

Anacardium occidentale Methanolic Nut Extract Attenuated Testicular Dysfunctions in High-fat Diet and Streptozotocin-Induced Diabetic Male Wistar Rats

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ABSTRACT

Background: Diabetes induces reproductive ailment and modern therapy is still unsatisfactory to combat diabetes-related reproductive impairment. This study investigated effect of *Anacardium occidentale* nut methanolic extract on testicular dysfunctions in high-fat diet (HFD)/streptozotocin-induced diabetic rats.

Materials and Methods: Forty adult male Wistar rats weighing (180 ± 20 g) were used for the experiment. Diabetes was induced with a repeated single dose of freshly prepared streptozotocin (35 mg/kgb.wt) injected intraperitoneally after fed with HFD for six weeks. The animals were randomly allotted into five groups, 8rats/group. Group I: control; Group II: diabetic control; Group III & IV: diabetic rats + low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* nut methanolic extract; Group V: diabetic rats + 200 mg/kg bwp.o metformin for 28 days. Daily food and water intake with weekly body weight and fasting blood glucose were recorded throughout the experimental phase. On the last day of the experiment, the animals were sacrificed, blood sample were collected and testes was harvested for biochemical assay.

Results: Reproductive hormones, sperm parameters, testicular antioxidants, testicular B-cell lymphoma-2, testicular enzymes, body and weight of reproductive organs were significant ($p < 0.05$) reduced in diabetic rats with significant ($p < 0.05$) increase in blood glucose, insulin, sperm abnormalities, testicular caspase-3, testicular malondialdehyde (MDA), and food intake. Low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* nut methanolic extract administration significantly ($p < 0.05$) improved the testicular biochemical changes.

Conclusion: *A. occidentale* nut attenuated testicular dysfunctions and could be a novel therapy for diabetes-induced reproductive dysfunctions.

Keywords: Diabetes mellitus, Testes, Male reproductive hormones, Sperm parameters, *Anacardium occidentale* nut.

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INTRODUCTION

Diabetes mellitus (DM) is one of the global fast-growing disease and according to International Diabetes Federation (IDF), the worldwide estimated number of diabetes sufferers in 2017 was 451 million and is predicted to escalate to 693 million by 2045.¹

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia and abnormalities in carbohydrate, protein and lipid metabolism resulting from deficiency of insulin secretion, insulin action or both.² The pancreatic β -cells synthesize and secrete insulin, which plays a vital role in regulating reproductive function. Reproductive function impairment in both sexes is one of diabetes mellitus complications and nearly 90% of patients with diabetes suffered reproductive dysfunctions complication.^{3,4}

Diabetes induces impairment in male reproductive functions both in experimental diabetic animal models and humans have been confirmed.⁵ Diabetes may be linked with hypothalamus-pituitary-testicular axis impairment leading to decline levels of serum testosterone (TST), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Also, diabetes mellitus induces spermatogenesis disorder, testicular

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apoptosis, change in sperm morphology and impecunious sperm quality. Furthermore, weak libido, erectile dysfunction and retrograde ejaculation in diabetes have been implicated in male infertility.^{6,7}

Additionally, oxidative stress is well known in the

pathogenesis of diabetes and its related complications via the production of high free radicals and weak antioxidant defense system.⁸ Oxidative stress significantly contributes to the pathogenesis of testicular apoptosis and atrophy, leading to male hypogonadism, bad sperm quality and infertility.^{9,10}

Diabetes therapies with many currently available anti-diabetic drugs associated with several adverse effects are ineffective in ameliorating the induced reproductive dysfunctions of diabetes.^{11,12} Numerous bioactive Phyto-constituents in medicinal plants have led to the development of novelty drugs.¹³ Bioactive components from plant extracts have been scientifically proven to boost the antioxidant defence system and ameliorate hyperglycemia and male reproductive functions in diabetic rats, proposing the pharmacological significance of phytomedicine therapy for diabetes and its associated male reproductive dysfunctions.^{14,15}

Anacardium occidentale is a universally known plant. The plant originated from Brazil and consumed naturally.¹⁶ Many parts of the plant are medicinally used as remedy for diverse ailments.¹⁷ Phytochemical analysis of *A. occidentale* plant evidences the presences of various bioactive compounds and other polyphenols.¹⁸ Although there's remarkable research on the hypoglycemic activity of these plant parts specifically, *A. occidentale* nut and no report from scientific research on any part of *A. occidentale* as therapy for reproductive dysfunction in diabetes. This study therefore investigated potential effect of *A. occidentale* methanolic nut extract on testicular reproductive function in high-fat diet/streptozotocin induced diabetic rats.

