Antioxidative stress and nephroprotective activity of leaf fractions of *Setaria megaphylla* in alloxan-induced diabetic Wistar rats

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Submitted: 16th may, 2022; Accepted: 14th June, 2022; Published online: 30th June, 2022

DOI: *https://doi.org10.54117/jcbr.v2i3.19*

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Abstract

Setaria megaphylla (Steud) Dur & Schinz (Poaceae), a medicinal plant used traditionally to treat diabetes was investigated for antioxidative stress and renoprotective potentials against alloxan-induced kidney injuries in diabetic rats. Standard biochemical and histological methods were used to determine oxidative stress markers levels, kidney function indices and kidney histopathology, which were used as parameters to evaluate antioxidative stress and renoprotective activities of the leaf fractions (200 mg/kg). Significantly ($p<0.05$ – 0.001) increased levels of kidney oxidative stress markers (SOD, CAT, GPx, GSH) and decreased MDA level were caused by the leaf fractions in the treated-diabetic rats. Creatinine and urea levels were also significantly ($p<0.05$-0.001) reduced, while electrolytes levels were not significantly ($p>0.05$) reduced when compared to control. Kidney histology revealed absence or significant reductions in pathological features in the treated diabetic rats relative to untreated diabetic rats. The results suggest that the leaf fractions of *Setaria megaphylla* possess antioxidative stress and nephroprotective potentials due to the antioxidant activities of their phytochemical constituents.

Keywords: *Setaria megaphylla*, medicinal plant, nephroprotection, antioxidant, oxidative stress.

Introduction

Setaria megaphylla (Steud) Dur & Schinz (Poaceae) is a pasture grass with broad leaves which are found in tropical and subtropical areas of Africa, America and India (Van Oudtshoorn, 1999). The plant is of high medicinal value in Ibibio traditional medicine in Akwa Ibom State, Nigeria in the treatment diabetes (Okokon *et al.*, 2007a). Previous reports showed that the leaf extract possesses antidiabetic and hypoglycaemic activities (Okokon and Antia, 2007; Okokon *et al.*, 2022). Other biological activities of the leaf reported include *in vivo* and *in vitro* antiplasmodial activities (Clarkson *et al.*, 2004; Okokon *et al.*, 2017; Okokon *et al.*,

2007b), anti-inflammatory and analgesic (Okokon *et al.*, 2006), cytotoxic, immunomodulatory and antileishmanial (Okokon *et al.*, 2013) and antidepressant (Okokon et al., 2016), inhibition of α-amylase and α-glucosidase (Okokon *et al.*, 2021) activities. The leaf extract with LD_{50} value of 2.4 ± 0.5g/kg has flavonoid, terpenes, saponins, tannins, anthraquinones and cardiac glycosides as secondary metabolites (Okokon and Antia, 2007). 8,11,14-eicosatrienoic acid (Z,Z,Z), phthalic acid, diisooctyl ester, Vitamin E, γ-Elemene, Urs-12-ene, bicyclogermacrene, α-muurolene, germacrene- A, and guaiol among others have been reported to be present in the hexane fraction of the leaf (Okokon *et al.*, 2013). 1-triacontanal, 1-triacontanol, 1-dotriacontanol, 1-triacontyl cerotate, and stigmasterol have also been isolated from the plant leaves (Okokon *et al.*, 2022). The antioxidative and nephroprotective potentials of the leaf fractions of *S. megaphylla* in alloxan-induced diabetic rats are reported in this study.

Material and methods

Plant material

The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The leaves were collected from a forest in Uruan, Akwa Ibom State, Nigeria in August, 2021 and a voucher specimen (FPHUU221) of the plant was deposited at the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

Extraction

The leaves were dried, chopped into small pieces and reduced to powder using electric grinder. The powdered plant material, (1.5 kg) was successively and gradiently soaked for 72 h in each of these solvents (2 × 2.5L); n-hexane, dichloromethane, ethyl-acetate and methanol to give corresponding fractions of these solvents. The liquid filtrates obtained were concentrated, evaporated to dryness *in vacuo* at 40 °C using rotary evaporator and were stored in a refrigerator at -4 °C, until they were used for the experiments reported in this study.

Experimental animals

Wistar rats of both sexes used in these experiments were obtained from the Animal House, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Nigeria. The animals were kept in standard cages and maintained on standard pelleted Feed (Guinea Feed) and water *ad libitum*. Animal handling and care was conducted as enshrined in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Furthermore, permission and approval for animal studies were obtained.

