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Cytoprotective activities of Anacardium occidentale (Linn) on the testis of streptozotocin-induced diabetic rats

Victor Ukwenya*[,1], Olumide Ashaolu[1], David Adeyemi[2], Efere Obuotor[2], Bernard Enaibe[3] and Ezekiel Caxton-Martins[3]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Bowen University, Iwo, Nigeria, [2]Obafemi Awolowo University, Ile-Ife, Nigeria and [3]University of Ilorin, Ilorin, Nigeria

SUMMARY

This study presents the protective effects of methanolic *Anacardium occidentale* leaf extract (AOLE) on the testes of streptozotocin (STZ)-induced diabetic rats. Forty male wistar rats (*Rattus norvegicus*) were randomly divided into four experimental groups A,B,C and D. Hyperglycaemia was induced by a single intraperitoneal injection of 0.1 M STZ (70 mg/kg b.w). Five days after the confirmation of hyperglycaemia by using a glucometer (Roche$^{(R)}$) and compatible glucose test strips, groups A and B were treated with 300 mg/kg b.w of the extract and 1 I.U/kg b.w insulin respectively. Groups C and D served as hyperglycaemic and normal controls and received 1 ml/kg b.w citrate buffer respectively.

After 16 days of treatment, blood was collected through retro-orbital puncture for insulin and reproductive hormone (FSH, LH and testosterone) analysis; the animals were then sacrificed and the testes were processed for histological staining. Data obtained were expressed as means of ten (10) replicates ± SEM and subjected to one-way analysis of variance (ANOVA) and the Scheffe's *post hoc* test for multiple comparisons.

Results showed that the levels of testosterone, FSH and LH in the AOLE- and insulin-treated rats increased significantly compared to the hyperglycaemic controls (P<0.05).

Histological sections revealed improved cellularity, germinal epithelium, tubular diameter, cross-sectional area and luminal spermatids in the AOLE- and insulin-treated rats when compared with the hyperglycaemic control.

AOLE improved the structural integrity of the testes, promoted spermatogenesis, and improved the profile of reproductive hormones.

Key words: *Anacardium occidentale* – Testes – Cytoprotective – Streptozotocin – Reproductive hormones – Hyperglycaemia

INTRODUCTION

Male reproductive alterations have been widely reported in individuals with diabetes. The administration of high doses of streptozotocin to male rats induced a decrease in testicular testosterone production (Sanguinetti et al., 1995).This decrease may be the result of both a decrease in the total number of Leydig cells and in the rhythm of androgen biosynthesis by the remaining functional cells (Orth et al., 1979; Paz and Homonnai, 1979; Hurtado de Catalfo et al., 1998). This alteration has been claimed to be responsible for the diabetic- related effects on the libido (Hassan et al., 1993).

Corresponding author: Victor Ukwenya. Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Bowen University, Iwo, Osun State, Nigeria
Tel: +2347061007589. E-mail: victorwyn@yahoo.com

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Diabetes also affects spermatogenesis. However, these disturbances are less consistent than those that affect Leydig cell function. Morphometric studies have shown significant differences in the diameter of the seminiferous tubules in diabetic individuals. Although a constant 20-25% total or subtotal block of spermatogenesis at spermatocyte stages II and III occurs in the small tubules of all animals after 4 weeks of STZ treatment (Anderson and Thliveris, 1986), a significant increase in degenerated germ cells at various stages of development has also been observed (Orth et al., 1979; Sanguinetti et al., 1995). The spontaneous diabetic (BB) rats also showed altered spermatogenesis, although in this case it was preceded by a primary dysfunctionality in Leydig cells (Murray et al., 1983).

This indicates that this process and other morphological alterations observed in the testes of STZ-diabetic rats are not caused by a direct effect of the drug, but rather by diabetes (Oksanen, 1975).

Anacardium occidentale, commonly known as cashew tree, is a multipurpose tree of the Amazon that grows up to 15 m high. It belongs to Anacardiaceae family, a native of Brazil, having great economic and medicinal value, and is composed of some 60 to 74 genera and 400 to 600 species. The tree is small and evergreen, growing to 10-12 m (~32 F) tall, with a short, often irregularly shaped trunk. The leaves are spirally arranged, leathery textured, elliptic to obovate, 4 to 22 cm long and 2 to 15 cm broad, with a smooth margin. The stem-bark and leaves of *Anacardium occidentale* have been reported to possess hypoglycaemic activities (Ojewole, 2003) while radical scavenging activities have been reported for the shoot (Roach et al., 2003) and the leaves (Abas et al., 2006). Phytochemical screening of the methanolic extract of *Anacardium occidentale* revealed that it contains substances with hypoglycaemic activities. These include α-glucosidase inhibitors (Toyomizu et al., 1993), kampferol, and quercetol rthamnosides (Arya et al., 1989).

