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# Evaluation of antioxidant potential of methanolic leaf extract of *Anacardium Occidentale* (Linn) on the testes of streptozotocin-induced diabetic wistar rats

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## SUMMARY

*Anacardium occidentale* is a plant with reported anti-diabetic and antioxidant properties. The objective of this work was to determine the effects of *Anacardium occidentale* leaf extract (AOLE) on the activities of glucose-6-phosphate dehydrogenase (G-6-PDH), thiobarbituric acid reactive substances (TBARS) and anti-oxidant enzymes (Glutathione peroxidase, GPx and superoxide dismutase, SOD) in the testicular homogenate of streptozotocin-induced diabetic rats. Forty (40) wistar rats (*Rattus norvegicus*) were randomly divided into four experimental groups. Diabetes was induced by a single intraperitoneal injection of Streptozotocin (70 mg/kg b.w.). Five days after the confirmation of hyperglycemia, 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 hyperglycemic and normal controls respectively. Animals were sacrificed 16 days after treatment. Our study showed that AOLE ameliorated the level of TBARS and improved the activities of G-6-PDH, SOD and GPx in the testes of extract-treated rats.

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## INTRODUCTION

Diabetic mellitus and other numerous pathological events such as atherosclerosis and inflammation processes are associated with the generation of reactive oxygen species (ROS), and consequently the induction of several chain reactions, among them lipid peroxidation (Cross et al., 1987).

Accumulating evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of diabetes mellitus (Bambolkar and Sainani, 1995). Moreover, diabetes also induces changes in the tissue content and the activity of the antioxidant enzymes (Genet et al., 2002).

"Oxidative stress" is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants commonly known as reactive oxygen species (ROS) (Sikka et al., 1995). ROS have been implicated in over a hundred of disease states which range from ar-

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thritis and connective tissue disorders to carcinogenesis, aging, toxin exposure, physical injury, infection, and acquired immunodeficiency syndrome (Joyce, 1987).

ROS are highly reactive oxidizing agents belonging to the class of free-radicals. A free radical is any compound (not necessarily derived from oxygen) which contains one or more unpaired electrons (Cotran et al., 1999). The most common ROS that have potential implications in reproductive biology include superoxide ($O^{2-}$) anion, hydrogen peroxide ($H_2O_2$), peroxyl (ROO-) radicals, and the very reactive hydroxyl (OH-) radicals. The nitrogen-derived free radical nitric oxide (NO.) and peroxynitrite anion (ONOO-) also appear to play a significant role in the reproduction and fertilization. The ultimate effects of (NO.) depend upon its concentration and interactions with hydrogen peroxide. Peroxynitrite (oxoperoxonitrate) anion may be formed in vivo from superoxide and nitrioxide and actively reacts with glutathione, cysteine, deoxyribose, and other thiols/thioethers (Koppenol et al., 1992).

The assumption that free radicals can influence male fertility has received substantial scientific support (Gagnon et al., 1991). The proposed mechanism for loss of sperm function upon oxidative stress has been shown to involve excessive generation of ROS (Aitken and Clarkson, 1987). The $H_2O_2$ has both beneficial and damaging effects on sperm and thus can influence the fertilization process. Hence, free radicals and ROS are associated with oxidative stress and are likely to play a number of significant and diverse roles in reproduction (Aitken et al., 1995).

*Anacardium occidentale*, commonly known as cashew tree, is a multipurpose tree of the Amazon that grows up to 15 m high. The tree is small and evergreen, growing to 10-12 m (~32 ft) 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 hypoglycemic activities (Ojewole, 2003) while radical scavenging activities has been reported for the nut and stem-bark (Korn-steiner et al., 2006; Trevistan et al., 2006), the shoot (Roach et al., 2003) and the leaves (Abas et al., 2006). Phytochemical study of the methanolic leave extract revealed the presence of phenolic, flavonoids, steroids and triterpenes (Fazali et al., 2011).

The methanolic and aqueous extracts of *Anacardium occidentale* have been reported to possess the ability to act as an antioxidant *in vitro* and *in vivo*; these were also able to increase the level of superoxide dismutase and catalase in experimental hypercholesterolemia (Fazali et al., 2011). However, the methanolic fraction has been reported to possess the most reducing and radical scavenging activities (Razali et al., 2008). It contained more than 7 fold of total phenolics content compared to the hexane and acetyl acetate extracts (Razali et al., 2008).