Induction of experimental diabetes using alloxan monohydrate

Forty Wistar rats (male and female) were fasted for 24 h, then weighed and induced by injecting freshly prepared solution of alloxan monohydrate (150 mg/kg; i.p) in ice cold 0.9% normal saline. Each animal received 2 mL of 5% dextrose solution orally post induction to overcome the drug induced hypoglycaemia (Pari and Saravanan, 2002). The animals were fed and allowed to rest for 72 h during which full development of diabetic condition ensued. Rats with blood glucose levels of 200 mg/dL and above were considered diabetic and selected for the experiments (Lenzen, 2008). The diabetic animals were then randomised and divided into six treatment groups of six rats each. They were treated with 200 mg/kg/day of

respective solvent fractions of *S. megaphylla* leaf orally for 14 days as follows. Group 1 was given 10 mL/kg/day of normal saline orally for 14 days. Group 2 was administered with 5 mg/kg/day of Glibenclamide orally for 14 days. Group 3 – 6 were respectively administered with 200 mg/kg/day of n-hexane, dichloromethane, ethyl acetate and methanol fractions of *S. megaphylla* leaf orally for 14 days.

Measurement of fasting blood glucose of alloxan-induced diabetic rats

The fasting blood glucose (FBG) levels of all the rats were determined post 14 days treatment by tail-tipping method using dextrostix reagent pad and a microprocessor digital blood glucometer to record the readings (WHO, 1980). Prior to each measurement, food was withdrawn from the experimental subjects for 12 h.

Body weight measurements

Body weights of the experimental rats were monitored and recorded at the following points; pre-induction of the diabetes, post induction, on stabilization of diabetes and after the prolonged (14 days) study.

Collection of blood samples and organs

On the 15th day, the experimental rats were weighed and sacrificed under diethyl ether vapour. Blood samples were collected by cardiac puncture into plain centrifuge tubes and centrifuged at 1500 rpm for 15 min to obtain the serum. This was used for biochemical assays. From each euthanized rats, the kidneys were eviscerated, blotted with tissue paper, weighed and sectioned. While some of the tissues were fixed in 10% formaldehyde for histological studies, others were stored at -4 ºC for tissue enzymatic studies (antioxidative stress assays).

Preparation of renal homogenate

The eviscerated kidney tissues were washed in 0.9% NaCl. One gram of wet tissues were homogenized in 3 mL phosphate buffer saline (PBS; 0.05 M, pH 7.4) using motor driven Teflon pestle and centrifuged at 3500 rpm for 10 min at ambient temperature to obtain the supernatants which were used for the determination of antioxidative stress markers.

Biochemical analysis
Kidney function test

The following biochemical parameters were determined as markers of kidney function using diagnostic kits at the Chemical Pathology Department of University of Uyo Teaching Hospital; Levels of electrolytes (Na, K, Cl, and HCO_3), Creatinine and Blood urea.

Antioxidative stress markers

The following antioxidative stress markers; malondialdehyde levels (Wilbur et al., 1949; Esterbauer and Cheeseman, 1974), superoxide dismutase (Marklund and Marklund, 1974), catalase (Sinha, 1972), glutathione peroxidase (Lawrence and Burk, 1976), and reduced glutathione (Ellman, 1959) were measured using colorimetric assay.

Statistical analysis

Data obtained from this study were statistically analysed using one-way ANOVA followed by Tukey-Kramer multiple comparison test. Differences between means were considered significant at 5% and 99% level of significance (i.e. $p < 0.05; 0.001$).

Results

Effect of fractions of S. megaphylla on fasting blood glucose levels of diabetic rats

S. megaphylla leaf fractions demonstrated significant ($p<0.05$-0.01) reduction in fasting blood glucose (FBG) levels of the treated diabetic rats relative to control. These effects were seen during the 14 days prolonged study with the methanol, dichloromethane and n-hexane fractions being the most potent (Figure 1).

Effect of fractions of S. megaphylla on body and kidney weights of rats

Change in body weights of the experimental rats are as presented in Table 1. Leaf fractions of *S. megaphylla* significantly administration produced body weight increases in diabetic rats which were significant ($p<0.05$-0.01) relative to control. Ethyl acetate fraction treated group produced the highest increase (9.64%), followed by n-hexane group (8.30%). Significant ($p<0.01$) decreases in kidney weights of the treated-diabetic rats compared to control were observed with the n-hexane fraction exerting the highest reduction (Table 1).