MATERIALS AND METHOD

Chemicals and drugs

Streptozotocin, ketamine, xylazine, methanol, metformin

A. occidentale nut collection

A. occidentale nuts were freshly harvested from Agricultural Research Farm, Ladoké Akintola University of Technology, Ogbomosho, Oyo State, Nigeria. The nut was identified, authenticated, and assigned a voucher specimen number LH0533 by Dr. A. T. J. Ogunkunle at Biology Department, Ladoké Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

A. occidentale nut Extraction

A. occidentale nuts were thoroughly washed, and air-dried at room temperature and the outer coated was removed. The nuts were then grinded into fine powder using an electric blender and stored in an air-tight container. 500 g of the fine powdered form was extracted in a Soxhlet apparatus with methanol (95%) as a solvent. The semi-solid methanolic extract recovered was kept in air-tight container at 4°C until used.

Experimental Animals

Forty matured male albino Wistar rats weighing (180 ± 20 g)

were procured and housed at Animal Research House of the Physiology Department, Ladoké Akintola University of Technology, Ogbomosho, Oyo State, Nigeria. The animals were kept in clean polypropylene cages, 8 rats/cage and had access to standard feed and water *ad libitum* under ventilated pathogen-free hygienic environment of temperature (25 ± 2°C), relatively humidity (45% ± 5%) and natural 12:12 hours light/dark cycle for a week acclimatization before the initiation of the experiment. All experimental procedures were conducted according to the protocol of National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the Research Ethical Committee of Ladoké Akintola University of Technology.

Experimental Diabetes Induction

Animals in groups II-V were fed a high-fat diet (HFD) for six weeks after acclimatization. At the end of high-fat diet feeding, the animals were fasted overnight (12 hours) and diabetes was induced by intraperitoneal injection of repeated doses of freshly prepared streptozotocin (STZ) (35 mg/kgb.wt) dissolved in 0.1 M citrate buffer (pH 4.5)¹⁹ and 20% glucose solution was given to the animals overnight to avert drug-induced hypoglycemic death. Fasting blood samples was taken from tail vein of the rats after 72 hours of STZ injection to authenticate diabetes induction using a digital glucometer (Accu-Chek) with test strips and animals with fasting blood glucose levels ≥ 200 mg/dL were considered diabetic model and selected for the experiment.

Experimental Design and Animals treatment

The forty (40) experimental animals were randomly separated into five groups; 8 rats each. The animals received different treatments for 21 consecutive days as follows:

Group I: Normal control rats + 0.5 mL/kgb.wt distilled water

Group II: Diabetic control rats + 0.5 mL/kgb.wt distilled water

Group III: Diabetic rats + 100 mg/kgb.wt *A. occidentale* nut methanolic extract (AONME)

Group IV: Diabetic rats + 200 mg/kgb.wt *A. occidentale* nut methanolic extract (AONME)

Group V: Diabetic rats + 200 mg/kgb.wt metformin (MET).

The animals' body weight and blood glucose were taken on the 1st, 7th and 14th days of the treatment period, while food and water intake were recorded daily.

Fasting blood glucose Estimation

Fasting plasma blood glucose level was determined based on the glucose oxidase-peroxidase (GOD-POD) method using active digital glucometer (Accu-chek) via fasting blood samples drawn from tail vein of the animals.

