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Research Article

Suadian Acacia Gerrardii: Antidiabetic Effect in Rats Suffering from Diabetic Nephropathy and DNA Fingerprinting Using ISSR

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Abstract

Background and Objective: There is a widespread use of medicinal herbs with beneficial uses against different diseased conditions. This study was carried out to identify and study the biological effect of *Acacia gerrardii* leaf extract on lowering blood sugar in rats suffering from diabetic nephropathy. **Materials and Methods:** It studied the effects of leaf extract at concentrations ranging from 100-500 mg kg^{-1} b.wt. per day for 4 weeks. Serum glucose levels, total lipids profile and kidney functions were estimated. Plasma levels of sodium and potassium as well as total bilirubin levels were assessed and kidneys from different groups were histopathologically examined. **Results:** The results showed that leaves were rich in the major compounds of phenolic acids, including salicylic acid and flavonoids with reduction of total lipids, triglycerides and total cholesterol in diabetic rats with renal failure together with reduction in uric acid, creatinine and urea with reduced vacuolar degeneration of tubules and basement membrane thickening. Additionally, the phylogenetic analysis using ISSR primers detected a genetic divergence among different samples. The results showed that the rich antioxidant content of *Acacia gerrardii* improved lipid, serum antioxidant and kidney function profiles in diabetic rats. **Conclusion:** *Acacia gerrardii* could be used as a safe source of antioxidants. Moreover, the ISSR assay proved its usefulness in detecting genetic variations among different *Acacia gerrardii* samples.

Key words: *Acacia gerrardii*, phenolic acid, lipid profile, serum antioxidant, liver and kidney functions

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Many of the Non-Communicable Diseases (NCDs) in today's world are caused by toxic chemicals in alcohol, smoking and industrial pollutants in the environment, as well as life style issues such as; stress, poor diet and lack of exercise[1]. The NCDs kill 38 million people each year, mostly in middle and low-income countries. Of these, 17.5 million people die from cardiovascular diseases, 8.2 million from cancers, 4 million from respiratory diseases and 1.5 million from diabetes[2].

Since the stone age herbs, which can contain supportive, restorative and defensive agents have been used to maintain human health[3]. The WHO reported that 80% of African and Asian countries depend on traditional medicines for primary health in their daily regime[4]. The Taif area in the Mecca province of Saudi Arabia has a large *Acacia* tree population, primarily made up of small to medium rather than larger individual plants[5].

The most significant group of secondary metabolites, including the strongly bioactive compounds: phenolic acids, flavonoids, tannins and phenols are widely distributed within the plant kingdom[6]. *Acacia* species contain many phenolic ingredients, including phenolic diterpenes, flavonoids, phenolic acids, tannins and coumarins, which are potential natural antioxidant exporters[7]. These natural antioxidants play a significant pharmacological role in anticancer, neuroprotective and anti-inflammatory pathways[8]. Antioxidant compounds play a role in disease prevention by scavenging free radicals or preventing oxidation of essential biological molecules[9].

Mammalian systems demonstrate a range of physiological and pathological processes that generate reactive oxygen compound species[10]. Plant-based foods that contain phenolic compounds exhibiting antioxidant activity might therefore inhibit the risk of potential health issues[11]. Such compounds can also prevent oil from becoming rancid and inhibit oxidation more generally, thereby enhancing the shelf-life and nutritional value of some foods[12]. Scientists have recently become more attentive to natural antioxidants due to the influence of toxic and potentially carcinogenic synthetic antioxidants, such as; butylatedhydroxytoluene[13].

Diabetes mellitus is a chronic hyperglycemic condition caused by problems with insulin secretion and/or insulin action, resulting in disorders of fat, protein and carbohydrate metabolism[14]. The most common form of diabetes is type 2 (non-insulin-dependent diabetes mellitus or NIDDM). It is estimated that 300 million people will have a type 2 diabetes diagnosis globally by 2025[15].

For many common ailments, herbal drugs and medicinal plants are often cheaper, safer and more effective than synthetic drugs and have a long history in traditional folk medicine[16]. Various medicinal plants contain antidiabetic compounds[16].

The antidiabetic properties of the aqueous extract of some *Acacia* species, such *Acacia Nilotica, Acacia meansii, Acacia tortilis* and *Acacia mearnsii* have been reported in many research articles. The antidiabetic activities may be due to increasing insulin levels, the expression of energy expenditure-related genes or the decrease of some harmful enzymatic activity[17-19].

The medicinal plants are important part of the ecosystem and their use by humans reflects effective adaption to the surrounding environment. They potentially hold the key to defeating biotic and abiotic stresses. Additionally, studying medicinal plant diversity could shed a light on their genetic background, yielding important information for drug design and manufacture.

One of the most useful tools for genetic studies is molecular marker technology, which uses PCR molecular markers to target plant genomic regions. Genetic diversity and population structure among plant species can be divulged through analysis of PCR fragments[20]. Additionally, advanced molecular marker analysis could be used to correlate between PCR markers and plant gene networks, providing medicinal plant researchers more control over their genetic resources[21].

The Inter-Simple Sequence Repeat (ISSR) assay is designed to target nucleotide redundancy inside plant genomic regions, producing high polymorphism and repeatability. The ISSR is suitable for studying intra and inter-species genetic variation in different plant species. Additionally, it has been successfully utilized for studying medicinal and endangered plant species such as; *Thymus daenensis*[22], *Tribulus terrestris*[23] and *Bacopa monnieri*[24].

Phenolic acids and flavonoid content of the extracts were explored using fractionation analysis by using HPLC and by examining the histopathological and biological effect on rats kidneys. Furthermore, the genetic diversity and DNA fingerprint for different *Acacia gerrardii* samples collected from different geographical locations were conducted using the ISSR molecular marker assay. Building on this rich seam of research, the present study aimed to explore the potential of aqueous extract of *Acacia gerrardii* in lowering blood sugar in rats with diabetic nephropathy using concentration ranges from 100-500 mg kg^{-1} b.wt. per day for four weeks.

MATERIALS AND METHODS

Materials: *Acacia gerrardii* leaves were collected from the Taif region, which lies south-east of Jeddah and Makkah. Gentamicin was used to induce Chronic Kidney Disease (CKD) among rats (Sigma Chemical Co., St., Louis, MO, USA). Streptozotocin-induced diabetic rats were purchased from Upjohn Company, USA. The study was carried out at Taif University, during the period of March, 2017 to June, 2019.