Effect of leaf fractions on kidney function parameters of diabetic rats

The elevated serum levels of creatinine, urea in the alloxan-induced diabetic rats were significantly ($p<0.05$-0.01) reduced by treatment with the leaf fractions when compared to control with the ethyl acetate and hexane fractions having the most significant ($p<0.05$-0.01) effect. There were insignificant ($p>0.05$) reductions in levels of bicarbonate, chloride, sodium and potassium in the fractions-treated groups when compared to control group (Table 2).

Effect of leaf fractions on kidney antioxidant enzymes

The kidney antioxidant enzymes (SOD, GPx, CAT) and GSH levels were significantly ($p<0.05$-0.001) elevated when compared to control due to the administration of the leaf fractions. The fractions-treatment also caused significantly ($p<0.001$) reduction in the level of MDA of the treated diabetic rats when compared to control (Table 3).

Histopathological assessment of the kidneys

Histological study of kidneys of untreated diabetic rats revealed pathological areas such as glomerular inflammation, vascular congestion, and cellular degeneration. However, normal cyto-architecture profile was observed in kidneys of diabetic rats treated with glibenclamide (10 mg/kg), and fractions (n-hexane, dichloromethane, ethyl acetate and methanol) at magnification A (×100) and B (×400) demonstrating a strong reversibility effect (Figures 2a – 2b).

Table 1: Effect of *S. megaphylla* fractions on body and kidney weights of alloxan-induced diabetic rats

Treatment	Dose mg/kg	Body weight (g)			Kidney weight (g)
		Day 0	Day 15	% Increase	
Control normal saline	-	154.6 ±11.18	148.3 ± 14.60	-4.07	1.12±0.17
Glibenclamide	10	147.3 ±0.85	157.68 ± 11.20	7.04	1.01±0.07 [a]
n- hexane fraction	200	144.96 ± 1.81	157.0 ± 24.61	8.30	0.88±0.11[b]
Dichloromethane fraction	200	145.26 ± 4.67	151.25 ± 10.84	4.12	0.96±0.04[b]
Ethyl acetate fraction	200	144.4 ± 8.50	158.33 ± 16.02	9.64	0.91±0.03[b]
Methanol fraction	200	152.46 ±12.10	159.33 ± 15.16	4.50	0.98±0.07[b]

Data are expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, when compared to control. (n=6).

Table 2: Effect of *S. megaphylla* fractions on renal function parameters of alloxan-induced diabetic rats

Treatment	Dose (mg/kg)	Creatinine (mMol/L)	Urea (mMol/L)	Bicarbonate (mMol/L)	Sodium (mMol/L)	Potassium (mMol/L)	Chloride (mMol/L)
Control	-	172.6 ± 10.97	8.63± 0.54	27.3± 1.76	113.3±2.72	2.93± 0.48	55.33± 1.76
Glibenclamide	10	115.0±9.71[c]	5.73± 0.50[c]	24.6± 1.76	105.3± 1.43	2.53± 0.26	53.0± 2.08
n- hexane fraction	200	134.0 ± 1.15[a]	6.70± 0.05[a]	27.3± 0.66	126.0±14.52	4.00± 0.65	51.0± 3.09
Dichloromethane fraction	200	149.6 ± 5.48	7.50± 0.28	28.6± 0.66	99.6± 5.04	2.13± 0.20	53.0± 2.08
Ethyl acetate fraction	200	130.0 ± 1.15[b]	6.50± 0.05[b]	24.0± 1.15	104.3 ±1.85	3.00± 0.17	42.0± 1.15
Methanol fraction	200	146.0 ± 2.30[a]	7.30± 0.11	26.0± 1.15	132.6± 9.59	4.63± 0.61	53.66± 0.88

Data is expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, [c]p< 0.001, when compared to control. (n=6).

