowski test was used. The presence of flavonoids was proved using the Lead acetate test and alkaline test. For saponins, foam test and hemolytic test were carried out. Tannins and phenol compounds were tested using the ferric chloride test and lead acetate test. Molisch reagent test was used for carbohydrates, while Keller-Killani test was used for cardiac glycosides.

2.6. Statistical analysis

Data obtained from the experiments are expressed as mean ± standard deviation (SD). For statistical analysis, data were subjected to one-way analysis of variance (ANOVA) followed by *post hoc* test. A level of $P<0.05$ was taken as significant. Graphpad Prism version 8.0 was used for these statistical analyses.

3. Results

3.1. Sub–acute toxic effect of aqueous extract of A. africanus

3.1.1. Effect of aqueous extract of A. africanus on the relative weight

Results showed that animals gained weight in all groups (Figure 1). There was no significant difference in weekly weight gained between the groups ($P>0.05$). Independent of sex, animals taking the extract at 1 000 mg/kg showed a significant weight gain ($P<0.05$) in the 4th week compared to the weight gain in the 3rd week.

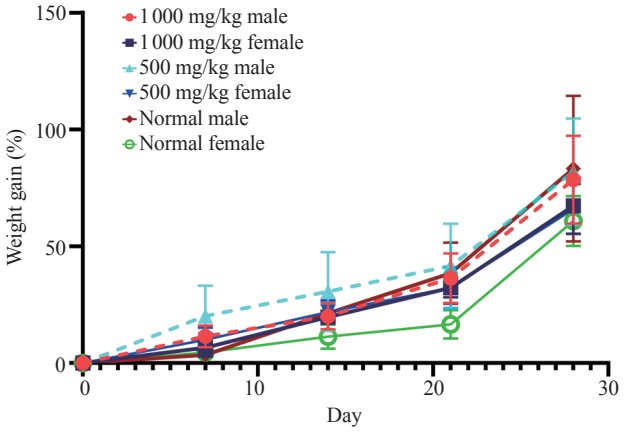


Figure 1. Weekly body weight gain (%) following treatment with *Asparagus africanus* extract.

Table 1. Liver, kidney, and heart relative weight and serum level of ALT, AST, urea, creatinine, and uric acid of the rats after 28 days of treatment with extract of *Asparagus africanus* (mean±SD, n= 6).

Parameters	Male			Female		
	Normal	500 mg/kg	1 000 mg/kg	Normal	500 mg/kg	1000 mg/kg
Relative organs weight						
Heart	0.48±0.07	0.46±0.04	0.45±0.03	0.45±0.05	0.46±0.01	0.45±0.04
Liver	4.19±0.37	4.31±0.35	4.20±0.16	3.80±0.34	4.31±0.27	4.21±0.23
Kidney	0.71±0.03	0.66±0.06	0.60±0.15	0.72±0.10	0.71±0.03	0.71±0.09
Biochemical parameters						
ALT (IU/L)	5.21±8.44	36.97±4.62	28.37±7.51	35.53±3.74	30.69±7.42	32.82±4.73
AST (IU/L)	11.31±5.63	15.76±3.18	9.30±4.39	17.53±8.56	13.61±5.21	10.29±4.34
Urea (mg/dL)	24.82±2.59	23.88±4.37	26.75±5.62	27.53±4.10	28.26±2.49	22.14±4.06
Creatinine (mg/dL)	0.73±0.16	0.67±0.15	0.75±0.35	0.72±0.10	0.77±0.35	0.69±0.37
Uric acid (mg/dL)	3.19±0.38	3.11±0.21	3.59±0.31	3.66±0.27	3.63±0.21	3.52±0.54

AST: aspartate aminotransferase, ALT: alanine aminotransferase.