In the light of the reported hypoglycemic and anti-oxidant activities of this plant, and the reported links between hyperglycemia, reactive oxygen species and reproductive alterations, the aim of this study was to: (i) determine the effects of AOLE on the activities of G-6-PDH, TBARS, GPx and SOD of the testes of (STZ)-induced hyperglycemic rats; (ii) determine if the extract has any differential effects on the histology of the testes of experimental rats that vary in their oxidative stress levels using the Feulgen's reaction.

## MATERIALS AND METHODS

### *Animal care*

Forty healthy normoglycemic adult male Wistar rats having fasting blood glucose level of 70-80 mg/dL and of average weight 162.5 g were used for this study. The animals were purchased from the animal holding unit of 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 pellet feed obtained from Bendel Feeds, Ilorin and water made available *ad libitum.*

### *Plant material*

Fresh leaves of *Anacardium occidentale* were collected 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 leave 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 admin-

istration. 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 0.1 M streptozotocin (Sigma Co., USA) in 0.1M citrate buffer, pH 4.5 (Ballester et al., 2004).

The 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 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 five 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 of *Anacardium occidentale*. Group B received 1 I.U/kg/day insulin; Group C-diabetic control, received 1 ml/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 days 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).

### *Tissue processing*

The animals were cut open by abdominopelvic incision after sacrifice and the testes were excised and processed for histological and histochemical evaluation. The right testes were stained for Feulgen's reaction while the left testes were homogenised for enzyme histochemistry.

The Feulgen's reaction is based upon the cleavage of the purine-deoxyribose bond by mild acid hydrolysis to expose a reactive aldehyde group. The aldehydes are then detected by the use of Schiff's reagent. The classical reagent leucofuchsin gives a reddish- purple colour in their presence, owing to the formation of a quinoid compound (Culling, 1974).

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

### *Glucose-6-phosphate dehydrogenase (G6PDH) activity assay*

The activity of G6PDH in the testicular homogenates were determined by the method of Dawson et al. (1958). The method reflects the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and since oxidised nicotinamide adenine dinucleotide phosphate (NADP) is the hydrogen acceptor for G6PDH, the readings are considered to be indicative of the enzyme activity. NADPH has a characteristic absorbance at 340 nm.

### *Oxidative stress markers*

The anti-oxidant effect of the extract were quantified by evaluating the oxidative stress status of the testes with respect to thiobarbituric acid reactive substances (TBARS), glutathione peroxidase (GPX), and Super oxide dismutase (SOD).

### *Assay for TBARS*

The testes were excised and rinsed in ice-cold phosphate buffered saline pH 7.4 (containing 0.16 M sodium dihydrogen phosphate ($NaH_2PO_4$), 0.121 M disodium hydrogen phosphate ($Na_2HPO_4$) and 0.154 M sodium chloride (NaCl). Tissue homogenate was prepared by weighing out 25 mg of tissue into a 1.5 ml centrifuge tube.

Thereafter, 250 µl of RIPA buffer was added. Homogenisation was carried out in a Potter-Elvejhem homogenizer with Teflon pestle (TRI-R STIR-R K43, USA).

The RIPA homogenizing buffer contained 150 mM sodium chloride, 50 mM tris buffer pH 8.0,

1.0 % triton X-100, 0.1 % sodium duodecyl sulphate (SDS) and 0.5 % sodium deoxycholate. The homogenates were centrifuged in Harrier 15/80 MSE ultracentrifuge at 5,350 revolutions per minute (rpm) for 10 minutes to obtain clear supernatant. The supernatant was then assayed for the level of TBARS using quantitative colorimetric TBARS detection kit (Bio Assays System, USA).

TBARS assay is based on the reaction of TBARS with thiobarbituric acid (TBA) to form a pink-coloured product (Okawa et al., 1979). The optical density of the pink-coloured product measured at 535 nm, is directly proportional to TBARS concentration in the sample.

