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Testiculo Protective Effects of Ethanolic Roots Extract of *Pseudocedrela kotschyi* on Alloxan Induced Testicular Damage in Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study and performed the statistical analysis. He equally wrote the first draft of the manuscript and undertook the final editing of the paper. Author OTO wrote the protocol, part of the draft and undertook in the initial editing of the paper. Authors OAO and BJD carried out most of the literature searches while author JAO handled the tissue processing for histology and author FAF performed the histological analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The study was designed to investigate the testiculo protective effects of ethanolic roots extract of *Pseudocedrela kotschyi* on alloxan-induced testicular damage in diabetic rats.
Study Design: Experimental diabetes using animal models.
Place and Duration of Study: Department of Anatomy, College of Medicine, Lagos

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State University, Ikeja, Lagos, Nigeria, between January, 2013 and May, 2013.
Methodology: Twenty male rats were divided into four groups: Group I consisted of non-diabetic rats that received only the vehicle; group II-IV was injected with a single dose of alloxan (ALX) of 150 mg kg^{-1} intraperitoneally; groups III and IV were given ethanolic roots extract of *Pseudocedrela kotschyi* orally, 3 days after the ALX administration, at daily doses of 250 and 500mg kg^{-1} respectively for a period of 30 days. After 4 weeks of treatments, all the rats were sacrificed.
Results: Administration of 150 mg kg^{-1} of alloxan to male rats induced diabetes and significantly reduced the body and testicular weights, testosterone levels, sperm count and motility, significantly increased the glucose level and decreased the levels of antioxidant enzymatic and non-enzymatic markers such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and while the level of malondialdehyde (MDA) was significantly increased. By contrast, rats given the ethanolic roots extract of *Pseudocedrela kotschyi* had significantly increase ($p<0.05$) in body weight gain, whereas the glucose levels significantly improved ($p<0.05$) in treated diabetic male rats. In addition, this extract improved the reproductive system of the diabetic male rats by significantly increasing the testis and epididymis weights, testosterone levels, sperm count and motility, reduced testicular GSH, CAT, SOD, GPx and significantly decreasing MDA.The extract had no deleterious effects and testicular cytoprotection damaged by ALX.
Conclusion: We concluded that the treatment with the ethanolic roots extract of *Pseudocedrela kotschyi* could reverse the adverse effects of ALX-diabetes on reproductive system of male rats which exhibits antihyperglycemic and fertility activities.

Keywords: Antihyperglycemia; antioxidants; spermatogenic activities; alloxan; rats; Pseudocedrela kotschyi.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder with developing pathological complications [1].DM is mainly due to relatively low level of production of insulin or an inability of the body to use insulin properly which leads to hyperglycemia [2].

Recently, hyperglycemia has been implicated in induction of oxidative stress which leads to initiation and development of diabetic complications. Diabetic complications are physical disability, kidney failure, visual impairment, cardiovascular disease and sexual dysfunction [3]. The direct effects of insulin depletion on male reproductive system of rats have been reported [4]. High doses of alloxan (ALX) administrated to male rats leads to a decrease in Leydig cell function, testosterone production, sperm output and fertility [5].

Many plants have been shown to possess antidiabetic and hypoglycemic properties by lowering the blood glucose levels and reducing the various complications associated with diabetes. For example, the antifertility activities of extracts from *Carica papaya*, *Quassia amara*, *Moringa oleifera, Azadirachta indica* and *Zingiber officinale* had been documented anti-diabetic properties had been reported [6-10].

Pseudocedrela kotschyi (PK) is a member of the family Meliaceae. The plant is widespread in savannah woodland [11-12]. It is a tree of up to 20 m high with a wide crown and fragrant white flowers [12]. 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 used as a febrifuge and for the treatment of gastrointestinal diseases and rheumatism [11]. The plant has also been reported to be used traditionally in the treatment of dysentery [12]. The analgesic, anti-inflammatory activities of plant [13], antiepileptic [14], and dental cleaning has also been reported [15-16]. This plant has demonstrated a wide range of biological effects such as antimalarial [17], anticonvulsant [18], antibacterial [19], antimicrobial [20], antipyretic [21] and antidiabetic activities [22-23].