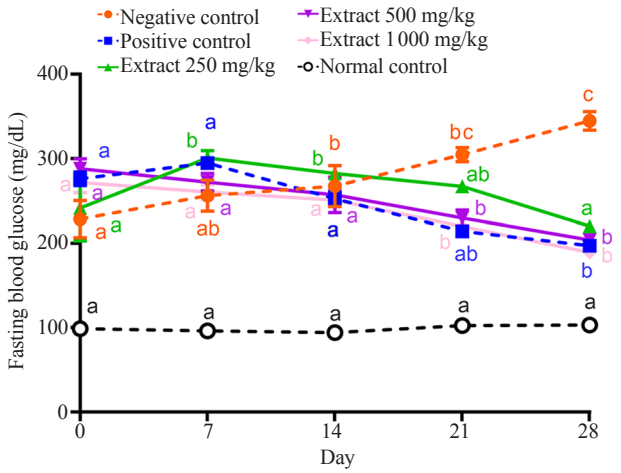


Figure 2. Weekly fasting blood glucose during treatment with *Asparagus africanus* aqueous extract and metformin in streptozotocin-induced diabetic rats. Same letters indicate insignificant differences. The normal control group: non-diabetic animals received normal saline at 2 mL/kg; the negative control: diabetic animals was given 2 mL/kg of saline solution; the positive control: diabetic rats received metformin (200 mg/kg) in saline.

3.1.2. Effect of aqueous extract of A. africanus on liver, heart, and kidney weight and serum level of some biomarkers of liver and kidney function

Results indicated no significant difference in the relative kidney, liver, and heart weight of the rats receiving extract at doses of 1 000 mg/kg and 500 mg/kg compared to those in the normal group for both males and females (P>0.05). Also, the results did not show any significant difference in the serum levels of ALT, AST, creatinine, uric acid, or urea in all animals receiving extract compared to those in the normal group (P>0.05) (Table 1).

3.2. Effect of A. africanus extract in diabetic rats

3.2.1. Hypoglycaemic effect of A. africanus extract in diabetic rats

The results revealed that the blood glucose levels of the three doses of extract and metformin treatment groups were significantly lower than those of the negative diabetic group at day 21 (P<0.05) and day 28 (P<0.05) after treatment (Figure 2).

3.2.2. Effect of aqueous extract of A. africanus on inflammatory cytokines in diabetes rats

Table 2 shows that the aqueous extract of *A. africanus* significantly reduced TNF-α, IL-1β, and IL-6 levels, and increased IL-10 levels in a dose-dependent manner with the maximum effect seen at 1 000 mg/kg (P<0.05). The effect of *A. africanus* aqueous extract on IL-6 and IL-10 levels was more significant than metformin.

3.2.3. Effect of aqueous extract of A. africanus on lipid profile in diabetic rats

Results showed that *A. africanus* aqueous extract significantly reduced TG, TC, and LDL-c levels while increasing HDL-c level with the maximum effect seen at 1 000 mg/kg (P<0.05). The effect of *A. africanus* aqueous extract was more significant than metformin (Table 3).

Table 2. Inflammatory cytokines of rats treated with EAA and metformin (mean±SD, pg/mL, n= 6).

Parameters	Normal control	Negative control	Positive control	EAA 250 mg/kg	EAA 500 mg/kg	EAA 1 000 mg/kg
TNF-α	155.11±12.10	365.37±10.59$^{\&}$	161.66±5.92*	283.04±8.28*	214.62±6.22*	210.44±5.56*
IL-1β	154.04±4.93	344.34±14.45$^{\&}$	151.52±8.64*	270.16±6.69*	221.23±7.45*	189.73±8.41*
IL-6	313.5±3.7	478.6±17.2$^{\&}$	311.8±6.5*	393.4±14.2*	318.0±3.9*	309.7±5.7*
IL-10	222.8±23.0	172.3±21.8$^{\&}$	197.4±11.4	257.4±52.1*	299.8±55.9*	326.8±74.1*

EAA: Aqueous extract of *Asparagus africanus*; TNF-α: tumour necrosis factor-α; IL: interleukin. $^{\&}$: P<0.05 compared with the normal control, *: P<0.05 compared with the negative control. The normal control group: non-diabetic animals received normal saline at 2 mL/kg; the negative control: diabetic animals was given 2 mL/kg of saline solution; the positive control: diabetic rats received metformin (200 mg/kg) in saline.

Table 3. Serum level of lipid profile of rats treated with EAA and metformin (mean±SD, mg/dL, n= 6).