In view of the link between diabetes and reproductive alterations, and the reported anti-diabetic effects of *Anacardium occidentale*, this research was designed to investigate the ameliorative effects of the extract on the testicular histology of streptozotocin-diabetic rats.

MATERIALS AND METHODS

Animal care

Forty healthy and normoglycaemic adult male Wistar rats having fasting blood glucose level of 70-80mg/dl and weighing between 150-175g were used for this study. The animals were purchased from the University of Ibadan, Ibadan. They were taken to the animal house of the Department of Anatomy, where the rats were kept in iron cages at controlled room temperature of about 30°C and photo-periodicity of 12L:12D. They were fed on rat pellets feed and water made available *ad libitum.*

Plant material

Fresh leaves of *Anacardium occidentale* were collected from Iwo town, Osun State, Nigeria, and identified by a plant taxonomist in the Department of Botany, University of Ilorin, Ilorin. The voucher specimen is deposited in the departmental herbarium (UIH/612).

Extract preparation

The leaves of *Anacardium occidentale* were washed and shade-dried to constant weight, and then ground into fine powder using a contact mill. 1,977.7 g of the leaf powder was macerated in 4,720 ml of methanol for 48 hours, and then filtered through Whatman filter paper at room temperature. The supernatant was concentrated under reduced pressure using a rotary evaporator (Laborato 4000, China). The final product was a solid, dark green sticky mass weighing 89.8 g, which represents a yield of 4.54%. This was refrigerated at 4°C before use.

The extract was dissolved in 1% dimethyl sulfoxide (DMSO) water solution (v/v) before administration. Stock solution was prepared by dissolving 42 g of the extract in 265 ml of physiological saline to give a concentration of 0.16 g/ml.

Induction / determination of diabetes

Diabetes was induced in the rats by a single intraperitoneal injection of 70 mg/kg body weight of 0.1M streptozotocin (Sigma Co., USA) in 0.1M citrate buffer, pH 4.5 (Ballester et al., 2004).

0.1 M citrate buffer was prepared by dissolving 2.1 g of citric acid and 2.94 g of sodium citrate in 100 ml of distilled water. The pH was adjusted to 4.5 by the proper addition of concentrated NaOH/ HCL using a calibrated pH meter (Adams Equipment, Belgium).

Accu-Check Glucometer (Roche, Germany) and compatible glucometer strips were used for the determination of blood glucose levels in overnight fasted rats 48 hours after induction of diabetes. Blood samples were obtained from the dorsal vein of the tail of conscious rats. Only rats with glucose level greater than 250 mg/dl were recruited into the study (Singh et al., 2007). Diabetes was allowed to stabilize for 5 days before the commencement of intervention.

Thereafter, the blood glucose level of all animals in each experimental group was assessed every other day after treatment with the extract and protamine zinc insulin (Insulatard®, Novo Nordisk, Denmark). The extract was administered through orogastric cannula. Animals were checked for clinical signs of drug toxicity such as tremors, diarrhoea, weakness, lethargy, poor wound healing, weight loss, hair loss, coma and death.

Experimental design

The animals were divided into four 4 groups of 10 rats each, and treatment commenced for a period of 16 days after 7 days in the diabetic state:

Group A were administered 300 mg/ kg/ day methanolic leaf extract. Group B received 1 I.U./ kg/day insulin. Group C -diabetic control-, received 1ml/kg of citrate buffer (placebo) and Group D -normal control-, received 1 ml/kg of citrate buffer (placebo).

The body weight measurements were taken at two-two day's interval and recorded, using a sensitive top loader balance (Adams Equipment, Belgium).

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Guiding Principles in the Care and Use of Animals (American Physiology Society, 2002).

Blood collection

Twenty four hours after the administration of the last doses of extract and insulin the animals were weighed and glycaemia was determined by means of a gluconometer (Accu-Check, Roche). Whole blood was collected by retro-orbital puncture from each animal into clean dry heparinised bottles. The blood was incubated for 2 hours at 37°C. Plasma was obtained from a portion of blood sample after centrifugation at 6000 rpm for 20 minutes. Plasma was extracted by aspiration with a micropipette, and was stored in refrigerator at 4°C before analysis.

Hormone assays

Plasma insulin concentrations were determined by an enzyme-linked immunosorbent assay (ELISA), using ELISA kit (BioVendor, USA). Plasma FSH, LH and serum testosterone were determined by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Teco Diagnostics, USA). Absorbance was read at 450 nm in a Plate Reader (Biotek, China).