Despite widespread use of *Pseudocedrela kotschyi* in folklore medicine for DM and other ailments, its protective effects on the reproductive system has not been elaborated. Alloxan (ALX) induced diabetes in rats provides a good model to study reproductive defect, as it exhibits a number of defects in reproductive organs that resemble those seen in diabetic humans. Therefore, testiculo protective effects of ethanolic roots extract of *Pseudocedrela kotschyi* on testicular damage in reproductive structures and functions of alloxan-induced rats.

2. MATERIAL AND METHODS

2.1 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).

2.1.1 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. 650 g of the root powder was soaked in 6500 ml of 96% ethyl alcohol in 3 cycles using soxhlet extractor. The crude extract was filtered through filter paper (Whatman No 4). The filtrate was concentrated and dried in a rotary vacuum evaporator at 30^0C to obtain 97.2g dry residue to yield an (14.9% vol.) viscous brownish-coloured extract which was stored in an air tight bottle kept in a refrigerator at 4^0C till used [24].

2.2 Experimental Animals

Twenty healthy male albino rats weighing between 160-180 g were obtained from the Laboratory Animal Center of College of Medicine, Lagos State University, Ikeja, Lagos, Nigeria. The rats were housed in clean cages with the filter tops under controlled conditions of 12 h light/12 h dark cycle, 50% humidity at 26±2ºC and kept in a well-ventilated room and allowed to acclimatize to the laboratory condition for two weeks before being used. They were maintained on a standard animal pellet (CHI Feeds Plc., Nigeria) and had free access to water *ad libitum*.

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.

2.3 Acute Toxicity Studies

The acute toxicity of ethanolic roots extract of *Pseudocedrela kotschyi* were determined by using thirty-five (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 14 h prior to the experiment with access to water only, the control group was administered with single dose of ethanolic roots extract of *P. kotschyi* with at a dose of 0.3ml of 2% acacia solution orally while each treated group was administered with single dose of ethanolic roots extract of *P. kotschyi* orally with at a doses of 1.0, 2.5, 5.0, 10, 15 and 20.0g/kg body weight respectively of 2% acacia solution. They were closely observed in the first 4 hours and then hourly for the next 12 hours followed by hourly intervals for the next 56 hours and continued for the next 2weeks after the drug administration to observe any death or changes in behaviour, economical, neurological profiles and other physiological activities [25-26].

2.4 Experimental Design

To induce diabetes, rats were first anesthetized with inhalation of gaseous nitrous. Alloxan® (Sigma, St, Louis, MO, USA) was purchased from representative of Sigma Company in Nigeria and was prepared in fresh normal saline. Diabetes was induced by intraperitoneal (ip) injection of alloxan monohydrate (150 mg/kg bwt) in a volume of 3 mL [27]. 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 [28].

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 $mLkg^{-1}$ body weight), and served as control.
Group II: Alloxan diabetic rats that received only vehicles (0.5 $mLkg^{-1}$body weight).
Group III: Alloxan diabetic rats treated with *P. kotschyiat* a dose of 250 mg/kg bwt
Group IV: Alloxan diabetic rats treated with *P. kotschyiat* 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.

2.5 Sample Collection

Samples were collected every week from each animal for blood glucose analysis and 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. Serum was collected and kept at -20°C for hormone determination. At the end of the experimental period, each rat was weighed and starved for 24 h. Then, blood samples were collected from each animal by cardiac puncture and rats were sacrificed under light ether anaesthesia. Epididymis and testes were carefully dissected out and rinsed in cold saline solution, weighed and processed immediately as described below.

After one month of treatment, the animals were sacrificed by decapitation. The trunk blood was collected and 2 ml of blood were distributed into tubes containing an anticoagulant agent, followed by centrifugation at 3000 rpm for determination of plasma glucose level. The remaining blood was placed in a dry tube, centrifuged and the serum was aliquoted into 1.5 ml vials, and frozen at -20°C for determination of testosterone level. Testis and epididymis were excised immediately, washed with ice-cold physiologic saline solution (0.9%, w/v), blotted and weighed. About 1 g of each organ was cut into small pieces homogenized with an Ultra Turrax homogenizer in 2 ml ice-cold appropriate buffer (pH 7.4) and centrifuged at 9000 rpm for 15 min at 4°C. Supernatants were collected, aliquoted and stored at - 60°C until use for enzyme assays.