Parameters	Normal control	Negative control	Positive control	EAA 250 mg/kg	EAA 500 mg/kg	EAA 1 000 mg/kg
TG	124.03±6.52	355.91±15.96$^{\&}$	200.04±18.97*	217.26±6.80*	125.64±55.96*	96.20±32.46*
TC	38.62±4.42	48.80±3.19$^{\&}$	37.23±3.74*	33.25±6.52*	37.46±2.67*	31.17±1.54*
HDL-c	22.45±1.16	8.47±0.58$^{\&}$	16.77±5.09*	17.02±5.59*	19.76±7.61*	21.55±2.40*
LDL-c	21.05±2.40	26.59±1.74$^{\&}$	20.29±2.04*	18.12±3.55*	20.42±1.46*	16.99±0.84*

TG: triglyceride; TC: total cholesterol; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol. $^{\&}$: P<0.05 compared with the normal control, *: P<0.05 compared with the negative control. The normal control group: non-diabetic animals received normal saline at 2 mL/kg; the negative control: diabetic animals was given 2 mL/kg of saline solution; the positive control: diabetic rats received metformin (200 mg/kg) in saline.

Table 4. Atherogenic index, Castelli risk index, and atherogenic coefficient of rats treated with EAA and metformin (mean±SD, n= 6).

Parameters	Normal control	Negative control	Positive control	EAA 250 mg/kg	EAA 500 mg/kg	EAA 1 000 mg/kg
AI	0.74±0.04	1.62±0.05$^{\&}$	1.09±0.13*	1.14±0.13*	0.79±0.22*	0.63±0.21*
CRI	1.73±0.27	5.79±0.68$^{\&}$	2.47±1.10*	2.08±0.63*	2.14±0.82*	1.46±0.13*
AC	0.73±0.27	4.79±0.68$^{\&}$	1.48±1.10*	1.08±0.63*	1.14±0.82*	0.46±0.13*

AI: atherogenic index; CRI: Castelli risk index; AC: atherogenic coefficient. $^{\&}$: P<0.05 compared with the normal control, *: P<0.05 compared with the negative control. The normal control group: non-diabetic animals received normal saline at 2 mL/kg; the negative control: diabetic animals was given 2 mL/kg of saline solution; the positive control: diabetic rats received metformin (200 mg/kg) in saline.

Table 5. Phytochemical screening of *A. africanus* root and aerial part aqueous extract.

Component	RE	APE
Tannins	+	++
Saponins	++	+++
Alkaloids	++	++
Glucosides	+	+
Anthraquinones	-	++
Terpenoids	+	+++
Carbohydrates	-	+
Steroids	++	++
Proteins	++	++
Coumarins	++	++
Reducing sugar	-	++
Flavonoids	++	+++

RE: root extract; APE: aerial part extract; +++: strong presence; ++: moderate presence; +: weak presence; -: absence.

3.2.4. Effect of aqueous extract of A. africanus on cardiovascular indices in diabetic rats

Results showed that *A. africanus* aqueous extract significantly reduced AI, CRI, and AC levels with the maximum effect seen at 1 000 mg/kg (P<0.05) in diabetic rats. The effect of *A. africanus* aqueous extract was more significant than metformin (Table 4).

3.3. Phytochemical screening

The results of the preliminary phytochemical screening of aqueous extract of *A. africanus* revealed the presence of the major pharmacological components. Both root and aerial parts of the plant contained phytochemicals such as flavonoids, alkaloids, tannins, saponins, terpenes, and steroids (Table 5).

4. Discussion

Plants with medicinal properties have long been used for the treatment and management of diseases with approximately 80% of people worldwide using medicinal plants for the needs of their health care[21]. The recent increase in the use of plants with medicinal properties for the treatment of ailments prompts studies on their safety and possible toxicity with various side effects if ingested plants are toxic. Biochemical parameters have been used for a long to determine the possible damage caused by medicinal plants and are good indicators for determining the possible toxicity and safety[22,23]. *A. africanus* extract was subjected to a sub-chronic toxicity study. The study revealed no significant difference in body, liver, heart, and kidney weights in treated animals. Also, there was no significant difference in concentration of AST, ALT, creatinine, and urea in serum of the rats receiving the extract. These results indicate that the aqueous extract of *A. africanus* is not toxic, which is similar to the findings of a previous study using the ethanolic extract from the root of the plant[21].

