

Research Article

Nephroprotective Activities of Ethanolic Roots Extract of *Pseudoedrela kotschy* against Oxidative Stress and Nephrotoxicity in Alloxan-induced Diabetic Albino Rats

¹A.O. Ojewale, ²A.O. Adekoya, ³F.A. Faduyile, ⁴O.K. Yemitan and ⁵A.O. Odukanmi

¹Department of Anatomy, Faculty of Basic Medical Sciences,

²Department of Medicine, Faculty of Clinical Sciences,

³Department of Pathology and Forensic Medicine,

⁴Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine,

Lagos State University, Ikeja, Lagos, Nigeria

⁵Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: Introduction: The present study was designed to evaluate the nephroprotective activities of ethanolic roots extract of *Pseudoedrela kotschy* against oxidative stress and nephrotoxicity in alloxan induced diabetic albino rats. Methodology: Diabetes was induced in Albino rats by administration of alloxan monohydrate (150 mg/kg, i.p). The ethanolic roots extract of *Pseudoedrela kotschy* at a dose of 250 and 500 mg/kg body weight was administered at single dose per day to diabetes induced rats for a period of 28 days. The effect of ethanolic roots extract of *Pseudoedrela kotschy* on blood glucose, Urea, Creatinine, renal oxidative stress markers and lipid peroxidation were measured in the diabetic rats. Results: The ethanolic roots extract of *Pseudoedrela kotschy* exhibited significant reduction of blood glucose ($p < 0.05$) at the dose of 250 and 500 mg/kg when compared with the standard drug Glibenclamide (10 mg/kg). Urea and Creatinine levels were significantly increased ($p < 0.05$) in diabetic group without treatment as compared to control. In addition, the level of oxidative stress markers such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione (GSH) were significantly decreased ($p < 0.05$) in diabetic rats as compared to normal rats while the lipid peroxidation (MDA) significantly increased ($p < 0.05$) in diabetic group without treatment as compared to control (normal) rat. Apart from these, histopathological changes also revealed the cytoprotective nature of the ethanolic roots extract of *Pseudoedrela kotschy* against alloxan induced necrotic damage of renal tissues. Conclusion: From the above results, we concluded that the ethanolic roots extract of *Pseudoedrela kotschy* can prevent renal damage from alloxan induced nephrotoxicity in rats and it is likely to be mediated through its antioxidant activities.

Keywords: Alloxan, glibenclamide, nephroprotective activity, oxidation stress markers, *pseudoedrela kotschy*, rats

INTRODUCTION

Diabetes Mellitus (DM) is clinical condition in which metabolic activity of the carbohydrate, lipid and protein metabolism that contributes to several kinds of complications including diabetic nephropathy. Diabetic nephropathy is one of the major complications of diabetes mellitus. Diabetic nephropathy is the most important cause of death in diabetics, of whom, 30-40% eventually develop end-stage renal failure (Giorgino *et al.*, 2004).

It has been reported that diabetic complications are associated with overproduction of Reactive Oxygen Species (ROS) and accumulation of lipid peroxidation by-products (Palanduz *et al.*, 2001). These complications are considered the leading causes for death among these patients. Oxidative stress is

generally considered as an imbalance between pro-oxidant and antioxidant (Lieber, 1997). Oxidation plays a major role in diabetes. The increase in free radical release accompanied by decrease in antioxidants is a major cause of diabetes (Mohamed *et al.*, 1999). In diabetes mellitus, there are usually alterations in the endogenous free radical scavenging defenses which leads to ineffective scavenging of reactive oxygen species resulting to oxidative damage (Oberley, 1988).

Experimental diabetes induced by alloxan, selectively destroys the β -cells of pancreas by generating excess reactive oxygen species and produces kidney lesions that are similar to human diabetic nephropathy (Boukhris *et al.*, 2012).

Thus, an early control of DM is recommended as one of main strategy to prevent these complications and increase the life span of these patients. The use of

Corresponding Author: A.O. Ojewale, Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Lagos State University, Ikeja, Lagos, Nigeria, Tel.: +2348023854621

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

herbal medicine for the treatment of DM has gained prominence because of the undesirable side effects of oral anti-diabetic drugs, coupled with more recurrent failure of beta cells to respond to treatment (Earl, 2005). The herbal approach received a boost following the WHO recommendation for research on the beneficial uses of medicinal plants in the treatment of DM (WHO, 1980) They were considered more potent over most western medicines that are often made of single chemical compounds effective for direct relief of the symptoms (Li *et al.*, 2004).

Pseudocedrela kotschy (PK) is a member of the family Meliaceae. The plant is widespread in savannah woodland (Hutchinson and Dalziel, 1958). It is a tree of up to 20 m high with a wide crown and fragrant white flowers (Shahina, 1989). It is commonly found in West and Tropical Africa and in abundance particularly in North Central Nigeria, It is commonly known as Emi gbegi among Yoruba's and Tuna among Hausa's. In Togo, the bark is not only being used as a febrifuge and it is also being used in the treatment of gastrointestinal diseases and rheumatism (Hutchinson and Dalziel, 1958). The plant has also been reported to be used traditionally in the treatment of dysentery (Shahina, 1989). The analgesic, anti-inflammatory activities of plant (Musa *et al.*, 2005), antiepileptic (Anuka *et al.*, 1999) and dental cleansing effect have also been reported (Akande and Hayashi, 1998; Okunade *et al.*, 2007) This plant has demonstrated a wide range of biological effects such as antimalarial (Asase *et al.*, 2005), anticonvulsant (Odugbemi, 2006), antibacterial (Koné *et al.*, 2004), antipyretic (Akuodor *et al.*, 2013), heamatinic (Ojewale *et al.*, 2013a) and antidiabetic activities (Georgewill and Georgewill, 2009; Bothon *et al.*, 2013).