Determination of relative organ weight

At sacrifice, the weights of the testes were determined using a top loader sensitive balance (Adams Equipment, Belgiun). To reduce the individual body weight differences, the relative weight (%) was calculated from the body weight at sacrifice and the absolute weights of each organ as follows:

$$Relative\ organ\ weight = \frac{Absolute\ organ\ weight}{body\ weight\ at\ sacrifice} x\ 100$$

Table 1. Effects of methanolic extract of *Anacardium occidentale* and insulin on the body weight and blood glucose.

Treatment groups	Body weight (g)		Blood glucose (mg/dl)	
	Initial	Final	Initial	Final
A	156.94 ± 3.69	145.32 ± 4.39	333.62 ± 15.19	102.78 ± 10.75[a]
B	167.36 ± 10.50	148.50 ±6.51	339.91 ± 9.99	112.89 ± 18.32[a]
C	174.88 ± 5.30	135.07 ± 8.97	358.64 ± 18.90	417.90 ± 33.39
D	148.77 ± 4.99	170.77 ± 10.16[a]	91.80 ± 3.81[b]	95.90 ± 4.16[a]

Group A: rats that had 70 mg/kg STZ + 300 mg/kg of methanolic leaf extract of *Anacardium occidentale.* B: rats that had 70 mg/kg STZ + 1.0 I.U/ kg insulin. C: rats that had 70 mg/kg STZ only. D: rats that had only 1 ml/kg citrate buffer. Values are mean ± SEM; n=10 in each group. [a]Statistically different from Group C at P <0.05. [b]Statistically different from Groups A, B and C at P <0.05

Table 2. Absolute and relative testicular weights of experimental rats.

Treatment groups	Absolute testicular weight (g)	Relative testicular weight (g / 100g)
A	0.85 ± 0.07 [a]	0.60 ± 0.05[+]
B	0.62 ± 0.05	0.43 ± 0.04[b]
C	0.56 ± 0.05	0.44 ± 0.05[b]
D	1.17± 0.07[*]	0.70 ± 0.05

Groups A: rats that had 70 mg/kg STZ + 300 mg/kg of methanolic leaf extract of Anacardium occidentale. B: rats that had 70 mg/kg STZ + 1.0 I.U/ kg insulin. C: rats that had 70 mg/kg STZ only. D: rats that had only 1 ml/kg citrate buffer. Values are mean ± SEM; n=10 in each group. [a]Statistically different from Group C at P <0.05. [b]Statistically different from Group D at P <0.05. *Statistically different from Groups A, B and C at P <0.05. [+]Statistically different from Groups B and C at P <0.01.

Tissue processing

After sacrifice by cervical dislocation, the animals were cut open by abdomino-pelvic incision and the testes were excised, trimmed of all fat and blotted dry. They were weighed using a sensitive scale (Adams Equipment, Belgium) and thereafter fixed in 10% formo-saline. The fixed tissues were transferred to graded series of ethanol and then cleared in xylene. The tissues were then infiltrated in molten paraffin wax in the oven at 58°C. Serial sections of 3 µm thick were obtained from a solid block of tissue, fixed on clean slides, stained with haematoxylin and eosin stains. Also, silver impregnation was carried out on tissues to demonstrate reticulin fibres (Gordon and Sweet, 1936). Silver-impregnated tissues were counter-stained with methyl red to indicate spermatogenic cells. Slides were examined with the light microscope.

Photomicrographs were taken with a high definition digital camera Leica ICC50 (Leica Microsystems, England) mounted on a microscope Leica DM 750 (Leica Microsystems, England).

Statistical analysis

Values were compared using computerized software: SPSS (USA). Values were recorded as mean ± standard error of mean (S.E.M). The statistical significance of difference in the mean and standard error of mean (P<0.05 and P<0.01) were analyzed by one-way analysis of variance (Anova) and the Scheffe's *post hoc* and LSD test for multiple comparison.

RESULTS

Blood glucose

STZ induced hyperglycaemia in all the experimental rats. Treatment with AOLE produced significant (P<0.05) reduction in the blood glucose levels of treated rats compared to the untreated animals (102.78 ± 10.75 mg/dl v 417.89 ± 33.39 mg/dl). Treatment with insulin also resulted in significant reduction of glycaemia at P > 0.05 (112.89 ± 18.32 mg/dl v 417.89 ± 33.39 mg/dl) (Table 1).