2.6 Glucose and Testosterone Determination

Glucose was measured by the glucose oxidase method using a commercially available kit (ACCU-CHEK, Roche Diagnostics). All serum samples were assayed for testosterone using tube-based enzyme immunoassay methodology (EIA) and the absorbency was read at 450 nm as described by Raji et al. [29]. The EIA kits were obtained from DiaSys (Germany).

2.7 Evaluation of Epididymal, Sperm Count and Motility

Epididymal contents were obtained after cutting the tail of epididymis, squeezing it gently on clean slide and the sperm progressive motility and cell count were determined according to the method described by Yokoi et al. [30]. Briefly, the cauda epididymis was minced with anatomical scissors in 2 ml of Earles buffer placed in a rocker for 10 min at 37°C. After dilution, the number of homogenization-resistant spermatozoon was counted in a haemocytometer and about 25 fields of view were examined under a light microscope at 40× magnification.

2.8 Evaluation of Testicular Enzymatic Antioxidants

2.8.1 Assay of catalase (CAT) activity

Catalase activity was evaluated according to the method described by Aebi [31]. Activity of catalase was expressed as units mg^{-1} protein.

2.8.2 Assay of superoxide dismutase (SOD) activity

Superoxide dismutase activity was evaluated according to the method described by Winterbourn et al. [32]. It was expressed as u mg^{-1}protein.

2.8.3 Assay of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was determined by the method described by Rotruck et al. [33]. The absorbance of the product was read at 430 nm and it was expressed as $nmol^{-1}$protein.

2.9 Evaluation of Testicular Non-Enzymatic Antioxidants

2.9.1 Assay of testicular reduced glutathione (GSH) activity

Reduced GSH was measured according to the method described by Ellman [34]. The absorbance was read at 412nm, it was expressed as $nmol^{-1}$ protein.

2.9.2 Assay of lipid peroxidation (Malondialdehyde)

Lipid peroxidation in the testicular tissue was measured colorimetrically by thiobarbituric acid reactive substance (TBARS) method described by Buege and Aust [35]. 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^{-1} protein.

2.10 Histological Analysis

This was done as described by Kusemiju et al. [36]. Briefly, after 48 h the organs were removed from Bouin's fluid and fixed in fresh Bouin's fluid for another 72 h. Each testis was sliced into slabs of about 0.5 cm thick and dehydrated in varying degree of alcohol (70%, 90%). From 90% alcohol to 3 changes of absolute alcohol for 1 hour each, then into chloroform for about 10 h and later transferred into fresh chloroform for about 30 min. The tissues were placed in 3 changes of molten paraffin wax for 30 min each in an oven at 57 °C. They were placed vertically in molten paraffin wax inside a plastic mould and left overnight to cool and solidify. They were later trimmed and mounted on wooden blocks. Serial sections were cut using a rotary microtome at 5 µm thickness. Sections were floated in a water bath and picked by albuminized slides and dried on the hot plate at 57 °C. To stain, the slides were de-waxed in staining racks and placed in staining wells containing xylene and rehydrated in varying degree of alcohol (absolute, 90%, and 70%) and then to water for 5 min after which they were stained with heamatoxylin for 3 min. Excess heamatoxylin was washed off with water and differentiated with 1% acid alcohol. Sections were rinsed under running tap water and then left for 5 min for blueing. Sections were counterstained with 1% eosin and washed off with water. They were dehydrated with 70%, 90% and absolute alcohol and cleared in xylene to remove all traces of water. A drop of mountant was placed on the surface of the slide and covered with a 22 by 22 cm cover slip. Light microscopy was used for the evaluations and the photomicrographs were taken.