However, despite of widespread use of *Pseudocedrela kotschy* as folk medicine to manage DM and other ailments, its protective effects on the renal system has not been established.

Therefore, the present study was designed to evaluate the nephroprotective effects of ethanolic roots extract of *Pseudocedrela kotschy* against nephrotoxicity and oxidative stress in alloxan induced diabetic rats.

MATERIALS AND METHODS

Collection of the Plant material: *Pseudocedrela kotschy* (PK) roots were collected from cultivated farmland at Kulende, Ilorin, Kwara State, Nigeria. The plant was identified and authenticated at Forestry Research Institute of Nigeria (FRIN), where voucher specimen has been deposited in the herbarium (FHI 108280).

Preparation of the plant extract: The roots of the plant were shade-dried at room temperature for 7 days and then powdered using mortar and pestle. 850 g of

the root powder was soaked in 96% ethyl alcohol in three cycles using soxhlet extractor. The crude extract was filtered through filter paper (Whatman No 4) and the filtrate was concentrated and dried in a rotary vacuum evaporator under reduced pressure in vacuole 30°C to obtain 105.2 g dry residue to yield an (12.3% vol.) viscous brownish-coloured extract which was stored in an air tight bottle kept in a refrigerator at 4°C till used.

Experimental animals: Twenty five healthy albino rats weighing between 160-180 g were obtained from the Laboratory Animal Center of College of Medicine, University of Lagos, Idi -Araba, Lagos, Nigeria. The rats were housed in clean metallic cages and kept in a well-ventilated room under 24±2°C with 12 h light/dark cycle throughout the experimental periods and allowed to acclimatize to the laboratory condition for one week before being used. They were fed with standard animal pellet (Livestock Feeds Plc., Nigeria) and had free access to water *ad libitum*.

The animals were carefully checked and monitored every day for any changes. The experiments complied with the guidelines of our animal ethics committee which was established in accordance with the internationally accepted principles for laboratory animal use and care.

Acute toxicity studies: The acute toxicity of ethanolic roots extract of *Pseudocedrela kotschy* were determined by using 35 male Swiss albino mice (20-22.5 g) which were maintained under the standard conditions. The animals were randomly distributed into a control group and six treated groups, containing five animals per group. After depriving them food with 12 h prior to the experiment with access to water only, the control group was administered with single dose of ethanolic roots extract of *Pseudocedrela kotschy* with at a dose of 0.3 mL of 2% Acacia solutions orally while each treated group was administered with single dose of ethanolic roots extract of *Pseudocedrela kotschy* orally with at a doses of 1.0, 2.5, 5.0, 10, 15 and 20.0 g/kg body weight respectively of 2% acacia solution. They were closely observed in the first 4 h and then hourly for the next 12 h followed by hourly intervals for the next 56 h and continued for the next 2 weeks after the drug administration to observe any death or changes in behavior, economical, neurological profiles and other physiological activities (Ecobichon, 1997; Burger *et al.*, 2005).

Experimental design: To induce diabetes, rats were first anesthetized with inhalation of gaseous nitrous. ALX was purchased from representative of Sigma Company in Nigeria and was prepared in freshly normal saline. Diabetes was induced by intraperitoneal (ip) injection of alloxan monohydrate (150 mg/kg bwt) in a volume of 3 mL (Ojewale *et al.*, 2013a). After 72 h,

blood was withdrawn for blood glucose estimation monitored with a glucometer (ACCU-CHEK, Roche Diagnostics). The animals with blood glucose level ≥ 250 mg/dl were considered diabetic and included in the experiment (Ojewale *et al.*, 2013b).

The diabetic animals were randomly distributed into three groups of five animals each while the last group, the positive control, had five normal rats. Treatments were as follows:

Group I : Normal rats received only vehicles (0.5 mL/kg body weight) and served as positive control.

Group II : Alloxan diabetic rats that received only vehicles (0.5 mL/kg body weight) (control negative).

Group III : Alloxan diabetic rats treated with glibenclamide at a dose of 10 mg/kg bwt

Group IV : Alloxan diabetic rats treated with *Pseudocedrela kotschy* at a dose of 250 mg/kg bwt

Group V : Alloxan diabetic rats treated with *Pseudocedrela kotschy* with at a dose of 500 mg/kg bwt.

Treatments were administered every day by intragastric gavage. Rats were maintained in these treatment regimens for four weeks with free access to food and water *ad libitum*. Every week measurements of body weight were recorded for 4 weeks.

Sample collection: Blood sample was collected every week from each animal and was used for glucose analysis. The remaining blood sample was put into sterile tubes and allowed to clot for 30 min and centrifuged at 4000 rpm for 10 min using a bench top centrifuge.