Methods

Methanol extraction from *Acacia gerrardii* leaves: Powdered extract of *Acacia gerrardii* leaves was obtained using a mill and stored in amber bottles to prevent degradation. About 5 g of the powder was dissolved in 50 mL of methanol water (4:1 v/v) at room temperature, overnight, using an orbital shaker. The filtrate was centrifuged at 5000 rpm for 10 min and the supernatant was concentrated under reduced pressure at 40°C by using a rotary evaporator. After solvent evaporation, the residue was dissolved either in distilled water and the final volume was recorded.

Estimation of total phenolic acids and total flavonoid compounds: The total phenolic content in the extract was measured by using Folin-Ciocalteu reagent[25]. The UV reading was 760 nm. Gallic acid was used as standard (1 mg mL^{-1}) and the results were expressed as gallic acid equivalents (GAE mg g^{-1} of dry weight).

The total flavonoid content was determined by the method used by Eghdami and Sadeghi[26]. Absorbance was measured against a blank solution at 510 nm and the total 143 flavonoid content was expressed in terms of milligrams of quercetin equivalent per gram dry weight (mgQE/g DW).

Quantitative determination of flavonoids by HPLC: The HPLC analyses were performed with Dionex Ultimate 3000 liquid chromatography (Germany), with four solvent delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 μL loop and Chromeleon 6.8 system manager as the data processor. The separation was achieved using a reversed-phase Acclaim TM 120 C18 column (5 μm particle size, 4.6×250 mm)[27].

Quantitative determination of phenolic compounds by HPLC: Phenolic compounds were determined by HPLC using the method described by Goupy *et al.*[28]. The HPLC Hewlett Packard (series 1050) equipped with auto-sampling injection, solvent degasser, ultraviolet detector set at 280 nm and quaternary HP pump (series 1050). Hewlett Packard using a column Altman C18, 5 mm (150×4.6 mm Alltech) the column temperature was maintained at 35°C. The G phenolic acid standard from sigma Co., were dissolved in a mobile phase and injected into HPLC.

Biological experimental: Male albino rats (n = 42) of 160-180 g were purchased from The Pharmacy College at King Saud University and delivered to the King Fahd Medical Research Center in Jeddah. The basal diet consisted of corn starch 65%, casein 15% (containing 12.6% protein), salt mixture 4%, vitamin mixture 1% and cellulose 5% was prepared according to AOAC[29]. Rats were housed individually in wire cages in a room maintained at 25±2°C and kept under normal healthy conditions. All rats were fed on the basal diet for one week for acclimatization before starting the experiment. The rats were then divided into two main groups; the first main group (6 rats) were fed on the basal diet and described as control -ve. The second main group (36 rats), control +ve was fasted overnight and injected with gentamicin at 100 mg kg^{-1} b.wt. twice per week for two weeks, to induce renal failure according to the method described by Farombi and Ekor[30]. The injected rats were then fed on the basal diet for 48 h, when renal failure developed. The same group was fasted overnight and injected in the leg muscle with streptozotocin (dissolved in 0.1 M citric acid buffer and adjusted at pH 4.5) at 5 mg/100 g b.wt., to induce diabetes according to Madar[31]. Forty eight hours after injection, the second main group was divided into six sub-groups (groups 2-7, 6 rats each) and fed on different diets for the experimental period of 4 weeks. The groups were as follows:

- **Group 1:** Basal diet (control -ve)
- **Group 2:** Basal diet (control +ve)
- **Group 3:** Basal diet and 100 mg/kg/day leaf extract
- **Group 4:** Basal diet and 200 mg/kg/day leaf extract
- **Group 5:** Basal diet and 300 mg/kg/day leaf extract
- **Group 6:** Basal diet and 400 mg/kg/day leaf extract
- **Group 7:** Basal diet and 500 mg/kg/day leaf extract

At the end of experimental period plus 12 h fasting, blood samples were collected by using the abdominal aorta and rats were scarified under ether anesthetic. Blood samples were withdrawn from the antecubital vein into glass centrifuge tubes, containing oxalate solution (1.34%) as anticoagulant. After centrifugation at 3000 rpm for 10 min, plasma was withdrawn for analysis. The levels of serum glucose, total

lipids, total cholesterol and triglycerides were determined according to various studies[32-35], respectively. High, low and very low-density lipoprotein cholesterol in serum was determined[36-38]. Kidney functions, in terms of serum creatinine, urea and uric acid concentrations were determined as described by different studies[39-41].

Sodium and potassium content in serum samples were determined by the adaptation method[42]. Moreover, total bilirubin is assessed using caffeine benzoate to split bilirubin from the unconjugated bilirubin protein complex[43].

Histopathological examination of rats kidneys: Kidney tissues were immediately preserved in 10% neutral buffered formalin, dehydrated through graded alcohol series, embedded in paraffin, cut into 5 mm sections and stained with hematoxylin and eosin (H and E). The slides were examined for alterations of pathological significance by using light microscopy at 400x magnification.

Statistical analysis: The obtained data analyzed for variance. Duncan's multiple range tests at $p \leq 0.05$ was used to compare between means, using the PRO ANOVA procedure of Statistical Analysis System[44].

Table 1: List of ISSR primers sequences used in this study

Primer name	Primer sequence (5'-3')
ISSR-2	ACACACACACACACACAC
ISSR-3	GAGAGAGAGAGAGAGACT
ISSR-5	GAGAGAGAGAGAGAGAGA
ISSR-7	GAGAGAGAGAGAGAGACA
ISSR-8	CCATGGCTACCACCGGCA
ISSR-9	GTGGTGGTGGTGGTG
ISSR-11	AGAGAGAGAGAGAGCCC
ISSR-12	AGAGAGAGAGAGAGAGC
ISSR-18	ACACACACACACACACG
ISSR-19	ACACACACACACACACG

DNA finger printing and diversity analysis using ISSR assay: Seven *Acacia gerrardii* plant samples were collected from different locations in Saudi Arabia. To retrieve intact and high quality genomic DNA suitable for PCR analysis, the commercial DNeasy Plant Mini Kit (Qiagen, New York, NY, USA) was used, along with a plant DNA extraction recommended by the manufacturer. DNA quantity and quality were identified using gel electrophoresis and DNA samples were stored at -20°C. Ten ISSR primers were utilized in this study (Table 1). ISSR PCR reaction composition and program cycles were performed[45]. The final products were stored until needed at 4°C. Electrophoresis analysis using agarose gel (8%) was used to separate different PCR fragments according to their molecular size. To observe PCR bands, agarose gels were ethidium bromide stained and documented using the Gel Doc XR system (Bio-Rad, Hercules, CA, USA).