### *Assay for superoxide dismutase (SOD)*

Tissue samples were perfused with Phosphate buffer saline (PBS) solution, pH 7.4 and blotted dry. Thereafter, a 10% homogenate was prepared with cold sucrose buffer (50 mM sucrose, 200 mM mannitol, 1 mM TRIS buffer pH 7.4) in a Potter-Elvejhem homogenizer with Teflon pestle (TRI-R STIR-R K43, USA). The homogenized sample was centrifuged at 10,000 g for 15 minutes at 4$^{o}$C. The supernatant was transferred into a new tube and stored at -80$^{o}$C prior to analysis. SOD was assayed using a commercial kit (Cell Technology, USA). SOD stock solution suspension in ammonium sulfate served as standard. The IC50 (50% inhibition activity of SOD was determined by colorimetric methods at 440 nm absorbance.

Superoxide Dismutase Activity was measured by the reduction of a superoxide anion by a highly water soluble tetrazolium salt, WST-1 according to the method of Ukeda et al. (1999). The rate of reduction with $O^{2-}$ is linearly related to the xanthine oxidase activity and is inhibited by SOD. The 50% inhibition activity of SOD was determined by colorimetry with absorbance measured at 440 nm using a Plate Reader (Biotek, China ).

### *Assay for glutathione peroxidase*

Glutathione is a tripeptide of glycine, glutamic acid and cysteine which is involved in protecting cells from oxidative damage. Glutathione is involved in detoxification of hydrogen peroxide through glutathione oxidase. Low levels of glutathione are found in deficiencies of key enzymes involved in glutathione metabolism, such as glucose-6-phosphate dehydrogenase, glutathione synthase and glutathione reductase.

### *Preparation of tissue homogenate*

The testes were excised, rinsed in ice-cold phosphate buffered saline pH 7.4 (containing 0.16M sodium dihydrogen phosphate ($NaH_2PO_4$), 0.121M disodium hydrogen phosphate ($Na_2HPO_4$) and 0.154M sodium chloride (NaCl). All subsequent procedures were done at 4$^{o}$C. The tissues were then homogenized in five volumes of homogenizing buffer per gram of tissue using a motor-driven glass-Teflon Potter-Elvejhem homogenizer (TRI-R STIR-R K43 USA). The homogenizing buffer contained 50 mM potassium phosphate (PH 7.0) and 1mM EDTA. The homogenates were centrifuged in Harrier 15/80 MSE ultracentrifuge at 13,018 revolutions per minute (rpm) for 15 minutes to obtain clear supernatant.

Glutathione peroxidase (GSH-Px) activity was indirectly measured by a coupled reaction with glutathione reductase (GR). Oxidised glutathione (GSSG), produced upon reduction of hydrogen peroxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of of NADPH to $NADP^{+}$ is accompanied by a decrease in absorbance at 340 nm (Paglia and Valentine, 1967).

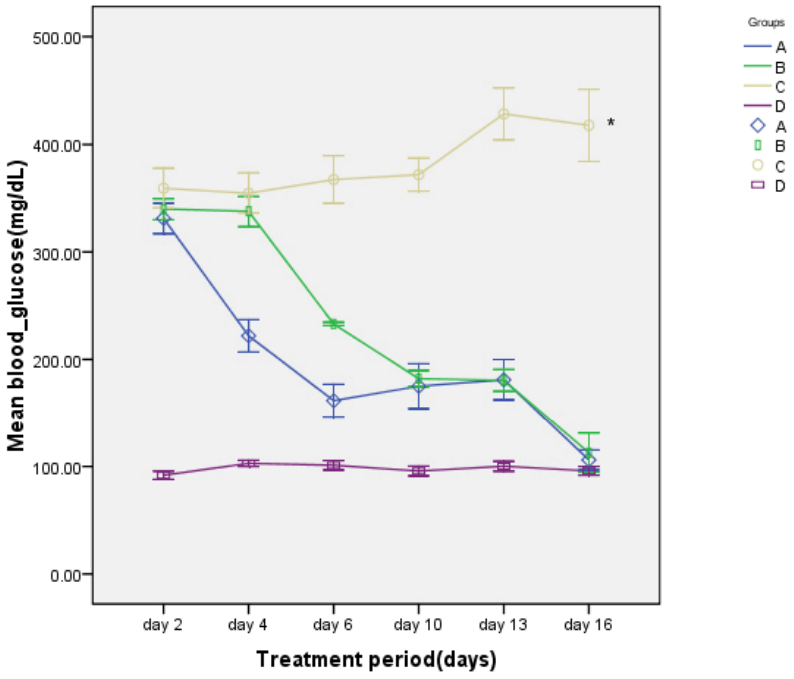


**Fig. 1.** Changes in blood glucose levels of treated and control rats. Bars represent ± SEM. * *P*< 0.05 compared to A, B and D.