2.11 Statistical Analysis

Data are presented as means ± SD. Student'st-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 ($p< 0.05$).

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity

The acute toxicity study result (Table 1), showed that four out of the five animals that received 20.0 g/kg bwt of the extract died within 4 h (80 % death) while the animals that received 2.5 g/kg body weight survived beyond 24 h. The LD_{50} of the drug was therefore calculated to be 15 g/kg bwt. The LD_{50} of the extract was determined by plotting a graph of probit on the Y-axis against the log dose on the X-axis.

Table 1. Acute toxicity of the Ethanolic roots extract of *Pseudocedrela kotschyi*

Groups	Dose (g/kg)	Log dose	24hr mortality	% Mortality	Probit
I	1.0	3.00	0/5	0.0	0.0
II	2.5	3.40	0/5	0.0	0.0
III	5.0	3.70	1/5	20.0	4.2
IV	10.0	4.00	1/5	20.0	4.2
V	15.0	4.18	2/5	40.0	4.7
VI	20.0	4.30	4/5	80.0	5.8

Control group received 0.3ml each of 2% Acacia solution

3.2 Effect on Body Weight of Male Rats

To determine the effect of treatment of *P. kotschyi* extract on body weight in the ALX-treated rats, the animals (Table 2) showed decrease in appetite and weight depreciation after alloxan induction. All rats were monitored for gain in body weight. The control group (I) gained weight over the four weeks of experimental period, with the mean body weight increasing by 29 g after 4 weeks (Table 2). In contrast, the untreated diabetic group (II) lost an average of -25.6 g after 4 weeks ($p<0.05$). Treatment with *P. kotschyi* resulted in significant weight gain to levels approaching the control group (Groups III and IV, versus Group I). In the untreated group, progressive weight decrease occurred while in the extract treated groups, there was weight appreciation after few days of treatment as well as showed increase in appetite.

Table 2. Effect of oral administration of ethanolic roots extract of *Pseudocedrela kotschyi* for four weeks on body weight (g) in ALX-diabetic male rats

Groups	Initial body weight (g)	Final body weight (g)	Difference in body weight (g)
I	164.3±6.2	183.3±10.8	29
II	168.4±9.3	142.8±10.1*	-25.6
III	172.6±8.6	186.4±7.5**	14.2
IV	174.2±5.1	195.0±8.5**	20.8

Values are the mean values ± standard deviation of 5 rats; Group I: consisting of control rats, Group II: consisting of diabetic rats, Group III: consisting of treated diabetic rats received 250mg of Pseudocedrela kotschyi, Group IV: consisting of treated diabetic rats received 500mg of Pseudocedrela kotschyi
**: Statistically significant when compared to control group (I) at p<0.05;*
***: Statistically significant when compared to diabetic untreated group (II) at p<0.05*

3.3 Effect on Serum Glucose Concentration

An increase in serum glucose concentration (mg dL^{-1}) was recorded in untreated diabetic group, relative to the control group (Table 3). After four weeks, the serum glucose concentration in untreated diabetic group increased to 362.2 mg dL^{-1}. In treated diabetic rats (Groups III/IV), the serum glucose concentration decreased to 254.2 and 242.7 mg dL^{-1}, respectively, after four weeks, which was significantly less than that in the untreated diabetic group ($p<0.05$).

Table 3. Effect of oral administration of ethanolic roots of extract *Pseudocedrela kotschyi* for four weeks on serum glucose concentration (mg dL^{-1}) in ALX-diabetic male rats serum glucose concentration (mg dL^{-1})

Groups	First week	Second week	Third week	Four week
I	83.8±4.8	85.2±7.4	91.4±7.8	87.4±4.1
II	343.4±20.2*	349.6±24.4*	357.1±15.8*	362.2±18.9*
III	298.2±21.4**	273.8±23.4**	269.5±25.4**	254.2±20.2**
IV	290.4±14.7**	269.3±17.8**	257.8±21.1**	242.7±18.4**

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 diabetic untreated group (II) at p<0.05*

3.4 Effect on Serum Level of Testosterone

The diabetic rats showed a decrease in serum testosterone levels compared to level of the control rats (Table 4). Treatment of the diabetic rats with *P. kotschyi* (Groups III/IV) caused a significant increase in the levels of testosterone in a dose-dependent manner ($p<0.05$).