Biochemical Assay

At the end of treatment period and after 24 hours of the last dose administration, the animals were sacrificed by cervical dislocation under ketamine-45 mg/kg and xylazine-5 mg/kg anesthesia. Fasting blood samples were collected via cardiac puncture into heparinized tube, centrifuged at 3500 rpm for

15 minutes and clear supernatant plasma retrieved were kept at -20°C for analysis.

The rats' testes were dissected and rinsed in normal saline. A minute portion of each testis was cut, and homogenized in an ice-cold phosphate buffer saline (PBS) P^H 7.4, and the homogenate was centrifuged at 10,000 rpm for 10 minutes at -20°C. The clear supernatant plasma obtained was used for biochemical estimations.

Enzyme-linked immunosorbent assay (ELISA) methods was used to determine the insulin, TST, FSH, and LH levels with each specific hormone rat ELISA kits according to protocol of manufacturers.

Semen samples collected from caudal region of the epididymis was used for sperm count and motility determination using adopted technique of Donnelly *et al.*²⁰ Sperm viability and abnormalities percentage were determined under microscopic examination (400x) using epididymial fluid stained with eosin-nigrosin stain according to Amann.²¹ Sperm viability percentage was calculated as (no of live spermatozoa / total spermatozoa counted) x 100.

Levels of testicular antioxidant enzyme superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) activity and oxidative stress marker malondialdehyde (MDA) level were estimated with corresponding assay kit based on the manufacturer's guideline.

Testicular alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and acid phosphatase levels were determined via colorimetric method.

The testicular pro-inflammatory cytokine levels, including tumor necrosis factor-alpha (TNF- α) and interleukin-1beta (IL-1 β), were determined using commercially available ELISA kits following the manufacturer's principle.

Apoptotic marker caspase-3 and anti-apoptotic marker B-cell lymphoma-2(Bcl-2) levels in the testicles were also determined by ELISA analysis with available commercial kits according manufacturer's instruction.

Statistical Analysis

Table 1: Effects of *A. occidentale* methanolic nut extract (AONME) on antioxidant activity, reproductive organs weight, body weight, food and water intake in high-fat diet and streptozotocin-induced diabetic rats

Groups Parameters	Normal control	Diabetic control	Diabetic + Low dose (100 mg/kgb.wt AONME)	Diabetic + High dose (200 mg/kgb.wt AONME)	Diabetic + 200 mg/kgb.wt Metformin (MET)
Body weight (g)	186 \pm 7.55	134.40 \pm 3.01*	185.40 \pm 5.40 [#]	181.00 \pm 9.88 [#]	188.00 \pm 6.78 [#]
Food intake (g/rat/day)	20.00 \pm 0.12	8.49 \pm 0.80*	15.47 \pm 3.13 [#]	16.43 \pm 1.34	16.59 \pm 1.14
Water intake (mL/rat/day)	35.20 \pm 1.79	50.61 \pm 3.00*	39.08 \pm 2.16 [#]	35.61 \pm 1.08 [#]	36.63 \pm 2.10 [#]
Testicular SOD (U/mL)	1.37 \pm 0.04	0.68 \pm 0.05*	1.19 \pm 0.03 [#]	1.38 \pm 0.01 [#]	1.34 \pm 0.06 [#]
Testicular CAT (Umol/mL/min)	18.89 \pm 0.08	14.47 \pm 0.33*	18.39 \pm 0.13 [#]	18.73 \pm 0.14 [#]	18.73 \pm 0.06 [#]
Testicular GSH (mM)	0.05 \pm 0.02	0.29 \pm 0.01*	0.54 \pm 0.05 [#]	0.57 \pm 0.03 [#]	0.58 \pm 0.03 [#]
Testicular MDA (mM)	6.29 \pm 0.71	9.18 \pm 0.52*	6.03 \pm 0.41 [#]	6.25 \pm 0.34 [#]	5.83 \pm 0.55 [#]
Testes (g)	1.25 \pm 0.05	1.07 \pm 0.03*	1.26 \pm 0.03 [#]	1.22 \pm 0.07 [#]	1.21 \pm 0.02 [#]
Epididymis (g)	0.39 \pm 0.01	0.25 \pm 0.03*	0.29 \pm 0.02 [#]	0.29 \pm 0.02 [#]	0.29 \pm 0.02 [#]
Seminar vesicle (g)	0.92 \pm 0.08	0.51 \pm 0.06*	0.66 \pm 0.09 [#]	0.76 \pm 0.08 [#]	0.77 \pm 0.07 [#]
Prostate gland (g)	1.01 \pm 0.04	0.41 \pm 0.07*	0.73 \pm 0.07 [#]	0.76 \pm 0.02 [#]	0.075 \pm 0.08 [#]