Regarding phylogenetic and diversity analysis, only scorable PCR fragments were counted as present (1) or absent (0). The unweighted pair group method with arithmetic averages (UPGMA) was utilized through Dice's similarity matrix coefficients to calculate similarity matrices between different samples and phylogenetic dendrogram was constructed using 'Past' software[46].

RESULTS

Polyphenolic fractions of *Acacia gerrardii* leaf extract: Polyphenolic compounds in *Acacia gerrardii* leaf extracts were determined through HPLC fractionation, with the results described in Table 2. Total phenolic acids were fractionated by using HPLC. The results showed that the alpha-coumaric, pyrogallol, protocatechuic, gallic and salicylic acids were the major compounds (19.41, 15.44, 9.92, 7.28 and 6.69 mg/100 g,

Table 2: Phenolic and flavonoids compounds (mg/100 g)

Phenolic acids	Quantification (mg/100 g dw)	Flavonoids compounds	Quantification (mg/100 g dw)
Gallic	7.28±0.58	Luteolin	2.53±0.26
Pyrogallol	15.44±1.24	Naringin	6.89±1.15
Protocatechuic	9.92±1.05	Rutin	9.25±2.67
Caffeic	3.16±0.18	Hesperidin	8.12±2.38
Sorbic acid	2.10±0.11	Quercetin	1.89±0.08
p-Coumaric	1.79±0.08	Kaempferol	3.02±0.35
Ferulic	3.97±0.21	Apigenin	5.36±1.46
Resveratrol	4.77±0.38	Quercetin-galloylglucoside	2.12±0.37
Sinapic	2.17±0.09	Kaempferol-3-O rutinoside	1.86±0.13
Alpha-coumaric	19.41±2.38		
Cinnamic	4.19±1.24		
Coumarin	1.48±0.07		
Salicylic	6.69±1.36		
3-Hydroxybenzoic acid	1.28±0.05		

Total phenolic acids 10.4±2.90 mg gallic acid/g, Total flavonoids compounds 6.38±1.31 mg Quercetin/g, Each value represents the Mean±SD, Mean followed by different superscript letters in each column are significantly different ($p<0.05$)

Table 3: Effect of *Acacia gerrardii* leaf extract on total lipids profile and total cholesterol fractions in diabetic rats with renal failure

Groups	Total lipids	Triglycerides	Total cholesterol	LDL	HDL	VLDL
	(mg dL^{-1})					
Control (-ve)	200±2.24^{e}	70±1.25^{e}	130±2.34^{e}	26±0.9l^{g}	90.0±2.15^{a}	14.0±1.59^{e}
Control (+ve)	370±6.11^{a}	140±2.14^{a}	210±4.59^{a}	112±5.13^{a}	70.0±1.95^{d}	28.0±3.21^{a}
Group 3	345±5.38^{a}	130±2.36^{a}	190±4.16^{a}	92±3.26^{b}	72.0±2.01^{d}	26.0±2.38^{a}
Group 4	295±7.12^{b}	115±1.79^{b}	170±3.14^{b}	70±3.24^{c}	77.3±1.73^{c}	23.0±1.17^{b}
Group 5	265±3.45^{c}	102±1.65^{c}	155±2.58^{c}	52±1.75^{d}	83.0±1.24^{b}	20.4±1.25^{c}
Group 6	231±3.75^{d}	90±2.13^{d}	145±2.67^{d}	40±1.49^{e}	87.0±1.26^{b}	18.0±1.13^{d}
Group 7	207±2.39^{e}	75±1.01^{e}	135±2.29^{e}	31±1.18^{f}	90.2±1.91^{a}	15.0±0.95^{e}

Each value represents the Mean±SD, Mean followed by different superscript letters in each column are significantly different (p<0.05)

respectively). The medium compounds from *Acacia gerrardii* leaf extract include: resveratrol, cinnamic, ferulic and caffeic at 4.77, 4.19, 3.97 and 3.16 mg/100 g, respectively. Minor compounds were sinapic, sorbic, p-coumaric, coumarin and 3-hydroxybenzoic acids.

Acacia gerrardii leaf extract had higher flavonoid content, with rutin, hesperidin, naringin and apigenin at 9.25, 8.12, 6.89 and 5.36 mg/100 g, respectively. Kaempferol, luteolin, quercetin galloyl glucoside and kaempferol-3-O rutinoside were the least prevalent flavonoids present.

Total lipids profile and total cholesterol fractions on diabetic rats with renal failure: Lipid profile and cholesterol fractions were determined in the serum of diabetic rats with renal failure, with results shown in Table 3. The greatest total lipid, triglyceride and cholesterol levels in the positive control group were 370, 140 and 210 mg dL^{-1}, respectively. The negative control levels were 200, 70 and 130 mg dL^{-1}, respectively. Different groups were fed on the basal diet and took 100, 200, 300, 400 and 500 mg/kg/day b.wt. of *Acacia gerrardii* leaf extract orally. Total lipid, triglyceride and cholesterol levels were reduced from 345-207, 130-75 and 190-135 mg dL^{-1}, respectively.

Acacia gerrardii leaf extract acted to improve LDL, HDL and VLDL levels in different groups. In comparison to the positive control group, the group treated with *Acacia gerrardii* leaf extract exhibited a significant reduction in cholesterol concentration, TG and serum LDL-C and a significant increase in serum HDL-C. As mentioned earlier, improvements in blood cholesterol, TG and LDL-C in diabetic renal failure rats might be due to *Acacia gerrardii* leaf extract having a direct effect on the lipid profile and also reducing blood glucose. These findings indicated the extract's capacity to decrease atherosclerosis, a complication arising from diabetes.

Kidney function in diabetic rats with renal failure: The kidneys remove toxic waste products and assist in maintaining appropriate levels of fluids and minerals in the body.