3.5 Effect on Sex Organ Weights, Sperm Count and Motility of Male Rats

Table 4 shows that the weight of testes and epididymis of diabetic untreated group were significantly lowered at the fourth week as compared with those of the control group ($p<0.05$).Treatment of the diabetic male rats with *Pseudocedrela kotschyi* (Groups III/IV) caused a significant increase in testis and epididymis weights ($p<0.05$). On other hand, sperm count and motility of untreated diabetic group were also lowered, as compared with those of the control group. This reduction was statistically significant ($p<0.05$).Furthermore, administration of ethanolic roots extract of *P. kotschyi* for 4 weeks showed a significant improvement in the sperm count and motility of treated diabetic groups (Groups III/IV) in a dose-dependent manner ($p<0.05$). However, the sperm count and motility of the treated diabetic groups (III and IV) were still lower than those of control group I.

Table 4. Effect of oral administration of *Pseudocedrela kotschyi* extract after 4weeks on testis and epididymis weights, serum testosterone level (ng mL^{-1}), sperm count and motility in ALX-diabetic male rats

Parameters	Groups			
	I	II	III	IV
Testis wt (g)	1.493±0.241	0.877±0.186*	1.203±0.103**	1.442±0.134**
Epididymis wt (g)	0.677±0.184	0.265±0.052*	0.582±0.155**	0.643±0.155**
Testosterone (ng mL^{-1})	0.754±0.087	0.387±0.029*	0.588±0.048**	0.678±0.076**
Sperm count x 10^6 (mL)	43.20±6.600	16.40±4.700*	25.20±6.700**	29.20±3.600**
Sperm motility (%)	87.90±9.400	26.70±10.700*	68.40±7.500**	79.60±8.700**

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 diabetic untreated group (II) at p<0.05*

3.6 Effect on Testicular Enzymatic and Non-enzymatic Antioxidants

Diabetic rats showed significant lowering in SOD, CAT and GPx compared to the control ($p<0.05$). Diabetic rats treated with the *P. Kotschyi* significant higher in testicular SOD, CAT and GPx levels compared to diabetic rats without treatment ($p<0.05$). Along the same line for the testicular GSH and MDA, GSH level in diabetic rats was significantly lower compared to normal rats ($p<0.05$). However the diabetic rats treated with the *P. kotschyi* showed significantly increase intesticular GSH compared to normal rats ($p<0.05$), on the other hand the level of MDA was significantly increased in diabetic rats without treatment compared to normal rats ($p<0.05$) while the level of MDA in diabetic treated groups with the *P. kotschyi* (Table 5) were significantly lower ($p<0.05$) compared to normal rats.

Table 5. Effect of oral administration of *Pseudocedrela kotschyi* extract after 4weeks on Testicular enzymatic and non enzymatic antioxidant in ALX-diabetic male rats

Parameters	Groups			
	I	II	III	IV
CAT (μmg^{-1} protein)	13.2±1.4	5.2±2.3*	12.5±1.9**	12.8±1.1**
SOD (μmg^{-1} protein)	48.7±3.0	12.4±1.3*	38.3±2.8**	42.5±2.5**
GPx (nmolmg-^{1} protein)	0.9±0.2	0.28±0.1*	0.65±0.2**	0.72±0.3**
GSH (nmolmg^{-1} protein)	2.6±0.2	0.89±0.2*	1.8±0.1**	2.3±0.2**
MDA (nmolmg^{-1} protein)	0.9±0.1	4.4±0.4*	1.4±0.2**	1.2±0.1**

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*

3.7 Morphology of Testis

The testis of control or normal rats (Fig. 1a) exerted different stages in seminiferous elements comprising germ cells, leydig cells and interstitial cells, which were normal in appearance compared to rats in the diabetic untreated group.