Values are expressed as mean \pm SEM (n=8). *significant at $p < 0.05$ compared against control; [#]significant at $p < 0.05$ compared against diabetic group.

Statistical Package for Social Science (SPSS version, 20.0) was used to analyze the data and results are expressed as standard error of means (mean \pm SEM). Statistical significant difference between groups was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc-test and $p < 0.05$ was considered statistical significant.

RESULTS

Effects of *A. occidentale* nut methanolic extract on body weight, food intake and water intake in high-fat diet/streptozotocin-induced diabetic rats

Body weight and food intake were significantly ($p < 0.05$) diminished in diabetic rats, while water intake increased ($p < 0.05$) significantly in comparison with control group. Administration of low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* methanolic nut extract significantly increased ($p < 0.05$) the body weight, food intake and reduced the water intake of diabetic rats as compared to diabetic control rats (Table 1).

Effects of *A. occidentale* nut methanolic extract on fasting blood glucose, and insulin, levels in high-fat diet/streptozotocin-induced diabetic rats

Diabetic control rats exhibited a significant ($p < 0.05$) increase in fasting blood glucose and insulin levels compared to the normal control rats. Administration of low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* methanolic nut extract to the diabetic rats significantly ($p < 0.05$) lowered the fasting blood glucose and insulin levels compared with diabetic control rats (Figure: 1A & 1B).

Effects of *A. occidentale* nut methanolic extract on reproductive hormones levels in high-fat diet/streptozotocin-induced diabetic rats

In comparison with normal control rats, FSH, LH and TST levels

in the diabetic control rats significantly ($p < 0.05$) decreased. Low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* methanolic nut extract administration to the diabetic rats significantly ($p < 0.05$) elevated the FSH, LH and TST levels as compared with diabetic control rats (Figure: 1C, 1D, & 1E).

Effects of *A. occidentale* nut methanolic extract on sperm parameters and abnormalities levels in high-fat diet/streptozotocin-induced diabetic rats

The sperm count, motility, and viability levels in diabetic rats were significantly ($p < 0.05$) lowered in comparison with normal control rats while, sperm abnormalities level increased ($p < 0.05$) significantly in the diabetic rats compared with normal control rats. Treatment with low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* methanolic nut extract significantly ($p < 0.05$) increased the sperm count, motility, and viability levels and significantly ($p < 0.05$) reduced the sperm abnormalities level as compared with diabetic control rats (Figure: 2A, 2B, 2C, & 2D).

Effects of *A. occidentale* nut methanolic extract on tumor necrosis factor-alpha and interleukin-1beta levels in testes of high-fat diet/streptozotocin-induced diabetic rats

In the testes of diabetic control rats, pro-inflammatory cytokines tumor necrosis factor-alpha (TNF- α) and interleukin-1 β (IL-1 β) levels were significantly ($p < 0.05$) increased in comparison with normal control rats. The diabetic rats administered with low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* methanolic nut extracts had a significant ($p < 0.05$) reduction in TNF- α and IL-1 β levels as compared with diabetic control rats (Figure: 3A & 3B).

Effects of *A. occidentale* nut methanolic extract on anti-apoptotic and apoptotic markers in testes of high-fat diet/streptozotocin-induced diabetic rats

Testicular anti-apoptotic marker B-cell lymphoma-2 (Bcl-2) level in the diabetic control rats significantly ($p < 0.05$) reduced while testicular apoptotic marker caspase-3 level elevated ($p < 0.05$) significantly in comparison with normal control rats. The level of testicular anti-apoptotic marker Bcl-2 increased ($p < 0.05$) significantly in the diabetic rats treated with low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* methanolic nut extract with significant ($p < 0.05$) reduction in testicular apoptotic marker caspase-3 level as compared with diabetic control rats (3C & 3D).