Table 3: Effect of *S. megaphylla* fractions on kidney antioxidative stress markers in alloxan-induced diabetic in rats

Treatment	Dose mg/kg	GSH (µg/mL)	GPx (µg/mL)	CAT (µg/mL)	SOD (µg/mL)	MDA (µmol/mL)
Control normal saline	10 ml/kg	0.75 ± 0.05	0.12± 0.01	0.47±0.05	0.44±0.04	0.55±0.01
Glibenclamide	10	1.10 ± 0.06^{c}	0.29±0.08^{c}	0.60±0.02^{c}	0.76±0.01^{c}	0.26±0.03^{c}
n -hexane fraction	200	1.16 ±0.05^{c}	0.32± 0.06^{c}	0.77±0.02^{c}	0.78±0.01^{c}	0.32±0.01^{c}
Dichloromethane fraction	200	1.04±0.06^{c}	0.30±0.02^{c}	0.60±0.08^{c}	0.66±0.02^{c}	0.36±0.02^{c}
Ethyl acetate fraction	200	0.98 ±0. 08^{c}	0.29±0.01^{c}	0.52±0.066^{c}	0.50±0.02^{c}	0.38±0.02^{c}
Methanol	200	0.78± 0.19^{c}	0.24±0.02^{c}	0.32±0.04^{c}	0.52±0.01^{c}	0.40±0.08^{c}

Data were expressed as mean ± SEM. significant at $^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$ when compared to diabetic control. n = 6.

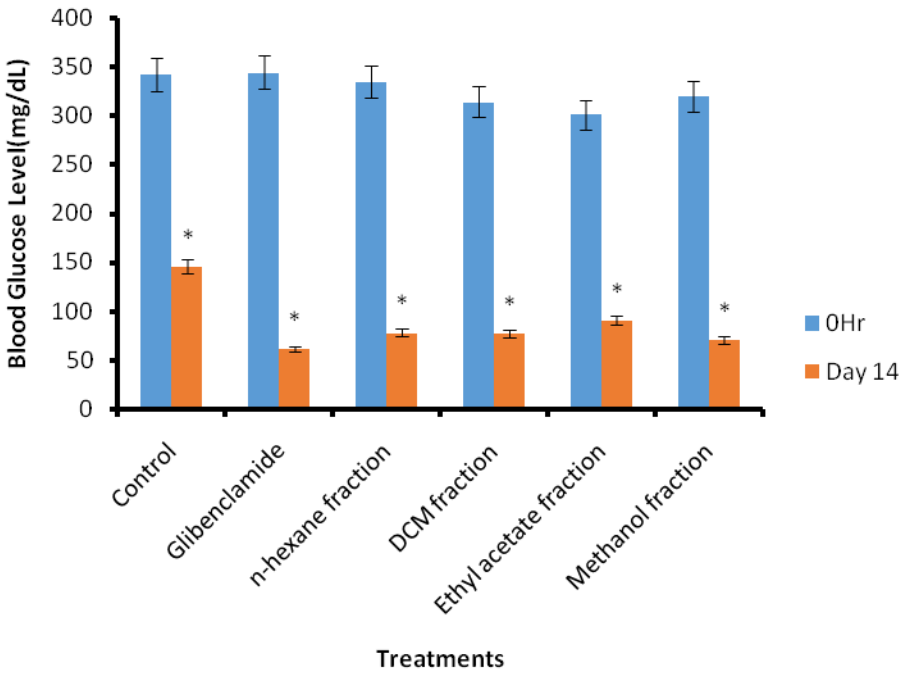


Figure 1: Effect of *S. megaphylla* fractions on blood glucose level of alloxan-induced diabetic in rats
Data were expressed as mean ± SEM. significant at *p< 0.01when compared to diabetic control. n = 6