At the end of the experimental period, each rat was reweighed and starved for 24 h. Then, blood sample was collected from each animal by cardiac puncture and rats were sacrificed under diethyl ether anesthesia. Kidney was carefully dissected out and rinsed in cold saline solution, weighed and processed immediately as described below.

Histological analysis: This was done as described by Ogunmodede *et al.* (2012). Briefly, the kidneys were cut on slabs about 0.5 cm thick and fixed in bouin's fluid for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57°C. Serial sections of 5 μ m thick were obtained from a solid block of tissue and were stained with hematoxylin and eosin, after which they were passed through a mixture of equal concentration of xylene and

alcohol. Following clearance in xylene, the tissues were oven-dried. Photomicrographs were taken.

Evaluation of biochemical parameters:

Glucose determination: Glucose was measured by the glucose oxidase method using a commercially available kit (ACCU-CHEK, Roche Diagnostics).

Creatinine and urea: Creatinine and Urea were determined using colorimetric assay kits from sigma (Lab-kit, Spain).

Determination of renal enzymatic antioxidants:

Assay of Catalase (CAT) activity: Catalase activity was evaluated according to the method described by Aebi (1984). Activity of catalase was expressed as units/mg protein.

Assay of Superoxide Dismutase (SOD) activity: Superoxide dismutase activity was evaluated according to the method described by Winterbourn *et al.* (1975). It was expressed as μ g⁻¹ protein.

Assay of Glutathione Peroxidase (GPx) activity: Glutathione peroxidase activity was determined by the method described by Rotruck *et al.* (1973). The absorbance of the product was read at 430 nm and it was expressed as nmol^{-1} protein.

Determination of renal non-enzymatic antioxidants:
Assay of renal reduced Glutathione (GSH) concentration: Reduced Glutathione (GSH) was measured according to the method described by Ellman (1995). The absorbance was read at 412 nm, it was expressed as nmol^{-1} protein.

Assay of lipid peroxidation (Malondialdehyde): Lipid peroxidation in the renal tissue was measured colorimetrically by Thiobarbituric Acid Reactive Substance (TBARS) method described by Ohkawa *et al.* (1979). Concentration was estimated using the molar absorptivity of malondialdehyde which is 1.56×10^5 $\text{M}^{-1} \text{cm}^{-1}$ and it was expressed as nmol/mg protein.

Statistical analysis: Data are presented as means \pm SD. Student's t-test analysis was applied to test the significance of differences between the results of the treated, untreated and control groups. The difference was considered significant at the conventional level of significance ($p < 0.05$).

RESULTS

Acute toxicity: The acute toxicity study result (Table 1), showed that five out of the five animals that received 20.0 g/kg bwt of the extract died within 4 h (100 % death) while the animals that received 5 g/kg body weight survived beyond 24 h. The LD₅₀ of the drug was therefore calculated to be 8.85 g/kg bwt.

Table 1: Acute toxicity of the ethanolic roots extract of *Pseudocedrela kotschy*

Groups	Dose (g/kg)	Log dose	24 h Mortality	% Mortality	Probit
I	1.00	3.00	0/5	0.0	0.0
II	2.50	3.40	0/5	0.0	0.0
III	5.00	3.70	0/5	00.0	0.0
IV	10.0	4.00	2/5	40.0	4.2
V	15.0	4.18	3/5	60.0	5.2
VI	20.0	4.30	5/5	100.0	8.7

Control group received 0.3 mL each of 2% Acacia solution

Table 2: The body weight (wt) variations

Groups	I	II	III	IV	V
Initial body wt (g)	165.4±0.7	172.8±2.0	175.6±1.4	169.2±2.5	165.6±1.8
Final body wt (g)	187.6±1.1	151.7±2.2	202.9±1.7	179.6±1.5	178.3±1.8
Diff in body wt (g)	22.2	-21.1	27.3*	10.4*	12.7*
Weight of kidney	0.86±0.32	0.68±0.24	0.82±0.20*	0.75±0.12*	0.77±0.10*

*: Statistically significant when compared to control group (I) at p<0.05

Table 3: Effect of ethanolic extract of *P. kotschy* for 4 weeks on plasma glucose concentration (mg/dl) in ALX-induced diabetic rats

Groups	1 st week	2 nd week	3 rd week	4 th week
I	83.5±5.5	79.7±6.2	82.3±6.0	86.4±4.5
II	340.4±11.2	348.1±16.6	352.9±20.5	356.1±18.6
III	291.6±16.5*	262.4±22.4*	252.8±21.7*	247.4±21.5*
IV	285.1±17.4*	261.3±15.8*	256.4±19.5*	240.2±21.0*
V	280.3±10.2*	261.4±14.2*	246.4±15.7*	233.7±18.1*

Mean±SD, n = 5, p<0.05 vs control group; *: Statistically significant when compared to control group (I) at p<0.05

Table 4: Effect of oral administration of *Pseudocedrela kotschy* extract after 4 weeks on renal biochemical parameters in ALX-diabetic male rats