Table 4: Effect of *Acacia gerrardii* leaf extract on kidney functions in diabetic rats with renal failure

Groups	Urea	Creatinine	Uric acid
	(mg dL^{-1})		
Control (-ve)	26.12±2.4^{e}	0.49±0.11^{e}	2.56±0.25^{e}
Control (+ve)	42.26±3.0^{a}	1.17±0.24^{a}	5.73±0.61^{a}
Group 3	39.59±1.83^{b}	0.63±0.32^{b}	3.65±0.31^{b}
Group 4	35.49±1.72^{b}	0.60±0.21^{b}	3.02±0.24^{b}
Group 5	33.96±1.94^{c}	0.55±0.22^{c}	2.91±0.45^{c}
Group 6	30.58±1.68^{d}	0.52±0.16^{d}	2.75±0.75^{d}
Group 7	27.24±1.35^{e}	0.49±0.12^{e}	2.58±0.38^{e}

Each value represents the Mean±SD, Mean followed by different superscript letters in each column are significantly different (p<0.05)

The results in Table 4 illustrated that the highest levels of urea, creatinine and uric acid in diabetic rats with renal failure in the positive control groups were 42, 1.17 and 5.73 mg dL^{-1}, respectively.

Moreover, the reduction of urea, creatinine and uric acid in the control groups ranged from 39.59-27, 0.63-0.49 and 3.65-2.58 mg dL^{-1} at different *Acacia gerrardii* leaf extract concentrations, respectively. The reduction of kidney functions may be caused by the amounts of phenolic and flavonoid compounds present in *Acacia gerrardii*.

Glucose and total bilirubin in diabetic rats with renal failure: Glucose and bilirubin were determined in the experimental group injected with streptozotocin and compared with the healthy normal control group. Table 5 showed that the positive control group had the highest serum glucose (240 mg dL^{-1}), while the negative control group had the lowest (105 mg dL^{-1}). The increase in glucose levels in the rats injected with streptozotocin may be because streptozotocin promotes mRNA expression of glucose-6-phosphate dehydrogenase in the liver, raising blood glucose levels. Moreover, diabetes has been implicated in dyslipidemia and fatty liver.

Acacia gerrardii leaf extract at concentrations from 100-500 mg/b.wt. per day appeared to reduce blood glucose levels to 131.2, 123.0, 115.0, 110.0 and 105.0 mg dL^{-1},

Table 5: Effect of *Acacia gerrardii* leaf extract on glucose and total bilirubin in diabetic rats with renal failure

Groups	Glucose (mg dL^{-1})	Bilirubin (mg dL^{-1})
Control (-ve)	105.0±5.00^{f}	0.30±0.01^{f}
Control (+ve)	240.0±10.00^{a}	0.70±0.02^{a}
Group 3	131.2±1.59^{b}	0.56±0.05^{b}
Group 4	123.0±3.00^{c}	0.50±0.04^{c}
Group 5	115.4±5.05^{d}	0.45±0.01^{d}
Group 6	110.0±2.11^{e}	0.38±0.03^{e}
Group 7	105.0±1.00^{f}	0.33±0.02^{f}

Each value represents the Mean±SD, Mean followed by different superscript letters in each column are significantly different (p<0.05)

Table 6: Effect of *Acacia gerrardii* leaf extract on serum sodium and potassium in diabetic rats with renal failure

Groups	Na (mmol L^{-1})	K (mmol L^{-1})
Control (-ve)	95±2.12^{f}	14.9±1.01^{a}
Control (+ve)	250±5.37^{a}	6.7±0.11^{f}
Group 3	215±3.78^{b}	8.8±0.40^{e}
Group 4	180±4.11^{c}	10.7±0.20^{d}
Group 5	152±3.51^{d}	11.3±0.30^{c}
Group 6	120±2.66^{e}	12.7±0.70^{b}
Group 7	100±2.99^{f}	14.1±1.00^{a}

Each value represents the Mean±SD, Mean followed by different superscript letters in each column are significantly different (p<0.05)

respectively. The gradual decrease in these groups may be due to the *Acacia gerrardii* leaves containing rich amounts of antioxidant.

The results from Table 5 showed that the experimental groups showed gradually decreased levels of bilirubin when the *Acacia gerrardii* leaf extract concentration increased. This means the *Acacia gerrardii* leaf extract prevents the red blood cells from dying in diabetic rats with renal failure.

Serum sodium and potassium in diabetic rats with renal failure: Potassium (K^+) is the dominant cation in intracellular fluid. Patients with chronic renal failure, whose regulatory mechanisms are impaired, are therefore prone to hyperkalemia. Sodium (Na^+), which plays a significant role in the balance of bodily fluids, assisting in the function of nerves and muscles, is dominant within extracellular fluid.

Serum sodium and potassium were determined in diabetic rats with renal failure (Table 6). These showed that sodium was the highest (250 mmol L^{-1}) and potassium was the lowest (6.7 mmol L^{-1}) in the positive control group compared with the negative control group (healthy rats), which were 95.0 and 14.9 mmol L^{-1}, respectively.

Diabetic rats with renal failure took 100-500 mg/b.wt. per day of *Acacia gerrardii* leaf extract orally. As these concentrations increased, sodium was decreased gradually from 215.0-100 mmol L^{-1}, respectively. Conversely, potassium levels increased gradually from 8.8-14.9 mmol L^{-1}, respectively.

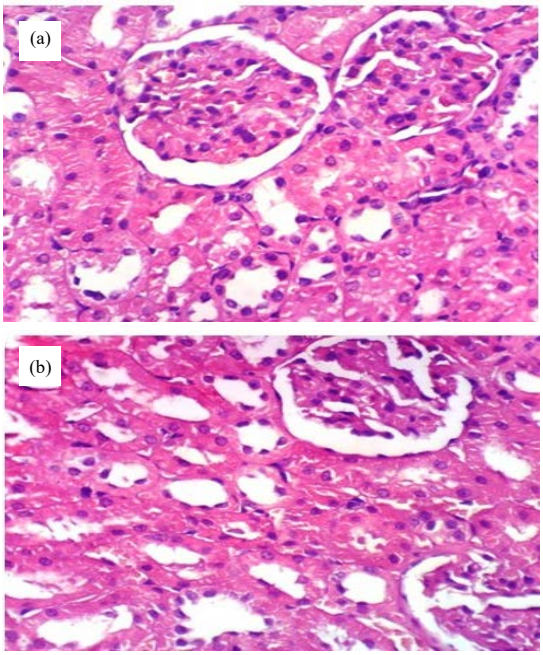

Fig. 1(a-b): Rat's kidney tissue (group 1) showing the normal histological structure of renal tissue (H and E X400)

Histopathological examination of kidneys: Microscopically, the kidneys of rats in the negative control group 1 revealed a normal renal histology (Fig. 1a-b). However, the kidneys of rats in the positive control group 2 showed vacuolar degeneration of the renal tubules epithelial lining (Fig. 2a-b), focal renal hemorrhage (Fig. 2b), parietal layer thickening of the Bowman's capsule and distension of the Bowman's space (Fig. 2c).