Body weight

Final body weights of Groups A and B (145.32 ± 4.39 g and 148.50 ± 6.51g) were significantly lower than D (170.77 ± 10.16 g) at P<0.05. However, both treated groups A and B showed final body weights that were higher than the untreated hyperglycaemic group C(145.32 ± 4.39 g; 148.50 ± 6.51 g vs 135.07 ± 8.97 g) at P <0.05 (Table 1).

Absolute and relative testicular weight

Data on testicular weight are presented in Table 2. STZ-induced hyperglycaemia was associated with a significant reduction at P<0.05 in both actual and relative mean testicular weight when compared to the non-hyperglycaemic controls (Table 2).

The mean absolute testicular

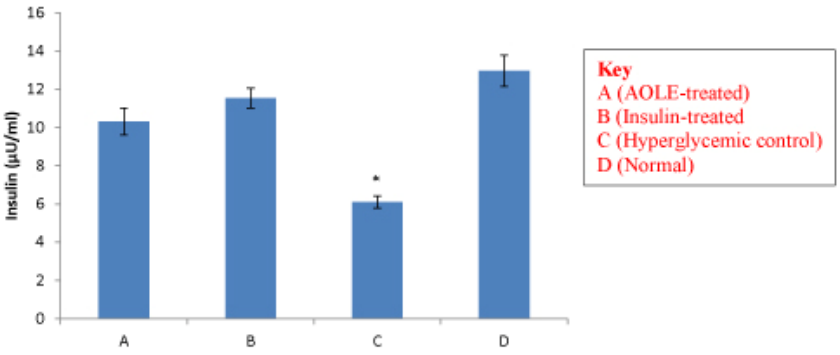

Fig. 1. Changes in the level of insulin of treated and control animals. Values are mean ± SEM, n= 10 in each group *statistically different from A, B and d (P< 0.05)

Table 3. Effects of methanolic extract of *Anacardium occidentale* on the reproductive hormones of experimental rats.

Treatment Groups	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/ml)
A	2.12 ± 0.14[a]	4.34 ± 0.32[a]	2.61 ± 0.53[a]
B	1.90 ± 0.06	3.53 ± 0.11[b]	1.60 ± 0.42
C	1.54 ± 0.04	3.30 ± 0.07	0.64 ± 0.07
D	1.96 ± 0.09[a]	4.04 ± 0.19	3.35 ± 0.50[a]

A: rats that had 70 mg/ k STZ + 300 mg/k of methanolic leaf extract of *Anacardium occidentale.* B: rats that had 70 mg/k STZ + 1.0 I.U/ k insulin. C: rats that had 70 mg/k STZ only. D: rats that had only 1 ml/k citrate buffer. *Values are mean ± SEM; n=10 in each group.* [a]*Statistically different from Group C at P <0.05.* [b]*Statistically different from Group D at P<0.05*

weight of the extract-treated rats (Group A, 0.85 ± 0.07 g) was significantly different (P<0.05) from those of the hyperglycaemic controls (Group C, 0.56 ± 0.05 g), though significantly lower than the positive controls (Group D, 1.17 ± 0.07 g). The mean absolute weight of the testes of Group D rats (1.17 ± 0.07 g) was significantly higher (P<0.05) than was obtained in Groups A, B and C (0.85 ± 0.07 g, 0.62 ± 0.05 g and 0.56 ± 0.05 g) (Table 2).

The mean relative testicular weight of the extract-treated rats (Group A, 0.60 ± 0.05) was significantly (P<0.01) different from those of the insulin-treated and hyperglycaemic controls (Group B, 0.43 ± 0.04 and Group C, 0.44 ± 0.05). The mean relative weight of the testes of Group D (0.70 ± 0.05) was significantly higher than Group B (0.43 ± 0.04) and C (0.44 ± 0.05) (Table 2).

Plasma insulin

Reduced plasma insulin levels were observed in the untreated hyperglycaemic rats compared to the normal control group. Plasma insulin assayed in the extract-treated group and insulin-treated group were significantly higher (P< 0.05) when compared with the hyperglycaemic group (Fig. 1).

Reproductive hormone profile

Data on reproductive hormones are shown in Table 3. The level of FSH was lowest in Group C (1.54 ± 0.04 mIU/ml), and values recorded for Groups A (2.12 ± 0.14 mIU/ml) and B (1.96 ± 0.09 mIU/ml) were significantly higher at P< 0.05 than in Group C (1.54 ± 0.04 mIU/ml).