Parameters	Groups				
	I	II	III	IV	V
Urea U/L	19.8±2.1	62.4±3.4*	18.3±2.7**	16.1±2.4**	15.4±2.2**
Creatinine U/L	0.92±1.0	1.5±1.1*	0.74±1.1**	0.78±1.5**	0.68±1.2**
CAT (µ/mg)	18.6±1.4	5.7±1.9*	17.6±2.4**	15.4±2.7**	16.8±1.8**
SOD (µ/mg)	46.2±2.8	14.4±1.6*	42.4±3.2**	39.4±2.6**	41.1±2.9**
GPx (nmol/mg)	0.92±0.4	0.34±0.2*	0.87±0.4**	0.63±0.1**	0.82±0.3**
GSH (nmol/mg)	2.85±0.5	0.63±0.1*	2.52±0.6**	1.76±0.7**	2.32±0.4**
MDA (nmol/mg)	1.1±0.10	4.6±1.0*	1.26±0.3**	1.62±0.2**	1.68±0.6**

Values are the mean values±standard deviation of 5 rats; *: Statistically significant when compared to control group (I) at p<0.05; **: Statistically significant when compared to untreated diabetic group (II) at p<0.05

Effect on body weight of rats: The control group (I) gained weight over the three weeks of experimental period, with the mean body weight increasing by 22.2 g after 4 weeks (Table 2). In contrast, the untreated diabetic group (II) lost an average of 21.1 g after 4 weeks (p<0.05). Treatment with glibenclamide and *Pseudocedrela kotschy* resulted in significant weight gain to levels approaching the control group (Groups III, IV and V, versus Group I). Mean kidney weight in the diabetic untreated group significant decreased as compared to that of control group while diabetic treated groups with *Pseudocedrela kotschy* and glibenclamide decreased by improving the restoring activity of *Pseudocedrela kotschy* extract to the weight lost due to alloxan administration.

Effect of the ethanolic roots extract of Pk on blood glucose level: The blood glucose level in diabetic group was significantly higher (p<0.05) than those of the control group (Table 3). On the other hand, administration of ethanolic roots extract of *P. kotschy* for 28 days was found out to lower blood glucose significantly in a dose dependent manner in treated diabetic groups (p<0.05) when compared with those of the diabetic nontreated (negative)group. The anti-

hyperglycemic effect of ethanolic extract (250/500 mg/kg) was found more effective than the reference drug, glibenclamide produced a significant reduction in blood glucose compare to diabetic control.

Effect of the ethanolic roots extract of Pk on Urea and Creatinine: The study also indicates that serum urea and creatinine levels significantly (p<0.05) increased in diabetic group when compared with the diabetic groups treated with the extract, the serum urea and creatinine levels reduced significantly (p<0.05) when compared to those of the diabetic group (Table 4).

Effect on renal enzymatic and non-enzymatic antioxidants: Diabetic rats showed significant lowering (p<0.05) in Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) compared to control animals. Diabetic rats treated with the *Pseudocedrela kotschy* significant higher (p<0.05) in kidney SOD, CAT and GPx activities compared to diabetic rats without treatment. Along the same line for the kidney content of Glutathione (GSH) and Malondialdehyde (MDA), GSH level in diabetic rats was significantly low (p<0.05) compared to normal rats. However the diabetic rats treated with the *Pseudocedrela kotschy* showed significantly increase

of ($p < 0.05$) the kidney content of GSH compared to normal rats, on the other hand the level of MDA was significantly increased ($p < 0.05$) in diabetic rats without treatment compared to normal rats while the level of MDA in diabetic treated groups with the *Pseudocedrela kotschy* (Table 4) were significantly lower ($p < 0.05$) compared to diabetic rats without treatment.

RENAL MORPHOLOGY

The biochemical results were also confirmed by the histological pattern of normal kidney showing normal

tubular brushborders and intact glomeruli and Bowman's capsule (Fig. 1), Diabetic untreated group showed severe tubular necrosis and degeneration is shown in the renal tissue (Fig. 2). Diabetic group treated with glibenclamide (10 mg/kg body weight) showed normal tubular pattern with a mild degree of swelling, necrosis and degranulation (Fig. 3). Diabetic group treated with the ethanolic roots extract of *Pseudocedrela kotschy* (500 mg/kg body weight) attenuated the toxic manifestations in the kidney caused by alloxan induction (Fig. 4).