### *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 analysed by one-way analysis of variance (Anova) and the Scheffe's post hoc and LSD test for multiple comparison.

## RESULTS

### *Blood glucose*

Data on blood glucose results are expressed in Fig. 1. STZ induced hyperglycemia in all the experimental rats. Treatment with AOLE and insulin produced reduction in the blood glucose levels of treated rats. Final glycemia of treated rats was significantly lower ($P$<0.05) compared to the untreated animals and was comparable to normal control rats. Hyperglycemia was sustained in the untreated hyperglycemic group for the duration of the experiment.

### *Anti-oxidant enzymes and G-6-PDH*

Data on G-6-PDH are expressed in Fig. 2. G-6-PDH was significantly high (P<0.05) in Groups A (71.82 ± 9.73) and B (72.06 ± 10.64) when compared with Groups C (34.15 ± 6.50) and D (37.58 ± 3.68).

Data on TBARS are expressed in Fig. 3. Reduction in the levels of TBARS was significant (P<0.05) for Groups A (5.50 ± 1.27), B (7.08 ± 1.30) and D (6.76 ± 1.37) when compared with the hyperglycemic control (15.34 ± 2.42) (Fig. 3).

Data on SOD are expressed in Fig. 4.The level of SOD assayed in the AOLE-treated and normal control groups was statistically different from Groups B and C at P<0.05 (Fig. 4).

Data on Gpx are expressed in Fig. 5. Groups A and B were found to be significantly different

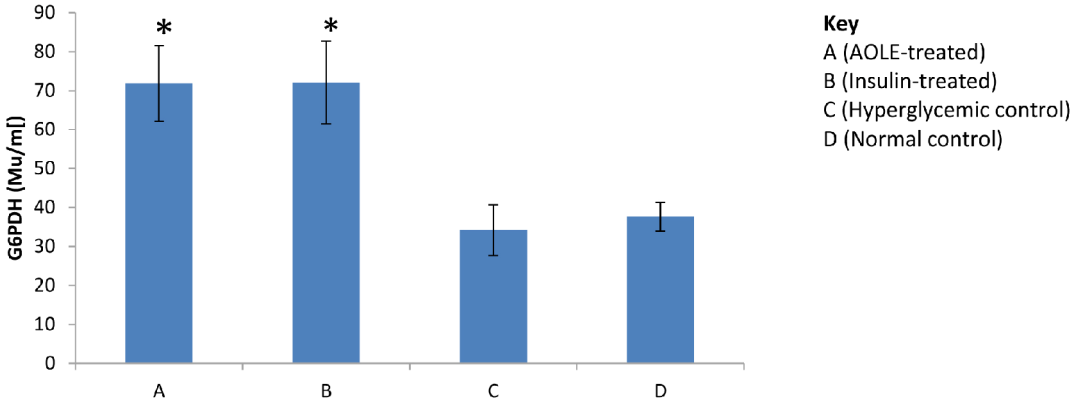


**Fig. 2.** Changes in the levels of glucose-6-phosphate dehydrogenase (G6PDH) of treated and control rats. Bars represent ± SEM. * $P$< 0.05 compared to C.