The kidneys of rats from group 3, who were fed on a basal diet with 100 mg/kg/day b.wt. of *Acacia gerrardii* leaf extract, taken orally revealed granular degeneration of renal tubules epithelial lining, slight glomerular tuft congestion (Fig. 3a) and slight renal blood vessel congestion (Fig. 3b).

Some kidney sections from group 4, who were fed on a basal diet with 200 mg/kg/day b.wt. of *Acacia gerrardii* leaf extract, taken orally showed granular degeneration of the epithelial lining in some renal tubules and slight vacuolation of the glomerular tuft (Fig. 4a), whereas other sections revealed no histopathological alterations (Fig. 4b).

A marked improved was noticed in sections from group 5 who were fed on a basal diet with 300 mg/kg/day b.wt. of *Acacia gerrardii* leaf extract, taken orally. The kidney revealed no histopathological alterations (Fig. 5a) except slight congestion of renal blood vessels (Fig. 5b) in some sections.

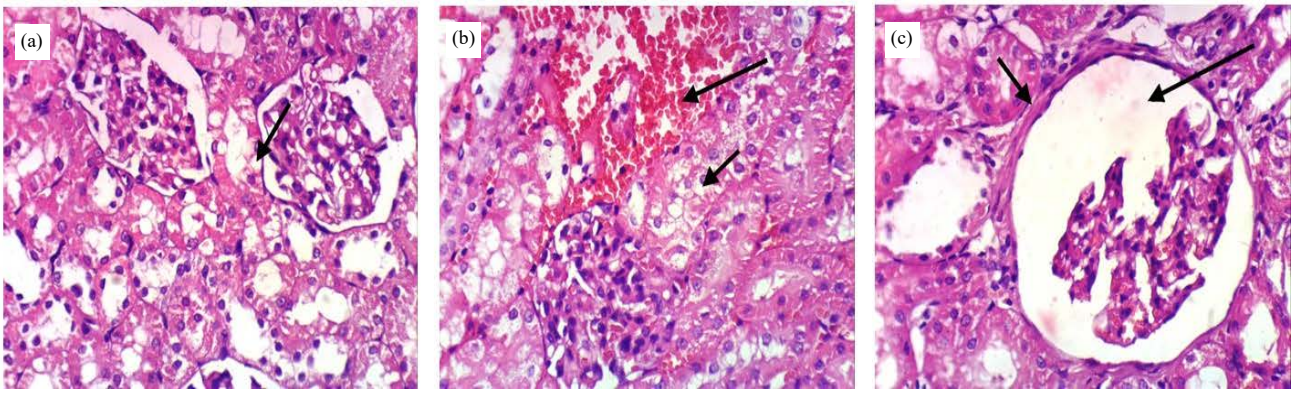

Fig. 2(a-c): Kidney of rat from positive control group 2 showing (a) Vacuolar degeneration of epithelial lining renal tubules (short arrows in a and b), (b) Focal renal hemorrhage (long arrow in b) and (c) Thickening of the parietal layer of Bowman's capsule (short arrow in c) and distension of Bowman's space (long arrow in c) (H and E X400)

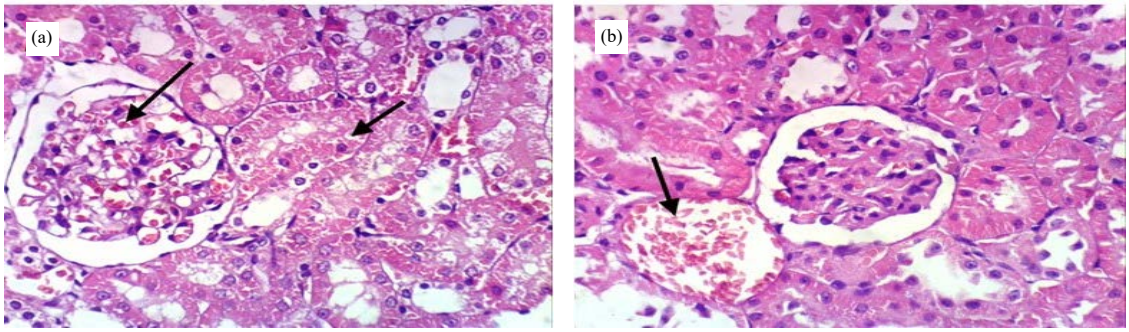

Fig. 3(a-b): Kidney of rat from group 3 showing (a) Granular degeneration of epithelial lining renal tubules (short arrow in a), slight congestion of glomerular tuft (long arrow in a) and (b) Slight congestion of renal blood vessel (arrow in b) (H and E X400)

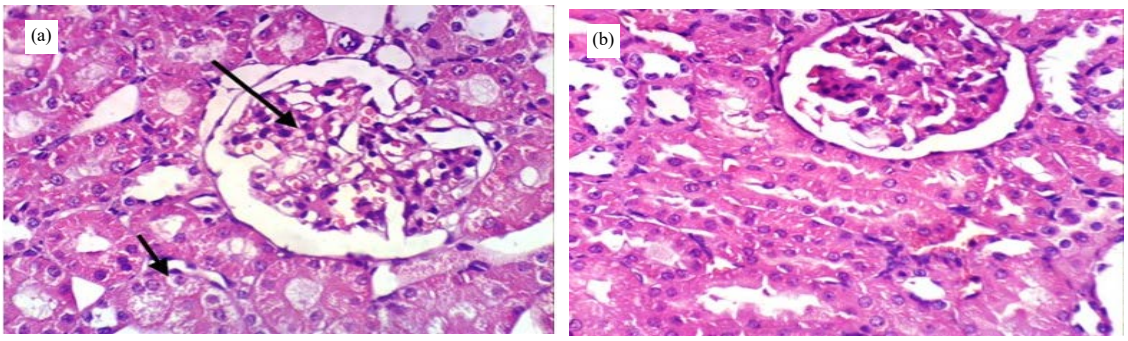

Fig. 4(a-b): Kidney of rat from group 4 showing (a) Granular degeneration of epithelial lining some renal tubules (short arrow in a), slight vacuolation of glomerular tuft (long arrow in a) and (b) No histopathological alterations (H and E X400)