The AOLE- and insulin-treated groups showed an increase in LH level when compared to the hyperglycaemic controls. This was significant for Group A at P < 0.05 (4.34 ± 0.32 mIU/ml vs 3.30 ± 0.07 mIU/ml). LH level was also higher in Group D (4.04 ± 0.19 mIU/ml) compared to C (3.30 ± 0.07 mIU/ml) at P < 0.05. However, the level of the hormone in the insulin-treated Group B was significantly lower (P < 0.05) than observed in the normal controls (3.33 ± 0.11 v 3.93 ± 0.17 mIU/ml).

Treatment groups showed differences in their testosterone levels with Group A and D significantly higher than Group C (2.61 ± 0.53 ng/ml and 3.35 ± 0.50 ng/ml vs 0.64 ± 0.07 ng/ml) at P < 0.05 (Table 3). Group B (1.60 ± 0.42 ng/ml) showed higher testosterone value than Group C (0.64 ± 0.07 ng/ml), though statistically lower than Group D (1.60 ± 0.42 ng/ml vs 3.35 ± 0.50 ng/ml).

Histological analysis

Histological examination of the testes of Groups A, B and D rats showed seminiferous tubules with regular configuration (Figs. 2a, b and d). The tubules from the three groups looked similar. The tubules appeared oval or rounded in cross-sections, and were separated by intact connective tissue and well-delineated angular interstitial spaces containing clusters of Leydig cells and blood vessels (Figs. 2a, b and d). They were lined with a complex stratified epithelium consisting of Sertoli cells and abundant spermatogenic cells.

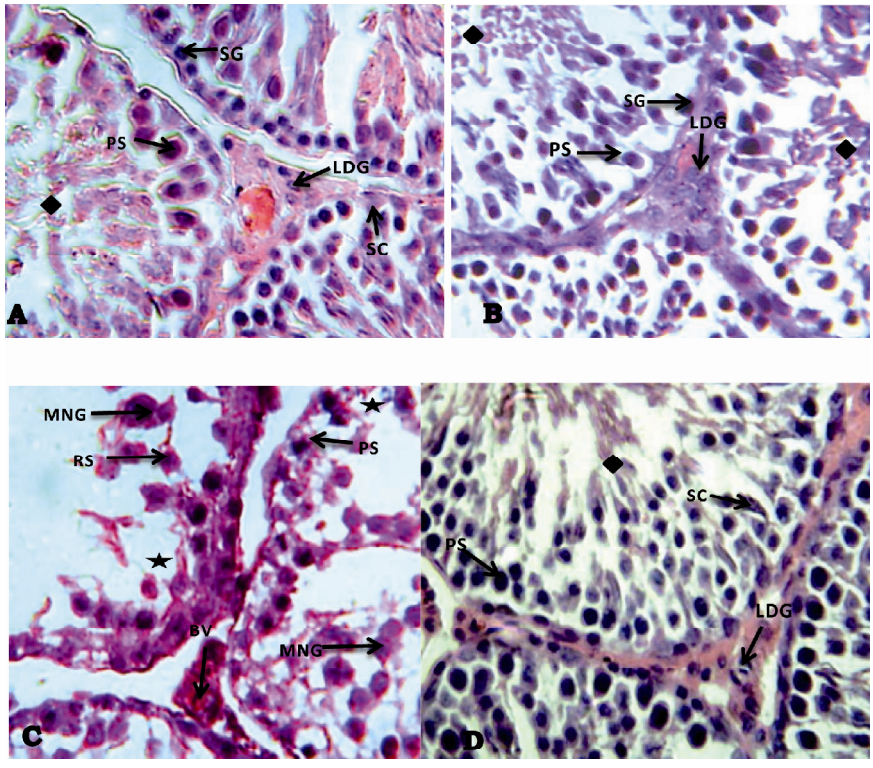

Fig. 2. Cross-section of seminiferous tubules of adult rats. Group **(A)** AOLE-treated, **(B)** insulin-treated, **(C)** hyperglycaemic control and **(D)**normal control. Stain: H&E. x 400. Rhomboids indicate luminal spermatids; stars indicate depleted germinal epithelium. SG, spermatogonia; PS, primary spermatocytes; SC, Sertoli cell; LDG, Leydig cell; MNG, multinucleated giant cell.