Towards the lumen, the primary spermatocytes, secondary spermatocytes, early spermatids and elongated spermatids were associated with leydig cells. Mature spermatozoa were also visible in the same region. The diabetic rats without treatment (Fig. 1b) showed testicular atrophy, and degeneration in the various stages of spermatogenesis. The basement membrane and tunica propria became thin and disrupted. Spermatogenesis was arrested either at the primary spermatocyte or at the spermatogonial stages. The sertoli cells showed vacuolization and cell debris due to cytolysis. The intercellular spacing became wider, Leydig cells reduced in numbers and the interstitium contained mostly fibroblasts. Diabetic rat treated with the ethanolic roots extract of *P. Kotschyi* 250 mgkg^{-1} (Fig. 1c) showed seminiferous tubules atrophy and presence of some germ cells in some tubules. Diabetic rat treated with the ethanolic roots extract of *P. kotschyi* 500 mgkg^{-1} (Fig. 1d) showed that the animals regenerated almost their normal general histology. Most of their seminiferous tubules were close together with regular outlines and narrow interstitium. A few tubules still had regeneration in their germinal epithelium, areas of epithelial separation, and a few sperm cells in their lumina were observed.

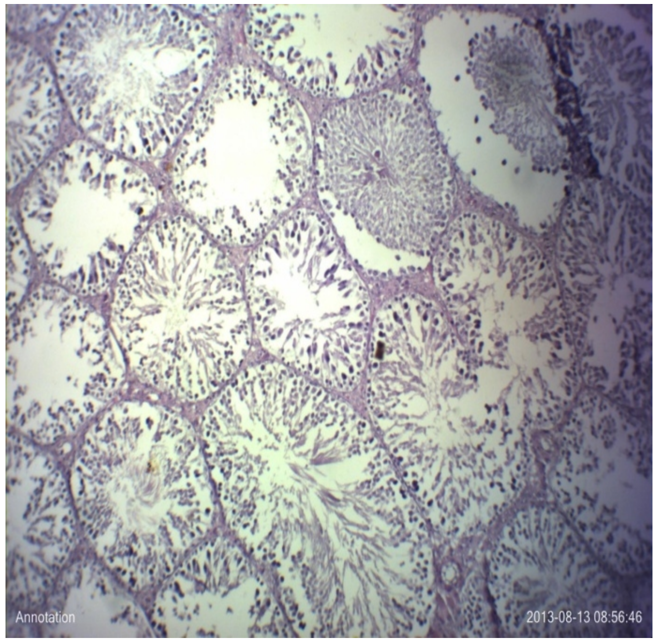

Fig. 1a. Cross section of control (normal) rat treated with distilled water 0.5 mlkg-1 b.w for 28days. It was stained with the heamatoxylin and eosin; Mag×400.

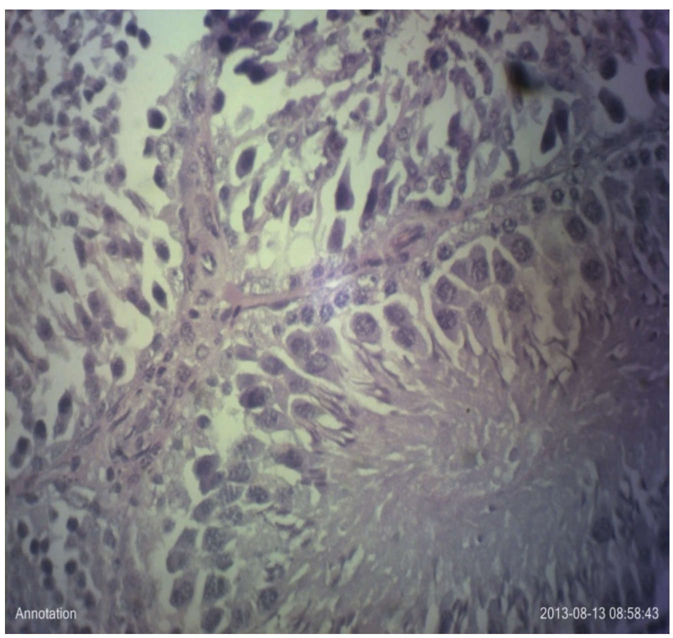

Fig. 1b. Cross section of diabetic rat (negative control) treated with normal saline 0.5 mLkg-1 b.w for 28days. It was stained with the hematoxylin and eosin; Mag