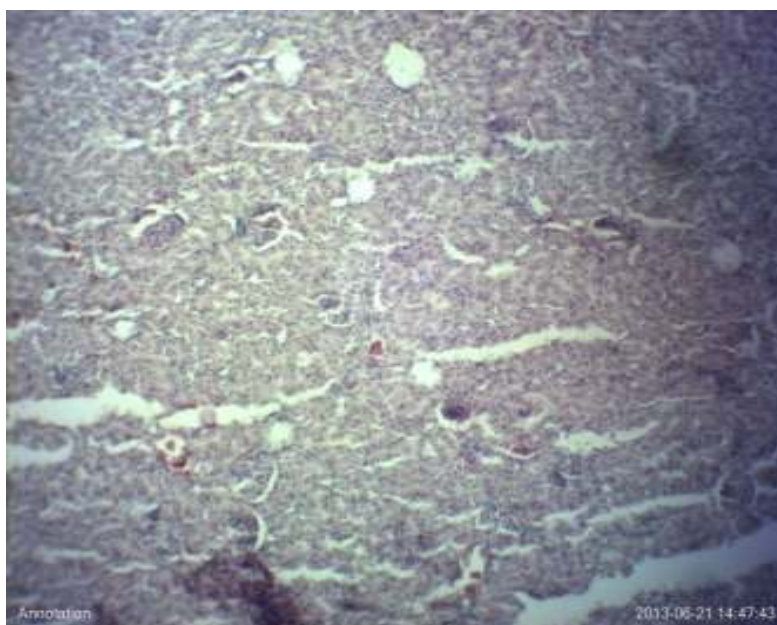


Fig. 1: Cross section of normal kidney stained with H & E, Mag: $\times 400$

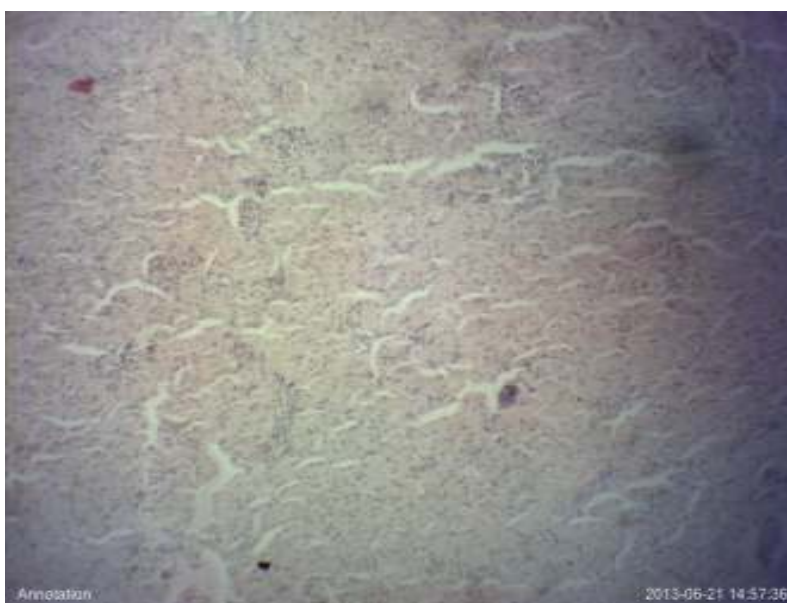


Fig. 2: Cross section of diabetic kidney stained with H & E, Mag: $\times 400$

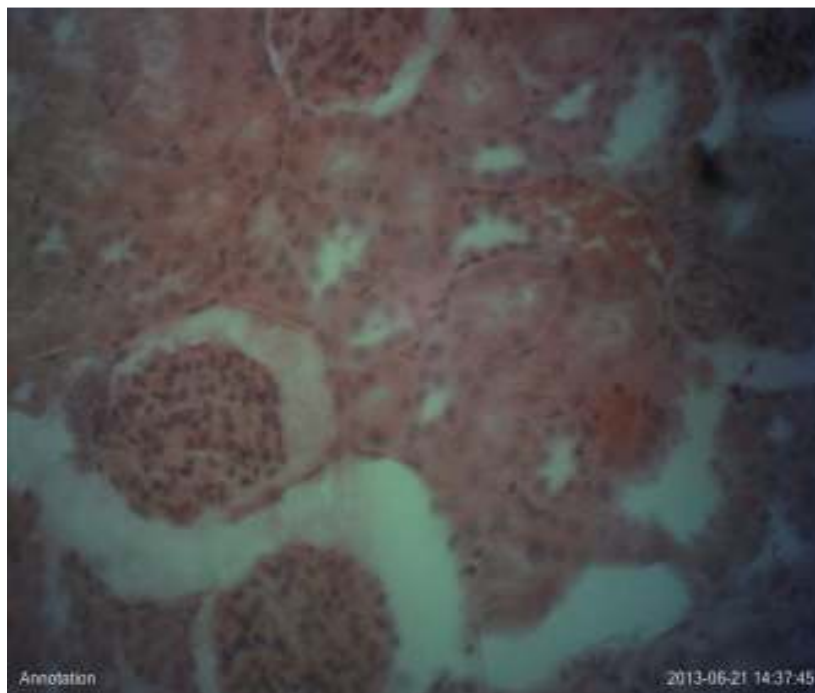


Fig. 3: Cross section of glibenclamide treated kidney stained with H & E, Mag: $\times 400$