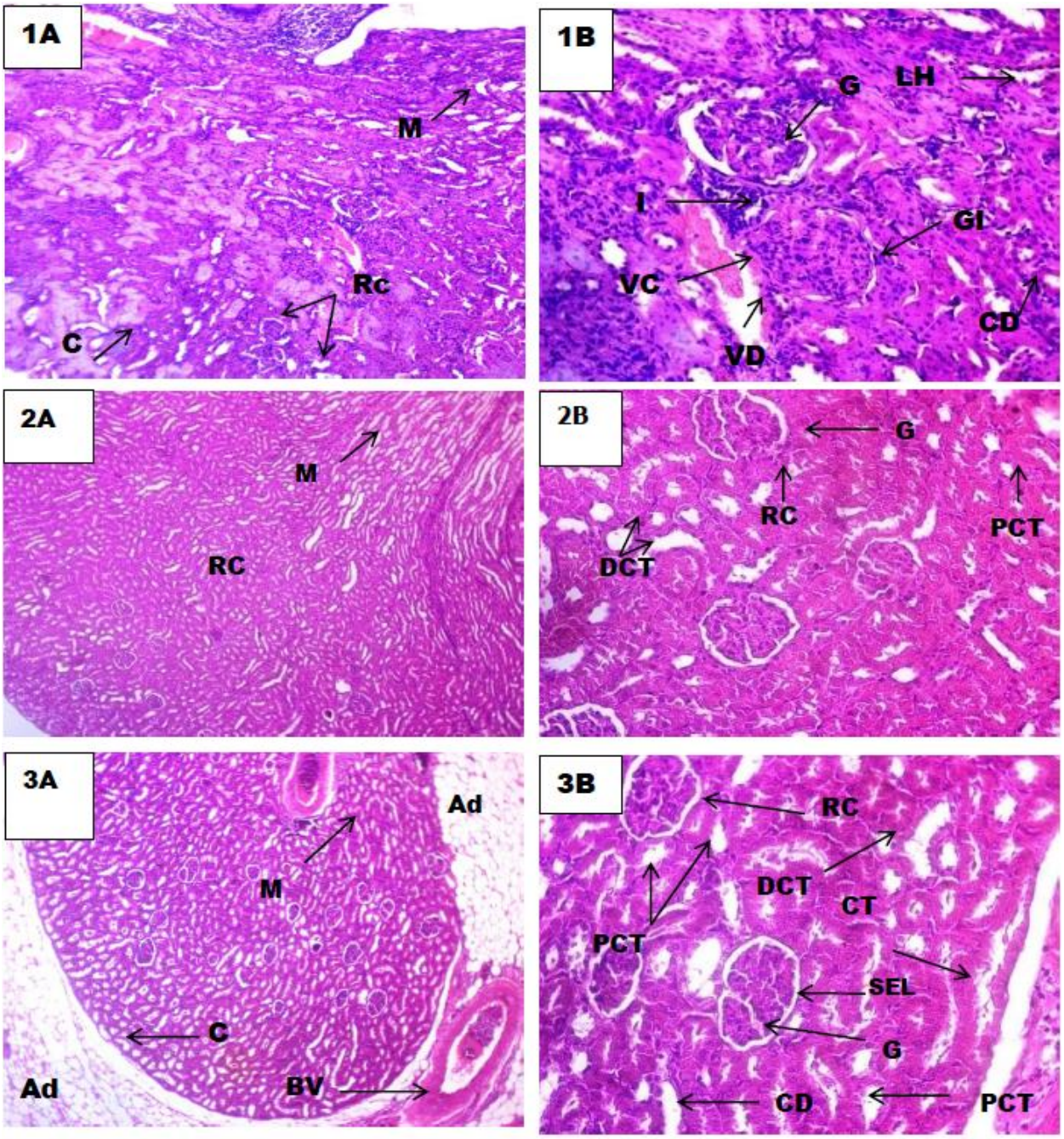


Figure 2a: Typical sections of kidney of alloxan-induced diabetic rats treated with 10 mL/kg body weight (bw) normal saline (1), 10 mg/kg bw glibenclamide (2), 200 mg/kg bw n-hexane fraction (3) stained with H & E at magnification A (×100) and B (×400). **Keys:** Cortex (**C**), Renal corpuscle (**Rc**), Medulla (**M**), Collecting duct (**CD**), Distal convoluted tubules (**DCT**), Proximal convoluted tubules (**PCT**), Lining hyperplasia (**LH**) and Squamous epithelial lining (**SEL**), Collecting duct (**CD**), Glomerulus (**G**), Connective tissue (**CT**), Blood vessel (**BV**), Adipocytes (**Ad).**