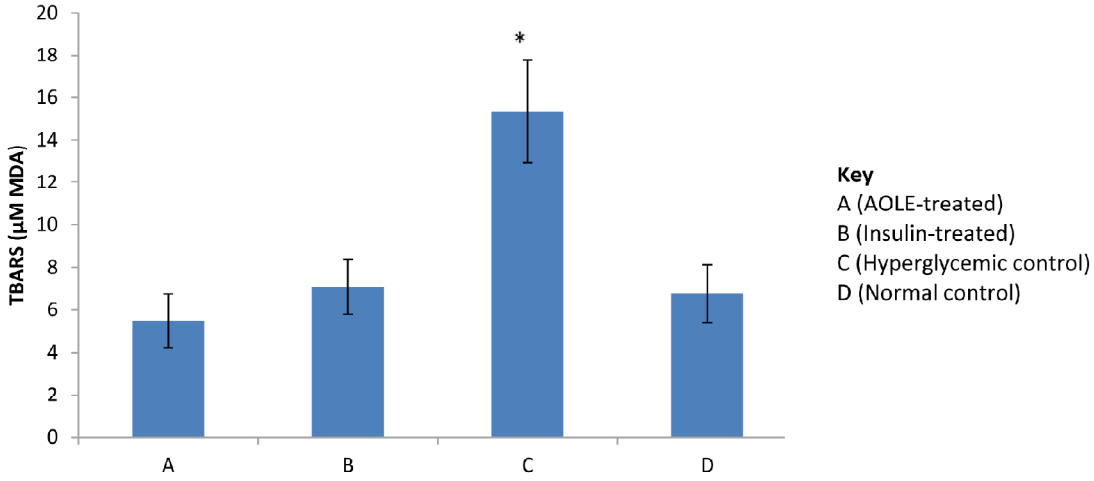


**Fig. 3.** Changes in the levels of thiobarbituric acid reactive substances (TBARS) in treated and control rats. Bars represent ± SEM. * $P$< 0.05 compared to A, B and D.

from Group C at P< 0.01. The normal control Group D was however found to be statistically higher from Groups A, B and C at P < 0.05.

### *Histopathological observations*

The Fuelgen reaction showed the intense cellularity of the treated and normal control groups (Fig. 6 A,B,D). The angular interstices, peritubular walls, connective tissue and tubules of these groups stained positive for DNA activity.

Sections from the hyperglycemic group (Fig. 6C) showed disruption of the integrity of the connective tissue (arrows) and the presence of amorphous lymphatic content within the tubules (stars).

## DISCUSSION

Glucose oxidation is believed to be the main source of free radicals. In its enediol form, glucose is oxidized to an enediol radical anion that is converted into reactive ketoaldehydes and superoxide anion radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide which can also lead to the production of highly reactive hydroxyl radicals (Jiang et al., 1990; Wolff and Dean, 1987). Hence oxidative stress is a prominent feature of the diabetic state because it is usually characterised by hyperglycemia.

Experimental induction of hyperglycemia in animal models has been shown to impair testicular function and decrease male fertility. This is because diabetogens such as streptozotocin enhance ROS generation and induce both lipid peroxidation and protein carbonyl expression in the testes (Aitken and Roman, 1995). This was manifest in this study by the reduced levels of Gpx and SOD and increase in the level of TBAR assayed in the testes of untreated hyperglycemic rats.

Peroxidative damage indicated by increased levels of TBARS as reported in this work has

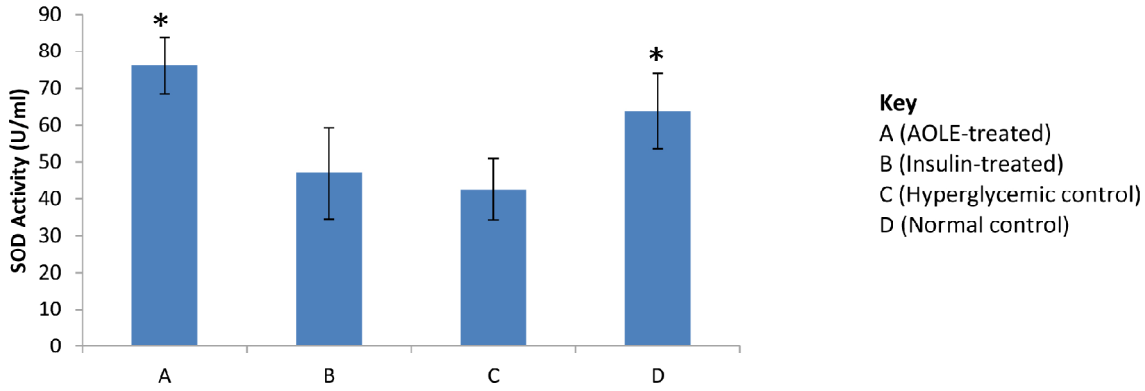


**Fig. 4.** Changes in the levels of superoxide dismutase (SOD) in treated and control rats. Bars represent ± SEM.

* *P*< 0.05 compared to B and C.