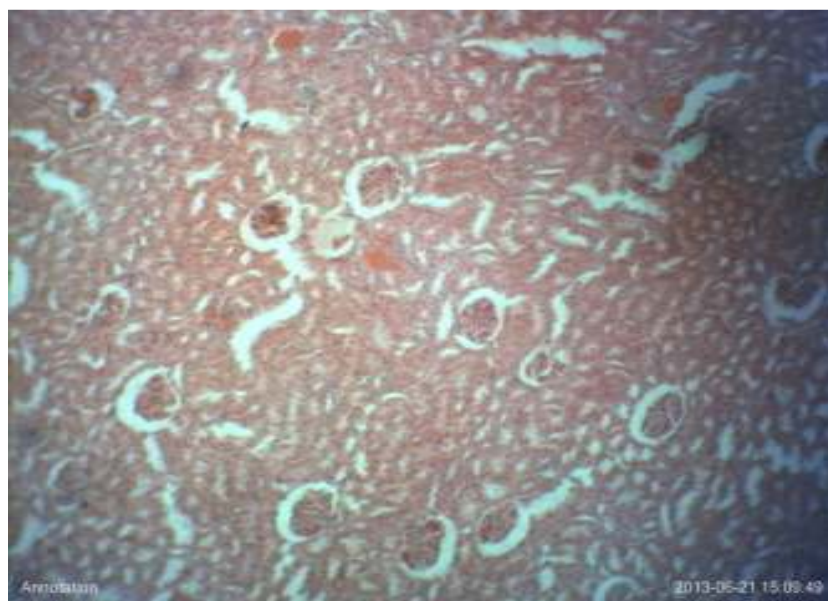


Fig. 4: Cross section of *Pseudocedrela kotschy* treated kidney stained with H & E. Mag: $\times 400$

DISCUSSION

This present study was aim to evaluate the nephroprotective effects of ethanolic roots extract of *Pseudocedrela kotschy* against nephrotoxicity and oxidative stress in alloxan-induced diabetic male albino rats.

Diabetic nephropathy is one of the major complications of diabetes that is associated with the

excretion of albumin in urine (Harcycy, 2002). Microalbuminuria and proteinuria typically reflect the presence of moderate and severed lesions, respectively, in kidney disease (Zipp and Schelling, 2003). However, the development of diabetic nephropathy is characterised by a progressive increase in urinary protein particularly albumin and a decline in glomerular filtration rate, which eventually leading to end-stage renal failure (Remuzzi *et al.*, 2006).

It could be interpreted from the result that the median acute toxicity (LD₅₀) value of the extract was 8.85 g/kg bwt. The extract can be classified as being non-toxic, since the LD₅₀ by oral route was found to be much higher than WHO toxicity index of 2 g/kg.

Our current data indicate that blood glucose level significantly increased, but body weight gain decreased after injection of ALX in albino rats.

Ethanollic roots extract of *Pseudocedrela kotschyi* normalizes the high blood glucose levels in diabetic rats. Glibenclamide was used as reference drug in diabetic models. It is interesting to note that the extract was more effective than reference drug.

However, the higher concentration of the extract used against the reference drug glibenclamide could be because of only a small amount of active substance present in the extract. Since, good activity has been seen in diabetic rats with damaged glomeruli therefore, it is likely to be expected that the ethanollic roots extract of *Pseudocedrela kotschyi* has some direct effect by increasing the tissue utilization of glucose (Ali *et al.*, 1993).

In the present study, a single dose of alloxan injection affected kidney functions and produced a marked increase in glucose, urea, creatinine, lipid peroxidation levels and decrease in oxidation stress markers over a period of 28 days. Treatment with both the ethanollic roots extract of *Pseudocedrela kotschyi* and glibenclamide prevented urinary excretion of glucose. Importantly, marked reduction in glucose urea, creatinine, oxidative stress, lipid peroxidation levels and increase in oxidative stress markers over a period of 28 days brought about by the ethanollic roots extract of *Pseudocedrela kotschyi* indicates its protective effect on the renal functions (De Zeeuw, 2007; Boukhris *et al.*, 2012).

An serum levels of urea and creatinine as a waste product formed during the digestion of proteins. An increase in serum of urea and creatinine levels in ALX-induced diabetic rats may indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine which is also another characteristic change in diabetes. The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt.

Furthermore, the results indicate that treatment of diabetic groups with ethanollic roots extract of *Pseudocedrela kotschyi* significantly reduced serum urea and creatinine levels. Based on these findings, the extract of this plant may enhanced the ability of the kidneys to remove these waste products from the blood as indicated by reduction in serum urea and creatinine levels and thus, confer protective effect on the kidney of diabetic rats.

In addition, administration of nephrotoxic doses of alloxan to rats resulted in development of oxidative

stress damage in renal tissue. In this study, alloxan induced nephrotoxicity showed a significant ($p < 0.05$) increase in the serum urea and creatinine concentrations in the Group II (diabetic untreated group) rat when compared to the normal group (Group I). Moreover, oral administration of ethanollic roots extract of *Pseudocedrela kotschyi* significantly ($p < 0.05$) decreased the levels of urea and creatinine in group IV and V when compared to the Group II. However the levels of urea and creatinine were significantly increased ($p < 0.05$) in the Group II rats when compared to Group I.

Thus, oxidative stress and lipid peroxidation are early events related to radicals generated during the renal metabolism of alloxan and also the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity (Roy *et al.*, 2005; Boukhris *et al.*, 2012).

Evaluation of SOD, CAT, GPx, lipid peroxidation, as well as GSH content and other antioxidant enzyme activities in biological tissue have been always used as markers for tissue injury and oxidative stress (Roy *et al.*, 2005; Boukhris *et al.*, 2012).

In view of the established role of oxidative stress and altered antioxidant levels in the pathogenesis of diabetic complications, we have evaluated the effect of the ethanollic roots extract of *Pseudocedrela kotschyi* on the levels of SOD, CAT, GPx, GSH and MDA in the kidney tissue of the rats. Like the ethanollic roots extract of *Pseudocedrela kotschyi*, glibenclamide also afforded antioxidant protection to the kidney.

Previous studies have clearly demonstrated that alloxan induction increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue (Boukhris *et al.*, 2012).