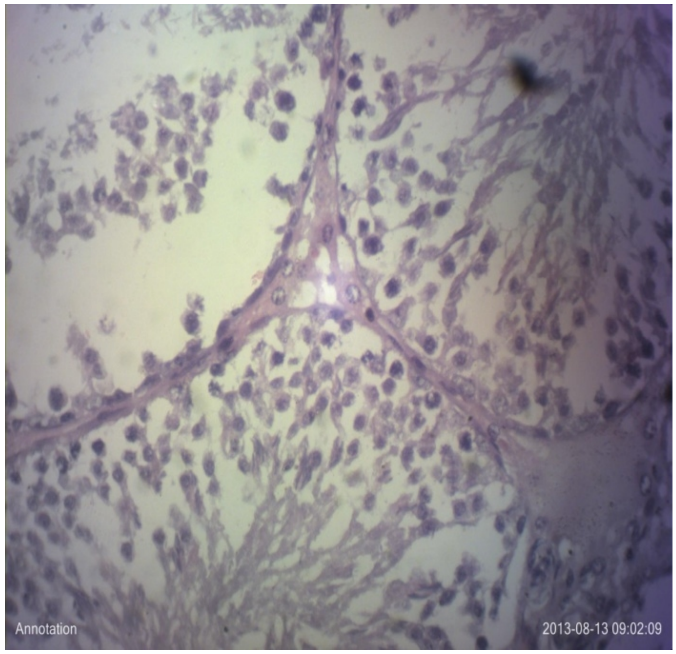

Fig. 1c. Cross section of diabetic rat treated with the *P. kotschyi* 250 mgkg^{-1} b.w for 28days. It was stained with heamatoxylin and eosin; Mag ×400

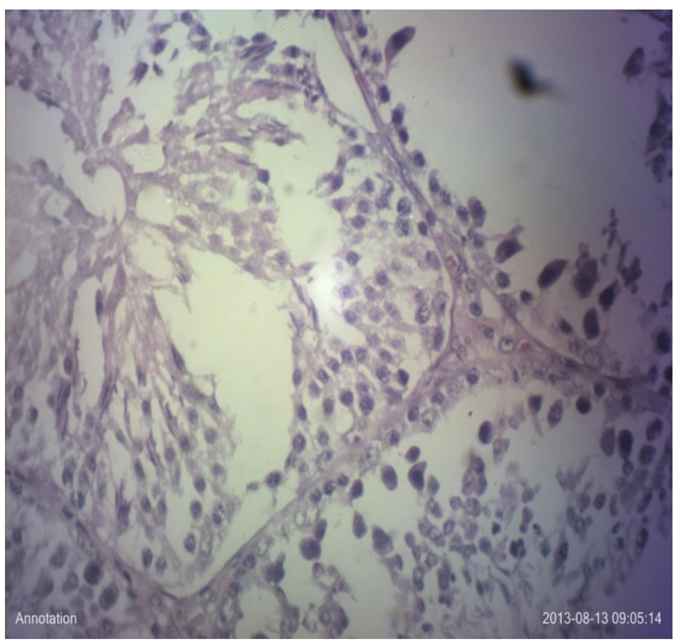

Fig. 1d. Cross section of diabetic rat treated with the *P. kotschyi* 500 mgkg^{-1} b.w for 28days. It was stained with heamatoxylin and eosin; Mag ×400.

4. DISCUSSION

Our current data indicate that blood glucose level significantly increased, but body weight gain decreased after injection of ALX to the male rats. Oral administration of ethanolic roots extract of *P. kotschyi* to the diabetic male rats at the dose of 250 and 500mgkg^{-1} for 30 days increased average weights of the bodies, testis and epididymis, with an improvement of semen quantity and motility, other than a decrease in blood glucose level, anincrease of serum testosterone level and the testicular oxidative stress markers as compared with the diabetic group.