Some sections from group 6 who were fed on a basal diet with 400 mg/kg/day b.wt. of *Acacia gerrardii* leaf extract taken orally, revealed no histopathological alterations (Fig. 6a), whereas other sections showed slight vacuolar degeneration of renal tubules epithelial lining and slight congestion of the glomerular tuft (Fig. 6b).

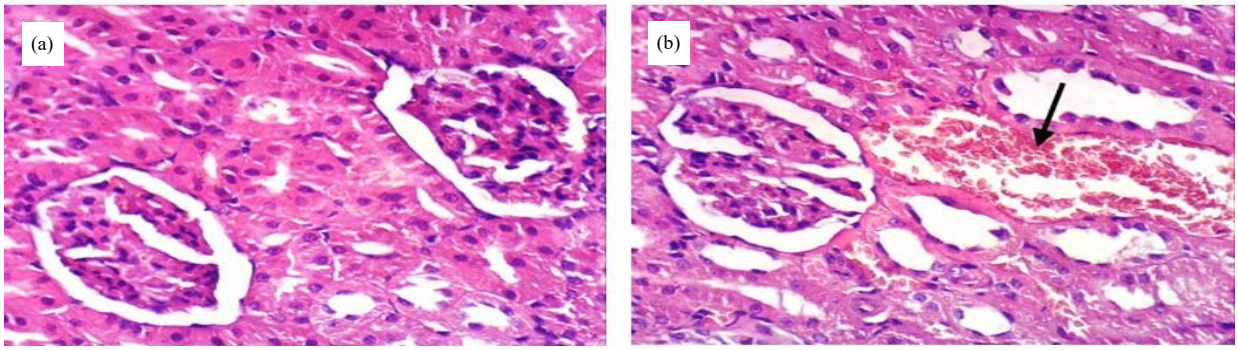

Fig. 5(a-b): Kidney of rat from group 5 showing (a) No histopathological alterations and (b) Slight congestion of renal blood vessel (arrow in b)(H and E X400)

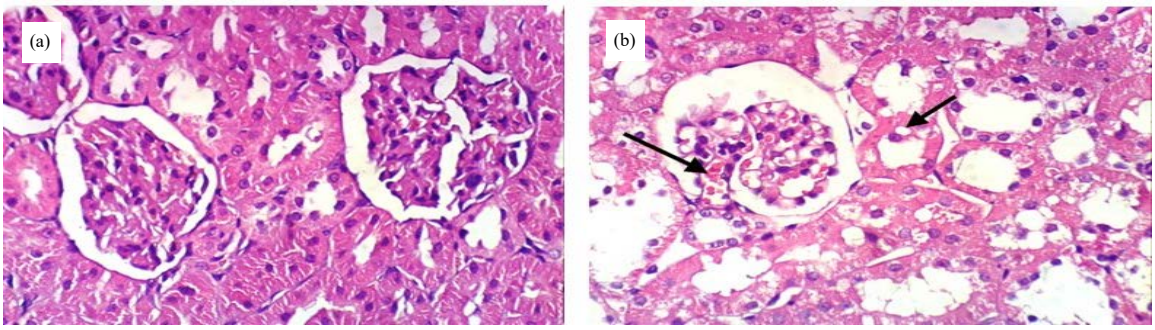

Fig. 6(a-b): Kidney of rat from group 6 showing (a) No histopathological alterations and (b) Vacuolar degeneration of epithelial lining renal tubules (short arrow in b) and slight congestion of glomerular tuft (long arrow in b)(H and E X400)

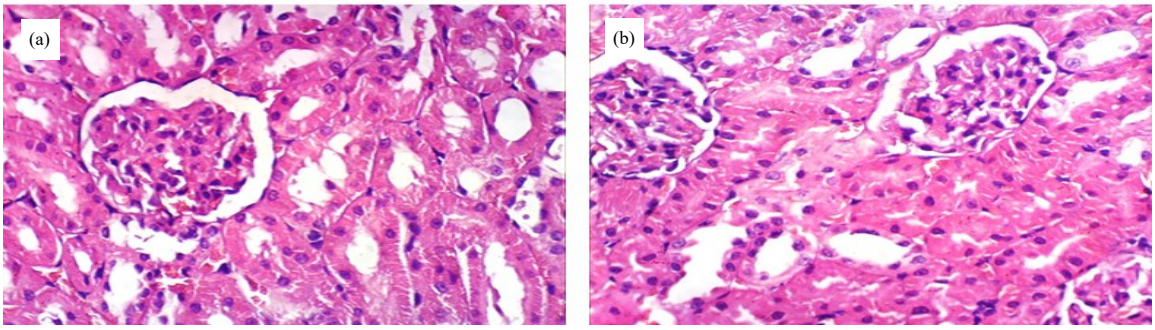

Fig. 7(a-b): Kidney of rat from group 7 showing (a-b) no histopathological alterations (H and E X400)

Group 7 who were fed on a basal diet with 500 mg/kg/day b.wt. of *Acacia gerrardii* leaf extract, taken orally showed no histopathological changes (Fig. 7a-b).

Histological evaluation STZ-diabetic rats treated with *Acacia gerrardii* revealed reduced vacuolar degeneration of tubules, Periodic Acid Schiff (PAS) base positivity staining intensity in glomeruli and thickening of basement membranes. Findings from this study provide experimental evidence that *Acacia gerrardii* leaves have potential antioxidant, antihyperglycemic and anti-glycation properties which might assist in slowing DNA progression.