During kidney damage, superoxide radicals are generated at the site of derangement and attenuate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism (Roy *et al.*, 2005; Boukhris *et al.*, 2012).

The present study also demonstrated that alloxan induction resulted in a decrease in the SOD, CAT, GPx and GSH activities, when compared to normal control rats. It is due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. When diabetic rat was treated with the *Pseudocedrela kotschyi*, the reduction of SOD, CAT, GPx and GSH activity was significantly decrease ($p < 0.05$) when compared with diabetic untreated group. However in the diabetic untreated animals the MDA levels are increased significantly, when compared to normal control rats. On Administration of ethanollic extract of *Pseudocedrela kotschyi*, the levels of MDA decreased significantly when compared to diabetic rats.

The histopathological studies of the kidney in this investigation provided additional evidence that damaged renal cells recovered with the treatment of ethanolic roots extract of *Pseudocedrela kotschyi*. The photomicrograph revealed severe degeneration of tubular and glomeruli, focal necrosis of tubules, cystic dilatation of tubules and fatty infiltration in diabetic control rats. These pathological conditions might be associated with increased diuresis and renal hypertrophy in the diabetic rats. Here, we demonstrated that injury to cells in the diabetic rats recovered by treatment of the ethanolic roots extract of *Pseudocedrela kotschyi* in 28 days. In this study, nephroprotective effects of ethanolic roots extract of *Pseudocedrela kotschyi* were observed in the photomicrograph that glomeruli appeared to be restored and tubules appeared to be regenerated.

CONCLUSION

We concluded that the ethanolic roots extract of *Pseudocedrela kotschyi* was found to effectively improve the renal function than the reference drug glibenclamide and ameliorate lesions associated with diabetic nephropathy in alloxan-induced nephrotoxicity rats. This was shown by improved activities of metabolic enzymes and recovered renal cells from injuries by ethanolic roots extract of *Pseudocedrela kotschyi* treatment in the diabetic rats. These results could further suggest that possible use of ethanolic roots extract of *Pseudocedrela kotschyi* as a nutraceutical supplement to cope with diabetic-induced detrimental effects and to protect renal cells from damages.

REFERENCES

- Aebi, H., 1984. Catalase. In: Bergmeyer, H. (Ed.), Methods of Enzymatic Analysis. Verlag Chemical, Weinheim, 3: 273.
- Akande, J.A. and Y. Hayashi, 1998. Potency of extract contents from selected tropical chewing sticks against *Staphylococcus aureus* and *Staphylococcus auricularis*. World J. Microb. Biot., 14: 235-238.
- Akuodor, G.C., A.O. Essien, G.A. Essiet, E. David-Oku, J.L. Akpan and F.V. Udoh, 2013. Evaluation of antipyretic potential of *Pseudocedrela kotschyi* Schweint Harms (Meliaceae). Eur. J. Med. Plants, 3(1): 105-113.
- Ali, L., A.K. Khan, M.I. Mamun, M. Mosihuzzaman, N. Nahar, M. Nur-E-Alam and B. Rokeya, 1993. Studies on hypoglycemic effects of fruit pulp, seed and whole plant of *Momordica charantia* on normal and diabetic model rats. Planta Med., 59: 408-412.
- Anuka, J.A., D.O. Ijezie and O.N. Ezebnik, 1999. Investigation of pharmacological actions of the extract *Pseudocedrela kotschyi* in laboratory animals. Abstracts of the Proceedings of XXV 11th Annual Regional Conference of WASP, pp: 9-10.
- Asase, A., A.A. Oteng-Yeboah, G.T. Odamtten and M.S.J. Simmonds, 2005. Ethnobotanical study of some Ghanaian anti-malarial plants. J. Ethnopharmacol., 99: 273-279.
- Bothon, F.D.A., E. Debiton, F. Avlessi, C. Forestier, C. Teulade and K.C. Sohounhlonge, 2013. In vitro biological effects of two anti-diabetic medicinal plants used in Benin as folk medicine. BMC Complem. Altern. M., 13(51): 1-8.
- Boukhris, M., M. Bouaziz, I. Feki, H. Jemai, A. El Feki and S. Sayadi, 2012. Hypoglycemic and antioxidant effects of leaf essential oil of *Pelargonium graveolens* L'Hér. in alloxan induced diabetic rats. Lipids Health Dis., 11: 81.
- Burger, C., D.R. Fisher, D.A. Cordenuzzi, A.P. Batschauer, V. Cechinel Filho and A.R. Soares, 2005. Acute and subacute toxicity of the hydroalcoholic extract from *wedelia paludosa (Acmela brasiliensis)* (asteraceae) in mice. J. Pharm. Pharm. Sci., 8(2): 370-373.
- De Zeeuw, D., 2007. Albuminuria: A target for treatment of type 2 diabetic nephropathy. Semin. Nephrol., 27: 172-181.
- Earl, M.A., 2005. Emerging therapies in the treatment of type 2 diabetes. Pharmacother. Update, 8: 1-8.
- Ecobichon, D.J., 1997. The Basis of Toxicology Testing. RC Press, New York, pp: 43-86.
- Ellman, G.L., 1995. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.
- Georgewill, O.U. and A.O. Georgewill, 2009. Effects of extract of *Pseudocedrela kotschyi* on blood glucose concentration of Alloxan-induced diabetic rats. Eastern J. Med., 14(1): 17-19.
- Giorgino, F., L. Lavidia, P.P. Cavallo, B. Solnica, J. Fuller and N. Chaturvedi, 2004. Factors associated with progression to macroalbuminuria in microalbuminuric type 1 diabetic patients: The EURODIAB prospective complications study. Diabetologia, 47: 1020-8.
- Harcycy, Y., 2002. Diabetic nephropathy. Biomed. J., 325: 59-60.
- Hutchinson, J. and J.M. Dalziel, 1958. Flora of West Tropical Africa. 2nd Edn., Crown Agents for Overseas Governments and Administrations, Millbank, London, pp: 702.
- Koné, W.M., K.K. Atindehou, C. Terreaux, K. Hostettmann, D. Traore and M. Dosso, 2004. Traditional medicine in North Côte d'Ivoire: Screening of 50 medicinal plants for antibacterial activity. J. Ethnopharmacol., 93: 43-49.
- Li, W.L., H.C. Zheng, J. Bukuru and N. De-Kimpe, 2004. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J. Ethnopharmacol., 92: 1-21.
- Lieber, C.S., 1997. Ethanol metabolism, cirrhosis and alcoholism. Clin. Chim. Acta, 258: 59-84.