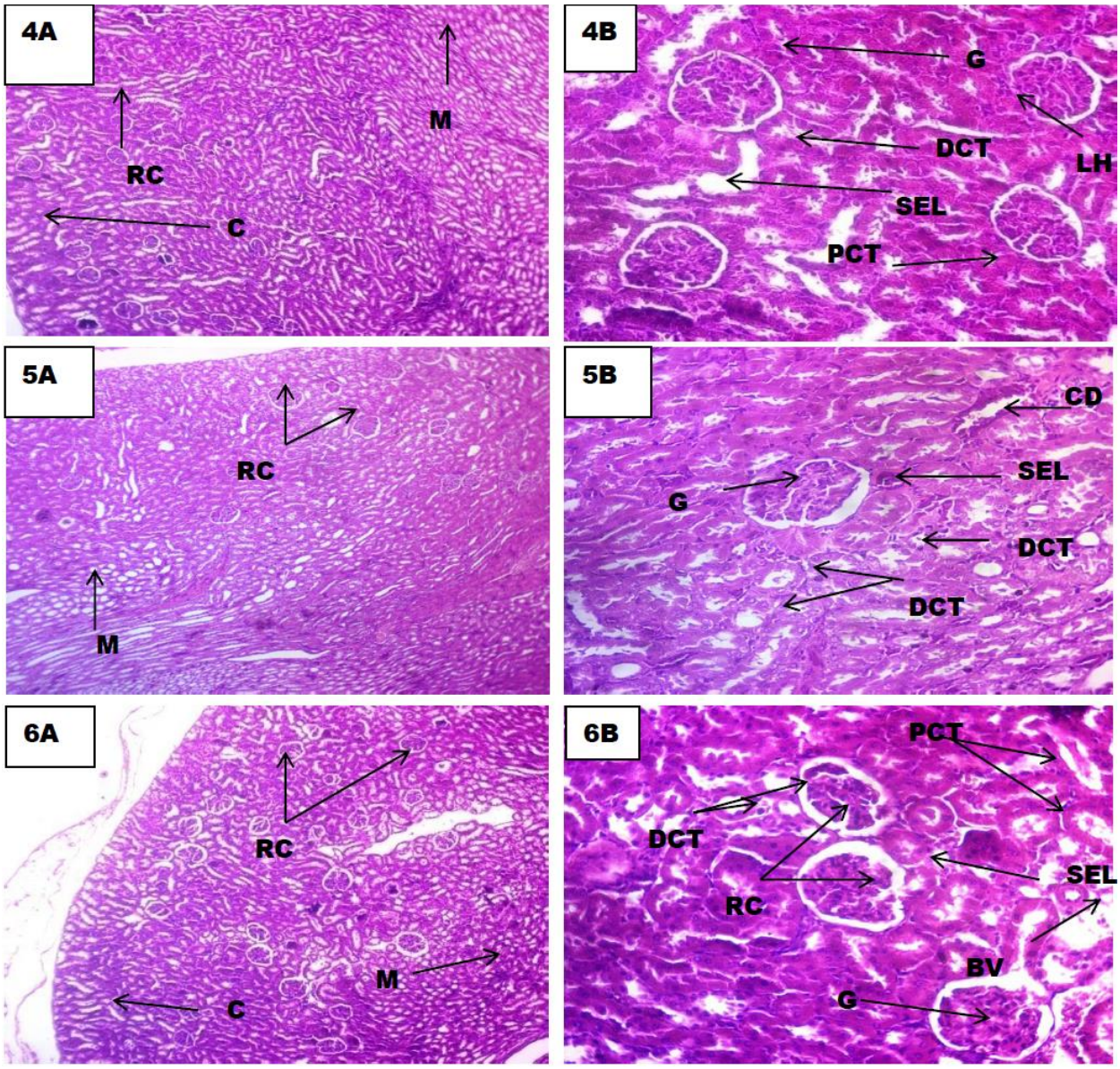


Figure 2b: Typical sections of kidney of alloxan-induced diabetic rats treated with 200 mg/kg body weight (bw) dichloromethane fraction (4), 200 mg/kg bw ethyl acetate fraction (5), 200 mg/kg bw methanol fraction (6) stained with H & E at magnification A (×100) and B (×400). **Keys:** Cortex (**C**), Renal corpuscle (**Rc**), Medulla (**M**), Collecting duct (**CD**), Distal convoluted tubules (**DCT**), Glomerulus (**G**), Lining hyperplasia (**LH**),Squamous epithelial lining (**SEL**). Collecting duct (**CD**), Proximal convoluted tubules (**PCT**), Glomerulus (**G**), Connective tissue (**CT**), Blood vessel (**BV**), Adipocytes (**Ad**).

Discussion

Diabetes mellitus is a chronic metabolic disorder which is associated with an increased generation of free radicals especially ROS (Okutana *et al.*, 2005). Alloxan is a hydrophilic compound whose biotransformation to dialuric acid is associated with the generation of hydrogen peroxide (H_2O_2), hydroxyl ion (•OH) and superoxide radical (Matthew and Leiter,

1999). Alloxan monohydrate causes diabetes by partial destruction of pancreatic β-cells of islet of Langerhans through free radical-mediated damage (Cakici *et al.*, 1994; Abdel-Bary *et al.*, 1997).