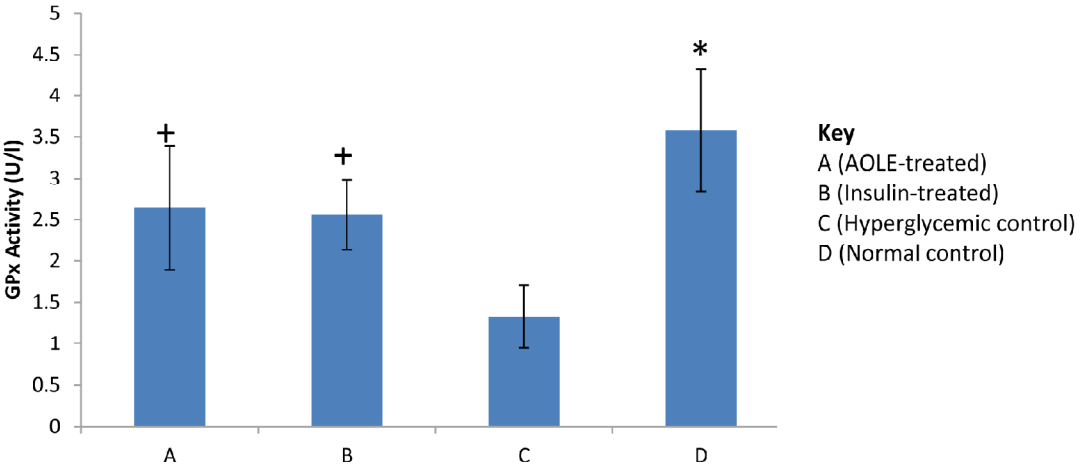


**Fig. 5.** Changes in the levels of glutathione peroxidase (GPx) in treated and control rats. Bars represent ± SEM.

* *P*< 0.05 compared to A, B and D; + *P* < 0.01 compared to C.

been described as the single most important cause of impaired testicular function underpinning the pathological consequence of diabetes (Aitken and Roman, 1995). The testes of untreated hyperglycemic rats showed tubules with attenuated germinal epithelium, as well as depleted lumen with poor differentiation of cells of the spermatogenic series (Fig. 6C). They also featured poor localisation and staining intensity for DNA, with the luminal spermatids also poorly differentiated. Both the interstitial spaces and lumen featured amorphous lymphatic content. This supports report that, at the level of the testes, oxidative stress is capable of disrupting the capacity of the germinal epithelium to differentiate normal spermatozoa (Naughton et al., 2001). This is more so considering that spermatogonia were observed in the periphery of the epithelium of this group, although the lumen showed very few spermatozoa. Such disruptions constitute spermatogenesis arrest and might be due to oxidative stress-mediated damage of the male germ cells reported by Kumar and Muralidhara (1999).

The testes of treated rats showed proper differentiation for DNA at the peritubular walls and angular interstitium, indicating the presence of Leydig cells. The ameliorative effects of AOLE treatment was further reflected in the distinct cellularity of their spermatogenic cells compared to the Group C testes (Fig. 6A).

The results for the Fuelgen's reaction appeared to be corroborated by the assay of G-6-PDH in the homogenates of rat testes. G-6-PDH is the first enzyme of the hexose monophosphate pathway that results in the production of ribose-5-phosphate, R-5-P. R-5-P is the precursor for nucleotide and nucleic acid synthesis (Nelson and Cox, 2005). G-6-PDH assayed in the homogenate of untreated hyperglycemic rats' testes was the lowest (Fig. 2). The positive results obtained for the Feulgen's reaction in the treated rats might be due to the enzymatic activities of G-6-PDH, resulting in increased synthesis of DNA.

Furthermore, in this experiment, increase in the levels of anti-oxidant enzymes was associated with increased levels of G-6-PDH. Since both spermatogenesis and Leydig cell steroidogenesis are highly prone to oxidative stress, the upregulation of this enzyme attains a significant proportion in maintaining the integrity of the testicular microenvironment.