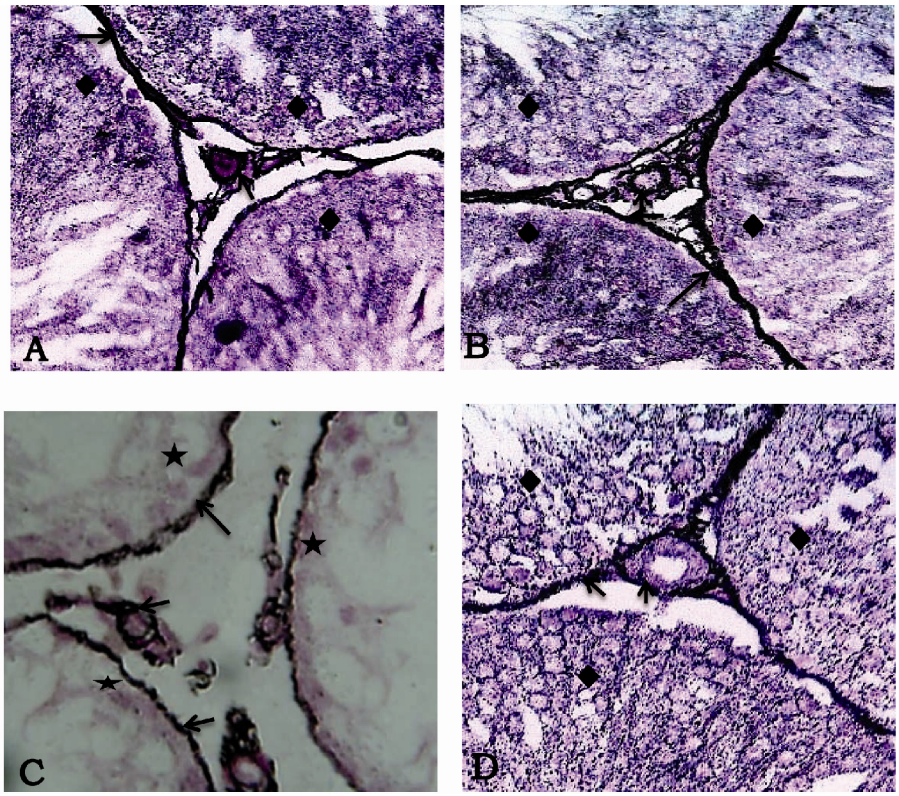

Fig. 3. Representative images showing the localisation of reticulin fibres in the tubules of Groups **(A)** (AOLE-treated); **(B)** (Insulin-treated); **(C)** (Hyperglycaemic) and **(D)** (Normal) rats. Staining procedure: Gordon and Sweet's. x 400. Arrows indicate reticulin fibres; rhomboids indicate spermatogenic cells; stars indicate amorphous lymphatic content.

The Sertoli cells were distinguishable as slender, elongated cells with irregular outlines that extend from the basal lamina to the lumen. Spermatogenic cells displayed various stages of maturation that characterises the cycle of a seminiferous epithelium. Spermatogenic cells were arrayed from the basal lamina to the tubular lumen as spermatogonia, spermatocytes, spermatids and spermatozoa (Figs. 2a, b and d). Located in the basal compartment immediately above the basal lamina are the spermatogonia cells. Other spermatogenic cells were found in the adluminal compartment (Figs. 2a, b and d).

Spermatids were present as small cells close to the tubular lumen. Early spermatids were identifiable by their central round nuclei, while late spermatids had elongated and condensed nuclei (Figs. 2a, b and d).

In contrast, the seminiferous tubules of untreated hyperglycaemic rats were irregular in outline and presented with a thickened basal lamina and congested blood vessels. The connective tissue was distorted with the occurrence of widened interstitial spaces with scattered Leydig cells (Fig. 2c). Sertoli cells were absent from many of the tubules. The tubules showed major reduction in the thickness of the spermatogenic cells, and did not show any ordered progression of spermatogenic cells (Fig. 2c).

Many of the tubules did contain neither sperm cells nor spermatids. Most of the spermatogonia were fixed at stages VIII and IX. In addition, the germinal epithelium featured prominent vacuoles. Conspicuous in the lumen were round spermatids and multinucleated giant cells (Fig. 2c).

Silver impregnation staining showed the presence of reticulin fibres around the peritubular walls of seminiferous tubules and blood vessels of all groups (Fig. 3a-d). The tubules of the untreated diabetic group, however, showed no evidence of spermatogenesis compared to the other groups (Fig. 3c), while the tubules of Groups A, B and D rats showed black stained reticulin fibres that form a continuous rim around the peritubular walls, extending intraluminally into the seminiferous tubules (Fig. 3d).

DISCUSSION

In this study, STZ-induced hyperglycaemia produced marked loss in body weight, hyperglycaemia and diminished insulin secretion, reduction in reproductive hormones, testicular atrophy, reduced germinal epithelium and disrupted spermatogenesis.