It has also been reported that the cytotoxic action of ALX is mediated by the formation of free radicals such as superoxide and catalase radicals [37].It has been reported that ALX induction in animals increased the level of lipid peroxidation an indication of tissue damage. Sperm plasma membrane has a high content of polyunsaturated fatty acids which is easily susceptible to lipid peroxidation caused by oxidative stress [38-39].

It could be interpreted from the result that the lethal dose (LD_{50}) value of the extract was 15g/kg bwt. According to Ghosh [40] and Klaasen et al. [41], the extract can be classified as being non-toxic, since the LD_{50} by oral route was found to be much higher than WHO toxicity index of 2 g/kg.

Excessive ROS production that exceeds critical levels can overwhelm all antioxidants defense strategies of spermatozoa and seminal plasma causing oxidative stress that damages the biological membranes in the testes. This in turn may cause the degeneration of the spermatogenic and Leydig cells, which disrupts spermatogenesis and reduces sperm counts [42-43].

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 [38,44-46].

In our study, testicular oxidative damage by ALX induction are also exhibited by a significant decrease in the activities of antioxidant enzymes, SOD, CAT, GPx and the testicular content of GSH and a significant increase of testicular content of MDA as compared with the normal group. However, diabetic rats treated with the *P. kotschyi* markedly attenuates the oxidative damage by ALX induced in rats. Therefore it is possible to suggest that this extract is safe and might confer protection against ALX induced testicular damage as evidenced by normal levels of antioxidant enzymes and testicular contents in treated diabetic groups.

The extract caused an increase in the weight of the testes, which was accompanied by an increase in the serum levels of testosterone. Similar reports have been made with the extract of *Quassia amara, Azadirachta indica, Morinda lucida*, *Zingiber officinale* and Nigella sativa in rats [7,9-10,47-48]. The present study shows that injecting of male rats with ALX compound reduced weights of testis and epididymis, testosterone production, sperm motility and count, suggesting a toxic effect of ALX in the cytoarchitecture and functional integrity of testicular tissues. It has been reported that induction of diabetes by high doses of ALX in male testes lead to reduction in testosterone production, suggesting a decrease in the function of both Leydig (testosterone producing cell) and Sertoli (supporting cell), which might be due to a reduction in insulin secretion [49-50].

It has been reported that glucose oxidation and utilization are important means by which spermatozoa derives energy for their motility [51].

It has also been reported that testicular weight reduction was accompanied by decreased serum testosterone levels in male rats treated with the ALX-induced diabetic rats [52].

Both *Cnidoscolus aconitifolius* and *Nigella sativa* have been demonstrated to possess potent antifertility properties [52,48]. Since all organs of male reproduction are androgen receptor, they serve as indicators of the Leydig cell function or androgen action, testosterone in association with follicle stimulating hormone normally acts on the seminiferous tubules to initiate and maintain spermatogenesis [47,53-54].

However, a significant decrease in the epididymal sperm count of rats treated with the extract for 4 weeks was recorded despite the high serum testosterone level. Testosterone is known to be critically involved in the development of spermcells and derangement results widely in leydig cell malfunction and testicular steroidogenic disorder [10].

This study suggests that *P. kotschyi* does not have spermatoxic effects but rather, it could potentially be further investigate for use in management of the development of diabetes and improved the performance of male reproductive system.

In view of these findings, further investigations into the effects of *P. kotschyi* with longer period of higher doses may show clearer features on male reproductive hormones and fertility will be required.

5. CONCLUSION

Oral administration of ethanolic roots extract of *P. kotschyi* exerted antihyperglycemic and fertility activities in ALX-induced diabetic male rats with reversing the negative effects of ALX compounds. It was also shown to be non-toxic at the doses used in this study. In addition, based on the fact that the possible consumption of ethanolic roots extract of *P. Kotschyi* exhibits a potent protection on the testis of laboratory animals induced with ALX, it could be a potential alternative to improve the performance of male reproductive system in animals and humans provided that further investigations also support these findings.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. Allex periments have been examined and approved by the appropriate ethics committee". All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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