ISSR molecular marker analysis: All ISSR primers produced clear and scorable PCR fragments. The total number of PCR fragments ranged from 6 (ISSR-8) to 19 (ISSR-3). Among these PCR bands, polymorphism ranged from 27% (ISSR-18) to 84% (ISSR-3), where the number of polymorphic bands was 3 and 16, respectively.

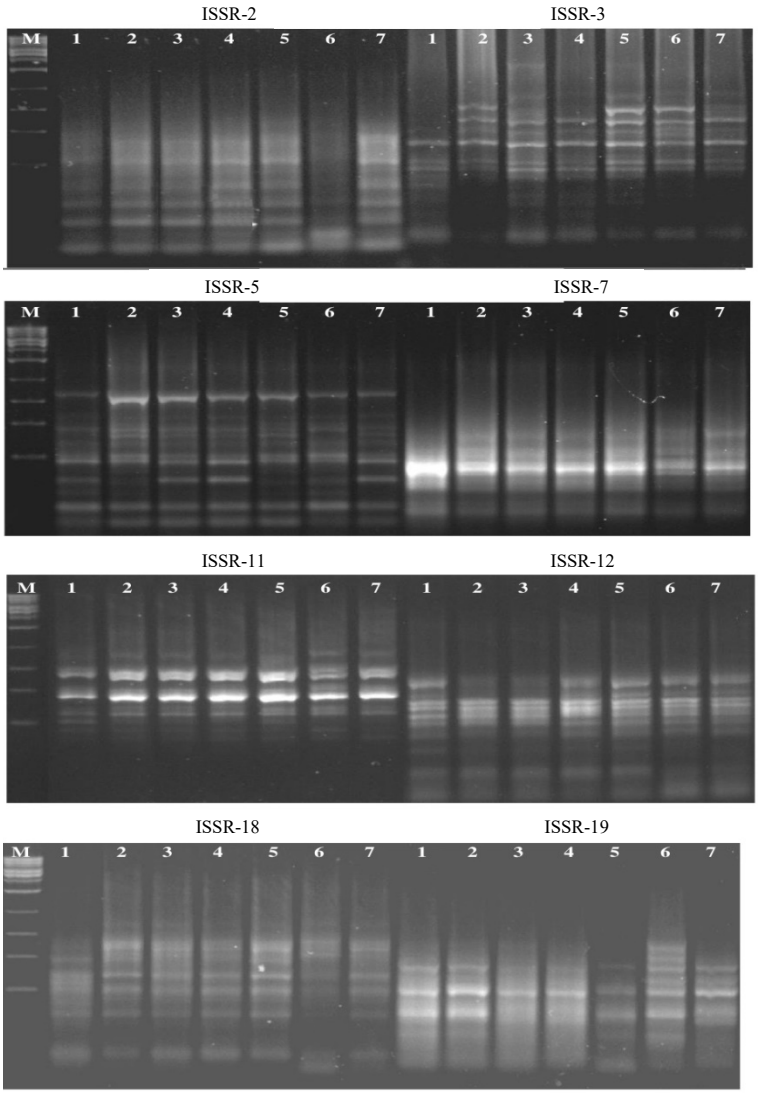

Fig. 8: Gel electrophoresis analysis results for some ISSR primers used in this study

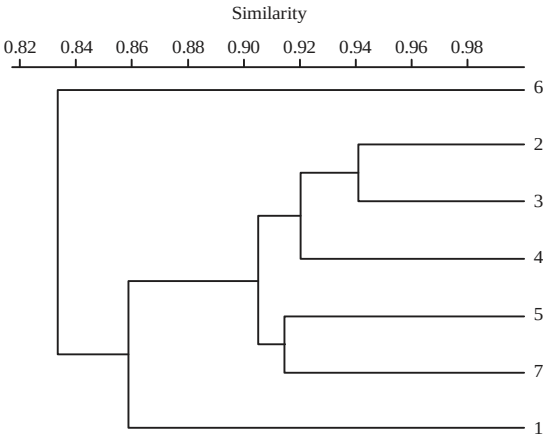

Fig. 9: Phylogenetic tree constructed using ISSR data for the 7 *Acacia gerrardii* accessions

Table 7: Primer Name (PN), Monomorphic Bands (MB), Unique Bands (UB), Polymorphic Bands (PB), Total Number of Bands (TNB), Polymorphism (%) (PP) and Mean of Band Frequency (MBF) for the ISSR primers

PN	MB	UB	PB	TNB	PP (%)	MBF
ISSR-2	5	1	7	12	58	0.8
ISSR-3	3	8	16	19	84	0.5
ISSR-5	8	1	5	13	38	0.8
ISSR-7	9	1	5	14	36	0.8
ISSR-11	7	1	6	13	46	0.7
ISSR-12	7	2	5	12	42	0.8
ISSR-18	8	1	3	11	27	0.8
ISSR-19	8	2	4	12	33	0.8
ISSR-8	3	0	3	6	33	0.7
ISSR-9	8	1	4	12	33	0.8

The mean band frequency was between 0.5 (ISSR-3) and 0.8 (ISSR-9, ISSR-5, ISSR-12, ISSR-18, ISSR-2, ISSR-7 and ISSR-19) (Table 7, Fig. 8).

The phylogenetic data analysis describes genetic diversity and evolutionary relationships among *Acacia gerrardii* samples. The phylogenetic tree was divided into three major clusters, two mono clusters, containing sample 6 and 1 separately, while the other clusters contained the remaining samples. This genetic divergence could indicate to what the genetic differences are and how different geographical locations may have affected the genomic content (Fig. 9).

DISCUSSION

The methanol extract, rich in polyphenolic compounds had potent antioxidant activity due to its abilities to donate hydrogen or electrons and to directly scavenge free radicals[47]. It possibly also caused the existence of the combined ring structures and hydroxyl groups. Many polyphenolic compounds are potential antioxidants due to their free radical scavenging properties, participation in oxidative processes and ability to hydrogenate oxidizing species[48].

Acacia spp. contained bioactive compounds including gallic acid, ellagic acid, isoquercetin, leucocyanidin, kaempferol-7-di-glucoside, glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6,8-bis-C-glucopyranoside, m-catechol and their derivatives[49]. Several parts of *Acacia* spp. have been found to contain high levels of tannins (ellagic acid, gallic acid and tannic acid), stearic acid, vitamin-C (ascorbic acid), carotene, crude protein, crude fiber, calcium, magnesium and selenium[50].