G-6-PDH is the first enzyme in the hexose monophosphate (HMP) pathway that produces ribose-5-phosphate (R-5-P) and NADPH. R-5-P is the precursor for nucleotide and nucleic acid synthesis, eventually leading to cell proliferation. NADPH on the other hand provides reducing power for biosynthetic reactions. It is necessary to maintain glutathione in its reduced form. Reduced glutathione protects cells from oxidative

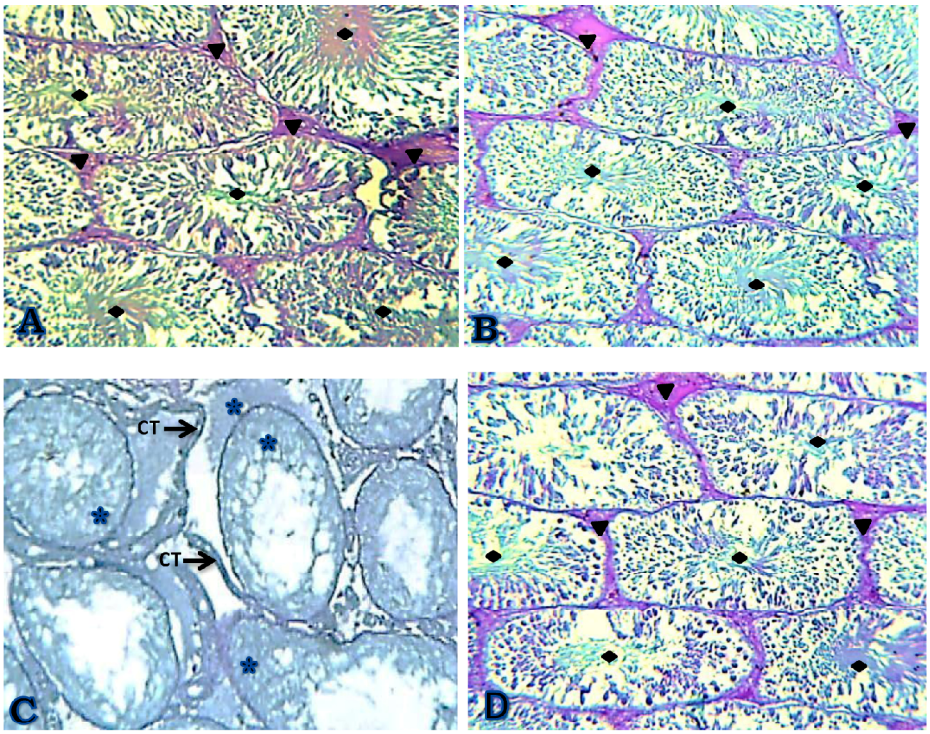


**Fig. 6.** Intensity and localisation of DNA in rat testes. Representative image showing the effects of Feulgen's reaction on the tubules of Groups **(A)** hyperglycemic + AOLE, **(B)** hyperglycemic + insulin, **(C)** hyperglycemic control and **(D)** normal control rats. x 250. Arrowheads indicate presence of Leydig cells in angular interstices; arrows indicate disrupted connective tissue (CT); rhomboids indicate luminal spermatozoa; asterisks indicate amorphous lymphatic content.

damage by hydrogen peroxide and superoxide free radicals (Sies, 1993).

Hence, the improved germinal epithelium, increased luminal spermatozoa, and decreased levels of TBARS observed in the testes of treated animals might be due to the potentiating stimuli of G-6-PDH. It is not surprising then that rapidly dividing organs like the testes send more glucose-6 -phosphate through the HMP pathway than others (Nelson and Cox, 2005). The up-regulation of G-6-PDH might have contributed to increased spermatogenesis evident by the increased germinal epithelial thickness of the extract-treated group.

In this study, treatment with AOLE produced significant increase in the testicular levels of SOD and GPx. The impact on SOD is of major importance. This is because hyperglycemia promotes lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals (Tsai et al., 1994; Kawamura et al., 1994). Also, glucose oxidation produce free radicals by generating superoxide anion which is converted to hydrogen peroxides and eventually hydroxyl radicals (Wolff and Dean, 1987). SOD, however, spontaneously dismutates ($O^{2-}$) anion to form $O_2$ and $H_2O_2$. Glutathione peroxidase, a selenium-containing antioxidant enzyme with glutathione as the electron donor then removes peroxyl (ROO.) radicals from various peroxides including $H_2O_2$ (Calvin et al., 1981). In this way these anti-oxidants protect the spermatozoa from oxygen toxicity.