Body weight loss is associated with alloxan-induced diabetes due to increased muscle wasting and loss of tissue proteins (Shirwaikar *et al.*, 2004). The leaf fractions in this study caused significant body weight increases especially ethyl acetate and hexane fractions. This activity suggests the involvement of some phytochemical constituents of these fractions with the ability to reduce hyperglycaemia as observed in this study, through various mechanisms such as increased glucose metabolism, inhibition of α-amylase and α-glucosidase enzymes as reported on the leaf extract (Okokon *et al.*, 2021) and stimulation of protein synthesis by controlling muscle wasting through reversal of gluconeogenesis (Singh *et al.*, 2007).

Conventionally, internal organs weights changes are regarded as indicators of injuries and toxicities (Farah *et al.*, 2013). In alloxan diabetic models, toxicity and damage to organs are commonly indicated by hypertrophy of the organs which is caused by free radicals generated by alloxan resulting in destruction of hepatic, pancreatic and kidney cells and tissues (Ping *et al.*, 2013).

The leaf fractions were observed to cause significant decrease in weight of organs. The decrease in weights of kidney especially in n-hexane fraction-treated group suggests that it possesses nephroprotective property. This may be as a result of the antioxidant activities as well as the hypoglycaemic and free radical scavenging activities of the phytoconstituents such as phenolic compounds and 1-triacontyl cerotate (Snehunsu *et al.*, 2015) in the leaf fractions.

The kidney functions to maintain the body homeostasis by reabsorbing important material and excreting waste products and is compromised during uncontrolled diabetes mellitus. Glycosuria during diabetes causes severe loss of electrolytes like potassium, sodium, chloride, calcium, and phosphates (Gaw *et al.*, 1995). However, this study recorded non-statistically significant reduction of serum electrolytes levels by the leaf fractions when compared to control. This may be a nephroprotective effect of the leaf fractions of *S. megaphylla* against acidosis secondary to diabetes. This is thought so as diabetic acidosis usually causes increase serum retention of sodium and decrease excretion of potassium in urine (Nduka, 1997). The recorded reduced serum levels of urea and creatinine in the fractions treated-diabetic rats underscores a protective effect of the leaf fractions especially as diabetes potentiates high urea levels. In insulin insufficiency, glycogenic amino acids circulate in plasma and are deaminated in the liver with corresponding increase in blood urea. Thus, the leaf fractions of *S. megaphylla* potentiate insulin effect, mopping up excess glucose from the blood with a resultant decrease in urea. Physiologically, the kidneys excrete excess creatinine, and increased levels thereof indicate renal dysfunction. Administration of the leaf fractions to alloxan-induced diabetic rats reduced serum creatinine suggesting a protection of the kidney against damage due to diabetes. The recorded reduction in serum creatinine and urea is adjudged as nephroprotective potential of *S. megaphylla* leaf fractions. These results corroborate the histopathology of the kidney that revealed significant kidney protective effects (Figure 2a -2b).

Oxidative stress caused by free radicals is seen in the aetiogenesis of virtually all diseases conditions including diabetes and it

associated nephropathy. Pathological hyperglycaemia potentiates oxidative damage due to reactive oxygen species (ROS) and increased lipid peroxidation in alloxan-induced diabetes. The untreated alloxan-induced diabetic rat had high MDA levels and reduced activities of the antioxidative enzymes (SOD, CAT, GPx and GSH) in the kidney homogenates. The recorded significant increase in SOD, CAT, GPx and GSH and reduction in MDA secondary to treatment with leaf fractions of *S. megaphylla* underscores antioxidative stress potential, which is attributed to phytochemicals present in this medicinal plant. Flavonoids and other phenolic compounds are known to exert free radical scavenging activities and are reportedly present in the leaf extract (Okokon *et al.*, 2013). Signs of pathological injuries found in the untreated diabetic rats were absent in the kidneys of leaf fractions-treated rats. This suggests significant kidney protective effect.

Conclusively, our findings suggest that the leaf fractions of *S. megaphylla* possess nephroprotective activity derived from its antioxidant properties and antidiabetic properties and may be exploited as an adjunct in the management of diabetic nephropathy.

Acknowledgements

The authors are grateful to Mr Nsikan Malachy of Pharmacology and Toxicology Department for providing technical assistance.

Conflict of Interest

Authors have declared that no competing interests exist.

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