STZ-induced diabetes has been reported to be associated with general weight loss (Dineshkumar et al., 2010; Mallick et al., 2006). This is because the body switches to burning fatty acids due to insulin shortage. The process of gluconeogenesis also converts glycogen stores in the liver and the muscles to glucose in the diabet-

ic state.

Increased body and testicular weights observed in the extract- and insulin-treated groups demonstrated that the extract and insulin stimulated weight recovery in these groups secondary to improved levels of insulin recorded in these groups. This is because insulin increases the activity of acetyl Co-A carboxylase and provides glycerol for the esterification of fatty acids to triacylglycerol. Insulin is also responsible for the formation of glycogen in skeletal muscles (Cotran et al., 1999). Moreover, insulin and testosterone are anabolic hormones that stimulate protein synthesis which might have contributed to increased body and testicular weight (Cotran et al., 1999).

In a previous work, Ukwenya et al. (2012), we had reported the ameliorative effects of the methanolic leaf extract of Anacardium occidentale on the histology of the pancreas of STZ-induced diabetic rats. We reported increased beta-cell density in extract treated rats compared to the diabetic controls. It is thus possible that increased plasma insulin observed in the extract-treated rats was secondary to improved cytoarchitecture of the pancreas.

The untreated hyperglycaemic rats had smaller testes compared to rats from the other groups (Table 2). Their interstitial tissue was also far less compact and irregular when compared with the control and treated groups (Figs. 2c and 3c). These morphological alterations are indicative of testicular atrophy.

Histological evidence suggests that the smaller testes observed in the untreated hyperglycaemic group is due to loss of interstitial tissue induced by reduced secretion of testosterone by the Leydig cells. A possible cause of reduced Leydig cell secretion is hypoinsulinemia. Khan et al. (1992) reported that insulin controls cell proliferation and metabolism within the Leydig cells.

Results also showed reduced quantities of testosterone, LH and FSH for untreated hyperglycaemic rats. The decrease in testosterone is in agreement with the finding of Paz and Homonnai (1979), who reported a reduction in the serum and testicular testosterone of Wistar rats after the induction of hyperglycaemia by streptozotocin. Similar decreases in the serum levels of FSH and LH in diabetes have also been reported (Benitez and Perez, 1985). Though histological evidence revealed the presence of Leydig cells, plasma assay showed reduction in the quantity of testosterone assayed in the untreated hyperglycaemic rats. Evidence, however, indicates that reduced insulin and LH observed in this group may be responsible.

Insulin has been reported to be related to the control of cell proliferation and metabolism (Ballester et al., 2004). Hurtado de Catalfo et al. (1998) reported that insulin restored alterations in lipid metabolism in cultured Leydig cells from hyperglycaemic rats; hence lipid metabolism is strongly related to androgen biosynthesis (Romanelli et al., 1995).

STZ is a diabetogen that acts by the oxidative destruction of the beta cells of the pancreas, incapacitating the ability of the organ to produce insulin (Junod et al., 1967). The absence of such stimulatory effect of insulin on the metabolism of the Leydig cells would affect the synthesis and release of testosterone by the cells.

LH stimulates the Leydig cells to produce testosterone; hence the low level of LH assayed in plasma samples of hyperglycaemic rats is a contributory factor to the low quantity of testosterone in this group.

It has also been postulated that LH mediates the proliferation of Leydig cells through a mechanism that involves insulin and IGF-1 signalling (Feng et al., 1999). The reduction of plasma insulin concentration in hyperglycaemic rats as recorded in this study will affect such mechanism. Thus reduction in LH and insulin has a negative correlation on testosterone levels.

There are conflicting results concerning the effects of insulin treatment on LH and FSH in diabetic rats. Hutson et al. (1983) and Sudha et al. (1999) reported a total recovery of LH and FSH respectively. Lack of recovery of LH (Hutson et al., 1983) and FSH (Seethalakshmi et al., 1999) have also been reported respectively.

The AOLE-treated group showed significant increase in LH, FSH and testosterone levels compared to the untreated hyperglycaemic group. This is due to the restorative effects of the extract on the pancreas and the synthesis of insulin as observed in this study. As mentioned above, LH mediates the proliferation of Leydig cells through a mechanism that involves insulin and IGF-1 signalling (Feng et al., 1999). In addition, the amount of testosterone produced by existing Leydig cells is under the control of LH which regulates the expression of 17-β hydroxysteroid dehydrogenase (Payne et al., 1996).

Also, there is a proportional relationship between FSH and testosterone. FSH stimulates the Sertoli cells to secrete a protein called androgen-binding protein (ABP) into the lumen of the seminiferous tubules. ABP binds to testosterone and increases their concentration. Hence, the increase in plasma samples of testosterone assayed in the treated groups is attributable to the increase in FSH.

