Research Article

Nephroprotective Activities of Ethanolic Roots Extract of Pseudocedrela kotschyi against Oxidative Stress and Nephrotoxicity in Alloxan-induced Diabetic Albino Rats

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Abstract: Introduction: The present study was designed to evaluate the nephroprotective activities of ethanolic roots extract of Pseudocedrela kotschyi 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 Pseudocedrela kotschyi 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 Pseudocedrela kotschyi on blood glucose, Urea, Creatinine, renal oxidative stress markers and lipid peroxidation were measured in the diabetic rats. Results: The ethanolic roots extract of Pseudocedrela kotschyi 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 Pseudocedrela kotschyi against alloxan induced necrotic damage of renal tissues. Conclusion: From the above results, we concluded that the ethanolic roots extract of Pseudocedrela kotschyi 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, pseudocedrela kotschyi, 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
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 kotschyi_ (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 (Akan 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., 2004), antibacterial (Bothon 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 kotschyi_ 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 kotschyi_ against nephrotoxicity and oxidative stress in alloxan induced diabetic rats.

**MATERIALS AND METHODS**

**Collection of the Plant material:** _Pseudocedrela kotschyi_ (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, Iddi -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 kotschyi_ 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 kotschyi_ 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 kotschyi_ 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 kotschyi* at a dose of 250 mg/kg bwt

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

Treatments were administrated 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 buin’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 µm thick were obtained from a solid block of tissue and were stained with heamatoxylin 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 u mg⁻¹ protein.

**Assay of Glutathione Peroxidase (GPx) activity**: Glutathione peroxidase activity was determined by the method described by Rotruck et al. (1973). The absorbance 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 absorptive of malondialdehyde which is 1.56×10⁵ M⁻¹ cm⁻¹ and it was expressed as nmol/mg protein.

**Statistical analysis**: Data are presented as means±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.
**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 kotschyi* 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 significantly decreased as compared to that of control group while diabetic treated groups with *Pseudocedrela kotschyi* and glibenclamide decreased by improving the restoring activity of *Pseudocedrela kotschyi* 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) compared to normal group. On the other hand, administration of ethanolic roots extract of *P. kotschyi* 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 kotschyi* extract showed significantly 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 kotschyi* extract 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 kotschyi (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 kotschyi (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: ×400

Fig. 2: Cross section of diabetic kidney stained with H & E, Mag: ×400
DISCUSSION

This present study was aim to evaluate the nephroprotective effects of ethanolic roots extract of *Pseudocedrela kotschyi* 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 severely 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.

Ethanolic 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 ethanolic 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 ethanolic 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 ethanolic 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 ethanolic 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 ethanolic 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 ethanolic roots extract of *Pseudocedrela kotschyi* on the levels of SOD, CAT, GPx, GSH and MDA in the kidney tissue of the rats. Like the ethanolic 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 ethanolic 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.

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