- Mohamed, A.K., A. Bierhaus, S. Schiekofer, H. Tritschlet, H. Ziegler and P.P. Nawroth, 1999. The role of oxidative stress and NF (B) activation in late diabetic complications. *Biofactors*, 10: 175-179.
- Musa, Y.M., A.R. Haruna, M. Ilyas, A.H. Yaro, A.A. Ahmadu and H. Usman, 2005. Analgesic and anti-inflammatory activities of the leaves of *Pseudocedrela kotschy* Harms (Meliaceae). Books of abstracts of the 23rd National Scientific Conference of the Nigerian Society of Pharmacognosy, pp: 88-89.
- Oberley, L.W., 1988. Free radicals and diabetes. *Free Radic. Biol. Med.*, 5: 1113-1124.
- Odugbemi, T., 2006. *Outlines and Pictures of Medicinal Plants from Nigeria*. Lagos University Press, Lagos, pp: 20-596.
- Ogunmodede, O.S., L.C. Saalu, B. Ogunlade, G.G. Akunna and A.O. Oyewopo, 2012. An evaluation of the hypoglycemic, antioxidant and hepatoprotective potentials of onion (*Allium cepa* L) on alloxan-induced diabetic rabbits. *Int. J. Pharmacol.*, 6: 1-9.
- Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95: 351-358.
- Ojewale, A.O., A.O. Adekoya, O.A. Odukanmi, O.T. Olaniyan, O.S. Ogunmodede and B.J. Dare, 2013a. Protective effect of ethanolic roots extract of *Pseudocedrela kotschy* (Pk) on some hematological and biochemical parameters in alloxan-induced diabetic rats. *World J. Pharm. Pharm. Sci.*, 2(3): 852-866.
- Ojewale, A.O., O.T. Olaniyan, O.K. Yemitan, O.A. Odukanmi, O.S. Ogunmodede, W.S. Nnaemeka, O. Omoaghe, A.M. Akingbade, B.J. Dare and A.O. Adebari, 2013b. Hypoglycemic and hypolipidemic activities of ethanolic roots extract of *Crossopteryx febrifuga* in alloxan-induced diabetic rats. *Mintage J. Pharmaceut. Med. Sci.*, 2(3):48.
- Okunade, M.B., J.A. Adejumobi, M.O. Ogundiya and A.L. Kolapo, 2007. Chemical, phytochemical compositions and antimicrobial activities of some local chewing sticks used in South Western Nigeria. *J. Phytopharm. Nat. Prod.*, 1(1): 49-52.
- Palanduz, S., E. Ademoglu and C. Gokkusu, 2001. Plasma antioxidants and type 2 diabetes mellitus. *Pharmacology*, 109: 309-318.
- Remuzzi, G., M. Macia and P. Ruggenenti, 2006. Prevention and treatment of diabetic renal disease in type 2 diabetes: The benedict study. *J. Am. Soc. Nephrol.*, 17: 90-97.
- Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, 179: 588-590.
- Roy, S., R. Sehga, B.M. Padhy and V.L. Kumar, 2005. Antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats. *J. Ethnopharmacol.*, 102: 470-473.
- Shahina, G., 1989. *Savanna Plants: An Illustrated Guide*. Macmillan Publishers Ltd., London, pp: 105.
- WHO, 1980. Expert committee on diabetes mellitus. Technical Report Series 646, World Health Organization, Geneva.
- Winterbourn, C.C., R.E. Hawkins, M.M. Brian and R.W. Carrell, 1975. The estimation of red cell superoxide dismutase activity. *J. Lab. Clin. Med.*, 85: 337-341.
- Zipp, T. and J.R. Schelling, 2003. Diabetic Nephropathy. In: Hricik, D.E., R.T. Miller and J.R. Sedor (Eds.), 2nd Edn., *Nephrology Secrets*. Hanley & Belfus Inc., Medical Publishers, Philadelphia, pp: 105-111.