Histological analysis showed that STZ-induced hyperglycaemia was associated with histopatho-

logical features such as reduced tubular diameter, sloughing and reduction in germinal epithelial thickness, and the presence of multi-nucleated giant cells. The germinal epithelium is the site of spermatogenesis and is sensitive to a variety of toxic agents, making it a major target for cytotoxic substances. Degenerating cells and the formation of multi-nucleate giant cells by coalescence of spermatids have been reported to be a prominent feature of these toxic effects (Fawcett, 1994), and are an indication of spermatid failure.

Similar reduction in tubular diameter was reported in adult STZ-diabetic Wistar rats (Altay et al., 2003), prepubertal STZ-diabetic rats (Soudamani et al., 2005) and in the offspring of diabetic female rats (Jelodar et al., 2009).

Reduction in the germinal epithelium would impair the process of spermatogenesis, resulting in a fewer luminal spermatozoa and a wider tubular lumen as seen in this work. It is noteworthy that whereas spermatogonia form the peribasal cells of hyperglycaemic testes, the epithelia and lumina still featured scanty spermatozoa compared to the treated and control groups. This suggests that the deleterious effect of hyperglycaemia on the testes of this group was not a complete cessation of spermatogenesis, but rather suspension of spermatogenesis. This agrees with the work of Fakoya et al. (2004), who reported a major reduction in the germinal epithelial thickness, dispersion of the germinal cells as well as spermatogenesis arrest in prenatal ethanol-exposed rats.

The mechanism of spermatogonia arrest is still unclear. According to Okanlawon, et al. (2001), it could have resulted from the disruption of Sertoli cell functions with or without a disruption in the physiology and/or morphology of the blood-testis barrier. It might also have resulted from interference of the signals between the germ cells and Sertoli cells. This is because germ cell and Sertoli cell functions are linked via local intratesticular or paracrine signals, which are suppressed or triggered at certain stages in the spermatogenic process (Kerr, 1995).

Sertoli cells were notably absent in most of the seminiferous tubules of the untreated hyperglycaemic group (Figs. 2c and 3c). Such absence of Sertoli cells would be unfavourable to spermatogenesis, as Sertoli cells provide structural and biochemical support to the developing sperms and they also secrete growth factors and transport proteins such as transferrin and androgen-binding protein (Xiong et al., 2005).

In addition, testosterone is necessary for normal sperm development; it activates genes in Sertoli cells, which promote differentiation of spermatogonia (Mehta et al., 2008). Therefore, reduction in the Sertoli cell population and peripheral testosterone as revealed in this study is expected to be detrimental to spermatogenesis.

The severity and duration of gonadal damage induced by cytotoxic substances correlate with the number of stem cells that are destroyed (Meistrich, 1986). Therefore, spermatogenesis is expected to recover if the stem cells are intact. Spermatogenesis was found to be restored in the AOLE-treated rats. This suggests that diabetes may not necessarily cause a permanent damage to the testes if intervention is initiated on time, and that the extract aided the recovery of the testes and restored reproductive capacity.

Compared with the untreated hyperglycaemic group, it can be deduced that AOLE possessed efficient spermatogenic properties as shown with increased germinal epithelial thickness, reduced luminal diameter and increased diameter of the seminiferous tubules. Increased germinal epithelium was associated with improved spermatogenesis which resulted in a narrower lumen observed in the treated rats.

Histological staining of the testes using silver impregnation techniques showed the extensive presence of reticulin fibres in the interstitium, peritubular walls and across the lumen of treated, hyperglycaemic and normal control rats. The peritubular walls consist of a single layer of myoid cells that are contractile in function and help expel spermatozoa out of the tubule. The presence of reticulin fibres along the peritubular walls is suggestive of the functional integrity of the myoid cells. This is an indication that hyperglycaemia was not injurious to the myoid cells. The interstitium of the hyperglycaemic rats however, stained poorly for reticulin fibres. The absence of reticulin fibres in the interstitial spaces of hyperglycaemic rats contributed to the scantiness of their interstitium and ultimately the low relative testicular weight observed in this group.

Our results show that reproductive alterations associated with STZ-induced diabetes are largely due to the deleterious effects of hyperglycaemia on the testes. Our results also show that the methanolic extract of *Anacardium occidentale* has a potent hypoglycaemic and anti-hyperglycaemic activity comparable to insulin, a reference drug. We conclude that *Anacardium occidentale* would serve as a good adjunct in the management of diabetes associated with reproductive alterations.

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