*Anacardium occidentale* leaves has been reported to contain several phenolics such as gallic acid, protocatehnic acid, p-hydroxybenzoic acid, cinnamic acid, p-coumaric acid and ferulic acid (Kogel and Zech, 1985). Since plant antioxidant activity is related to compounds such as vitamins and phenolics which play effective role in free radical scavenging (Abreu et al., 2009), reducing agents and hydrogen donators (Pietta et al., 1998), the foregoing suggests that the antioxidant effects of AOLE, indicated by increased levels of SOD and GPx of extract-treated animals recorded in this experiment, could be linked to its active components and its ability to bring about upregulation of glucose-6-phosphate dehydrogenase.

Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage (Salah et al, 1995; Del et al., 2005; Okwu, 2004).

Ascorbic acid (vitamin C) is another component of the extract and it is an effective scavenger of superoxide radical anion, hydroxyl radical, singlet oxygen and reactive nitrogen oxide (Tannenbaum et al., 1991; Weber et al., 1996).

Vitamin C (ascorbic acid) also contributes to the support of spermatogenesis, at least in part through its capacity to reduce Vitamin E (α-tocopherol) and maintain this antioxidant in an active state. Vitamin E is a powerful lipophilic antioxidant that is absolutely vital for the maintenance of mammalian spermatogenesis (Johnson, 1979). Vitamin C is itself maintained in a reduced state by a GSH-dependent dehydroascorbate reductase, which is abundant in the testes (Paolicchi et al., 1996).

Deficiency of vitamin C leads to a state of oxidative stress in the testes that disrupts both spermatogenesis and the production of testosterone (Johnson, 1979). Conversely, ascorbate administration to normal animals stimulates both sperm production and testosterone secretion (Sonmez, 2005). This vitamin also counteracts the testicular oxidative stress induced by exposure to prooxidants such as arsenic, PCBs (Arochlor 1254), cadmium, endosulfan and alcohol (Senthil et al., 2004; Maneesh et al., 2005).

Most GPx are selenium dependent; animals fed on selenium-dependent diets are known to exhibit a significant reduction of testicular GPx activity and an accompanying loss of germ cells from the germinal epithelium of the testes (Kaur and Bansal, 2004). On the other hand, selenium administration prior to the creation of oxidative stress has been found to suppress lipid peroxidation and improve histopathological profile in the testes (Avian et al., 2005). The selenium content of this extract thus contributes to its ability to mitigate oxidative stress.

The results of this experiment clearly showed that STZ-hyperglycemia is associated with elevated blood glucose and increased oxidative stress in the testes. The increased oxidative stress disrupted the capacity of the germinal epithelium to differentiate normal spermatozoa.

Results also showed that the extract has potent anti-oxidant effects; by potentiating the stimulation of G-6-PDH and inhibiting lipid peroxidation, the extract stimulates the endogenous production of antioxidant enzymes (SOD and GPx) inherent to the testes. Improvement in the oxidative stress status (OSS) of the testes promoted its structural and functional integrity.

Research efforts focusing on the use of antioxidants to treat diabetes are ongoing. There are reports that antioxidants such as ascorbic acid, melatonin, taurine or an herbal mixture containing extracts from *Musa paradisiaca, Tamarindus indica, Eugenia jambolana and Coccinia indica* are capable of mitigating oxidative stress induced by hyperglycemia (Mallick et al., 2007; Armagan et al., 2006). *Anacardium occidentale* could be useful as a natural source of anti-oxidants.

We conclude that the methanolic extract of *Anacardium occidentale* possesses anti-diabetic activities; and the hypoglycemic and antioxidant effects of AOLE appears to be due mainly to the synergistic effects of its constituents, namely α-glucosidase inhibitors, kampferol, quercetol rthamnosides, saponin, quercetin glycoside, flavonoids, phenolics and the antioxidants (vitamins A and C).

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