The findings of the present study indicate that the aqueous extract of *A. africanus* decreased the FBG in diabetic animals. This may justified by the presence of the phytochemicals, with a mechanism of action through insulin-like activity and inhibition of gluconeogenesis and glycogenolysis or by inhibiting the α-amylase and α-glucosidase activities as earlier reported[21,24,25]. Inflammation in tissues occurs as a response to harmful stimuli or damage to cells accounting for one of the critical risk factors of type 2 diabetes by inducing insulin resistance[26,27]. An increase in the levels of IL-1β, IL-6, and TNF-α is crucial in hyperglycemia-induced injuries and strongly associated with diabetic complications[28]. In the pathogenesis of diabetes, adipocytes and immunocytes produce various proinflammatory cytokines including IL-6 and TNF-α[29,30]. These cytokines through the activation of the NF-κB pathway lead to serine phosphorylation of insulin receptor substrate resulting in the insulin receptor[30]. The high concentration of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, has been shown to lead to the destruction of insulin produced by the pancreatic beta cells and also result in insulin resistance through activation of NF-κB pathway[31]. In the present study, diabetic rats showed an increase in levels of TNF-α, IL-6, and IL-1β and treatment with *A. africanus* aqueous extract showed a marked decrease in these levels. This may indicate the anti-inflammatory potentials of *A. africanus*, which was also indicated by an increase in IL-10 levels and may further demonstrate the therapeutic importance of the plant in treatment of diabetes.

In diabetes, insulin deficiency or insulin resistance is also aligned with dyslipidemia characterized by elevated serum levels of TG, TC, and LDL-c and a decrease in HDL-c level[32]. In this study, we observed a noticeable dyslipidemia in untreated diabetic rats as opposed to animals receiving *A. africanus* aqueous extract. Once more, this justifies the effectiveness of the plant in treatment of diabetes and its related complications. A suggested mechanism of action for *A. africanus* aqueous extract may be through induction of oxidation of fatty acids or inhibition of lipogenesis enzymes by activating AMPK which helps lower blood TG levels[33]. In addition, *A. africanus* aqueous extract may inhibit the activity of key enzymes in cholesterol biosynthesis and decrease the ability of LDL to transport free cholesterol to tissues, hence reducing cholesterol as demonstrated for other drugs in treatment of diabetes[34]. This study also showed an increase in cardioprotective lipid (HDL-c) which is evidence of a possible preventive effect of *A. africanus* aqueous extract against the formation of arteriosclerosis, which was further, justified by the decrease in AI, AC, and CRI[35].

In the qualitative analysis, the phytochemical analysis indicated that aqueous extract of *A. africanus* contains metabolites such as terpenoids, tannins, flavonoids, alkaloids, and saponins. Such compounds notably terpenoids have been demonstrated to reduce blood glucose through insulin-like activity and inhibition of gluconeogenesis and glycogenolysis[2,21]. Saponins have been also demonstrated to alleviate hyperglycemia-associated oxidative

stress in type 2 diabetes. This may justify the hypoglycemic effect of *A. africanus*. In addition, tannins, flavonoids and alkaloids were demonstrated to exhibit α-amylase and α-glucosidase inhibitory activities[2,25]. As demonstrated by this study, the therapeutic activities of *A. africanus* in diabetes might be attributed to its phytochemicals, nevertheless, each of the molecules can contribute, and further investigation is needed for more evidence. Bioactive compounds such as flavonoids and phenols have hypolipidemic activity and its presence may justify the use of *A. africanus* in the treatment of type 2 diabetes.

In this study, we focused on demonstrating the effect of *A. africanus* on the management of inflammatory reactions and cardiovascular disorders in diabetes in continuation of our previous study[36]. However, further investigations are required to validate the application of *A. africanus* preparations in treatment of diabetes. These investigations need to take into account the study of the effect of *A. africanus* on metabolic enzymes, inflammatory mechanisms, and cardiovascular systems.

In conclusion, the present findings show that *A. africanus* is not toxic and plays a therapeutic role in diabetes mellitus. Subacute administration of *A. africanus* aqueous extract to normal rats does not affect liver and renal functions. In diabetic rats, *A. africanus* aqueous extract could attenuate hyperglycemia and their risk of inflammation by reducing the proinflammatory cytokines. *A. africanus* aqueous extract also reduces the risk of atherogenesis in diabetic rats by reducing the cholesterol and LDL as well as by increasing HDL-c levels. *A. africanus* may contain phytochemicals such as flavonoids, saponins, and alkaloids that justify its therapeutic activity in diabetic rats.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

FEN was involved in collection, interpretation of data, and manuscript drafting. OM and TC participated in conception of the research idea, designing, data analysis, and revising manuscript. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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