Table 2 also showed that the total phenolic acid and total flavonoid compounds were 10.4±2.90 mg gallic acid/g and 6.38±1.31 mg quercetin/g, respectively. These results correspond with the study of Lee *et al.*[51], who reported a total phenol concentration of 13505 μmol gallic acid/100 g dry weight from *Acacia nilotica* leaf extract as well as Song *et al.*[52] who found the phenol concentration of *Acacia nilotica* leaf extract was 59.43 mg g^{-1} dry weight.

Flavonoids are a group of secondary plant phenolics that are potentially beneficial to human health due to their considerable antioxidant qualities and chelating properties. They are also active in some medicinal plants. Flavonoids antioxidant activity efficiently traps superoxide anion ($O2^{\bullet-}$), hydroxyl ($OH^{\bullet}$), peroxyl ($ROO^{\bullet}$) and alcohol ($RO^{\bullet}$) radicals[53].

Whereas cholesterol is troubled, the two lipoproteins most generalities with its carry out are the High Density Lipoproteins (HDL) and the Low Density Lipoproteins (LDL). The LDL deposits cholesterol to cells, sometimes unnecessarily, so is associated with atherosclerosis. The HDL meanwhile, deposits cholesterol in the liver, where it is processed and removed. Atherosclerosis can be indicated by the ratio of HDL to LDL[54].

Increased blood glucose may damage cells and micro-blood vessels in the kidneys[55].

This may be caused by hyperglycemia, which increases the formation of advanced glycation end-products causes oxidative stress and activates the polyol pathway and hexosamine flux causing inflammation and renal damage[56].

These results are confirmed by Zare *et al.*[57] who found that the intake of antioxidants in diabetic rats decreased renal injury.

Diabetic Nephropathy (DN) recognized for a long time as the major reason for end-stage renal disease, is still poorly understood in terms of its prevention[58]. It is also the most significant complication arising from diabetes. Abnormal Glomerular Basement Membrane (GBM) perm selectivity plays a significant function in DN pathogenesis. Heparan Sulfate (HS) is a major polysaccharide in GBM and is broken down by heparinase. Gil *et al.*[59] found that the crucial role played by heparinase in DN pathogenesis makes it a highly plausible target for DN-related interventions.

This might be due to insulin reduction raising the flow of fatty acids to the liver and lipoprotein secretion from the liver caused by a deficiency of apolipoprotein B[60]. These results are confirmed by Eidi *et al.*[61], who reported that the *Acacia gerrardii* leaf extract used to treat diabetes frequently exerts its influences when insulin secretion is greater and when glucose re-absorption by skeletal muscle and adipose tissues increases, preventing intestinal glucose absorption and hepatic glucose production. The major active constituents for diabetes are alkaloids, glycosides, steroids, carbohydrates, glycopeptide, terpenoids, amino acids and inorganic ions. Moreover, flavonoids have predominately useful influences on diabetes by inhibiting the enzyme aldose reductase[62], which is implicated in complications of diabetes[63].

The death of red blood cells produces bilirubin, which can exist in insoluble unconjugated (indirect bilirubin) or soluble glucuronide conjugated (direct bilirubin) forms. Direct bilirubin is transported to the liver's bile canaliculi, then to the gall bladder. After eating, the conjugated bilirubin, along with bile is excreted into the small intestine and converted into urobilinogen. When too much hemoglobin is degraded or when bilirubin extraction is not functioning properly, this may cause high levels of bilirubin in the body, leading to jaundice[64]. Sodium levels are regulated by the kidneys, so impaired renal functioning can cause fluid loss through vomiting, diarrhea or pyrexia, potentially leading to rapid hypovolemia and hypotension[65].

These results was in accordance with Ruiz-Gutierrez *et al.*[66], who studied disturbances of the membrane lipid organization that explain the decrease in Na+-K+-ATPase activity. One significant cause of DN is a dysfunction in Na+-K+-ATPase.

These results agreed with Fila *et al.*[67], who studied massive proteinuria and sodium retention, which encourages as cites formation in nephrotic syndrome. In the puromycin amino nucleoside-induced rat model of nephrotic syndrome, sodium retention in the collecting duct is a driving force for potassium secretion. Nephrotic patients whose plasma potassium levels were in the normal to high range were recommended both low sodium and controlled potassium diet. In addition, low serum potassium is implicated in the progression of Chronic Kidney Disease (CKD) damaging kidneys by modulating renal inflammation and impairing angiogenesis[68]. Hypokalemia is implicated in higher mortality rates in patients with heart failure and acute myocardial infarction[69].

Sugano *et al.*[70] reported similar histological findings showing that treated diabetic rats exhibited features similar to that of a normal kidney.

Unique bands are those that reflect an ability to distinguish specific plants or species using the PCR procedure[71]. The number of unique bands ranged from 1 (ISSR-2, ISSR-5, ISSR-7, ISSR-11, ISSR-18 and ISSR-19) to 8 (ISSR-3). Band frequency is the relative frequency of PCR bands at a specific locus in a population, exhibited as a fraction[72].

ISSR assay was used to assess genetic diversity in *Podophyllum hexandrum.* Eleven ISSR primers were used, generating 68 ISSR loci, with an average of 6.18 loci per primer and 83.82% polymorphism[73]. Additionally, the ISSR-PCR system was used to screen rhubarb species, which is an important herb in China. From the 150 bands generated, 107 (71.3%) exhibited polymorphism[74].

CONCLUSION

Based on these results, it can be seen that *Acacia gerrardii* leaves are rich in phenolic and flavonoid compounds, which reduce liver and kidney damage, improve the lipid profile and lower blood sugar in diabetic rats with renal failure, using concentration levels from 100-500 mg/b.wt. per day taken orally. The ISSR assay proved its efficiency in studying the genomic variability of *Acacia gerrardii* and successfully detected some genetic variation among different samples, potentially indicating its usefulness in studying this species in near future.

SIGNIFICANCE STATEMENT

This study was carried out to identify and study the biological effect of *Acacia gerrardii* leaf extract on lowering blood sugar in rats suffering from diabetic nephropathy. *Acacia gerrardii* could be used as a safe source of antioxidants. Moreover, the ISSR assay proved its usefulness in detecting genetic variations among different *Acacia gerrardii* samples.

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