Effects of *A. occidentale* nut methanolic extract on testicular enzymes in high-fat diet/streptozotocin-induced diabetic rats

Testicular alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and acid phosphatase (ACP) levels were significantly ($p < 0.05$) reduced in the diabetic control group as compared with normal control group. As compared with diabetic control

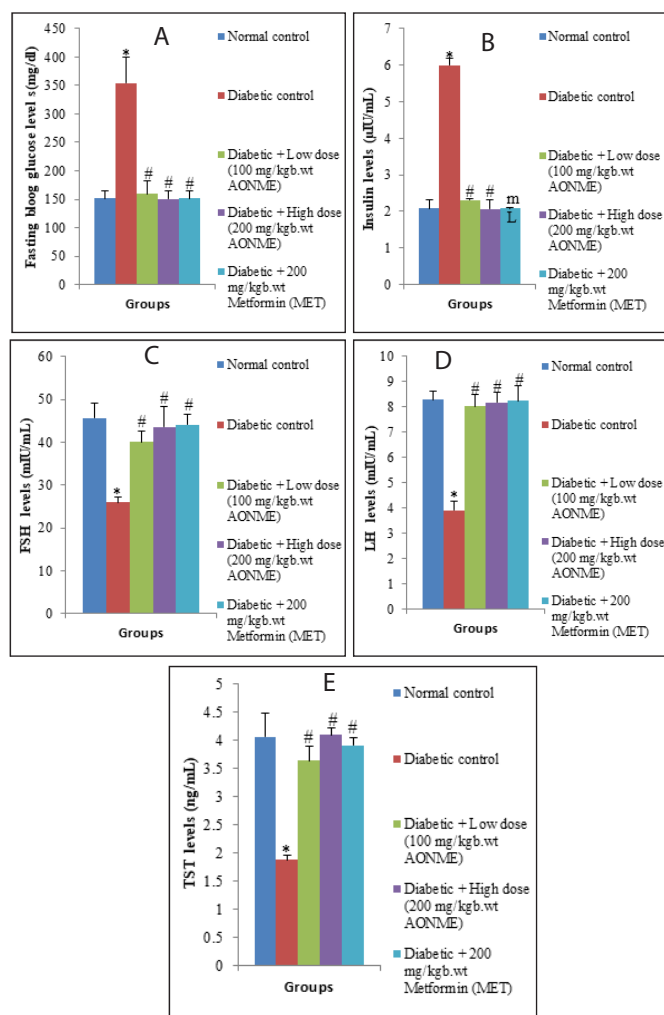


Figure 1: Effects of *A. occidentale* methanolic nut extract (AONME) on (A) fasting blood glucose, (B) insulin, (C) follicle-stimulating hormone (FSH), (D) luteinizing hormone (LH), (E) testosterone (TST) levels in high-fat diet and STZ-induced diabetic rats. Values are expressed as mean \pm SEM ($n=8$). *significant at $p < 0.05$ compared against control; #significant at $p < 0.05$ compared against diabetic group.

rats, testicular enzymes ALP, LDH, ACP levels were significantly ($p < 0.05$) elevated in the diabetic rats administered with low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* methanolic nut extracts (Figures 3 E-G).

Effects of *A. occidentale* nut methanolic extract on oxidative stress marker and antioxidant enzymes activities in testes of high-fat diet/streptozotocin-induced diabetic rats

Activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in the testes of diabetic control rats significantly ($p < 0.05$) diminished with significant ($p < 0.05$) elevation in oxidative stress marker malondialdehyde (MDA) level in comparison to the normal control rats. Testicular antioxidant enzymes SOD, CAT, GSH activities in the testes of diabetic rats administered with low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) *A. occidentale* methanolic nut extract significantly ($p < 0.05$)

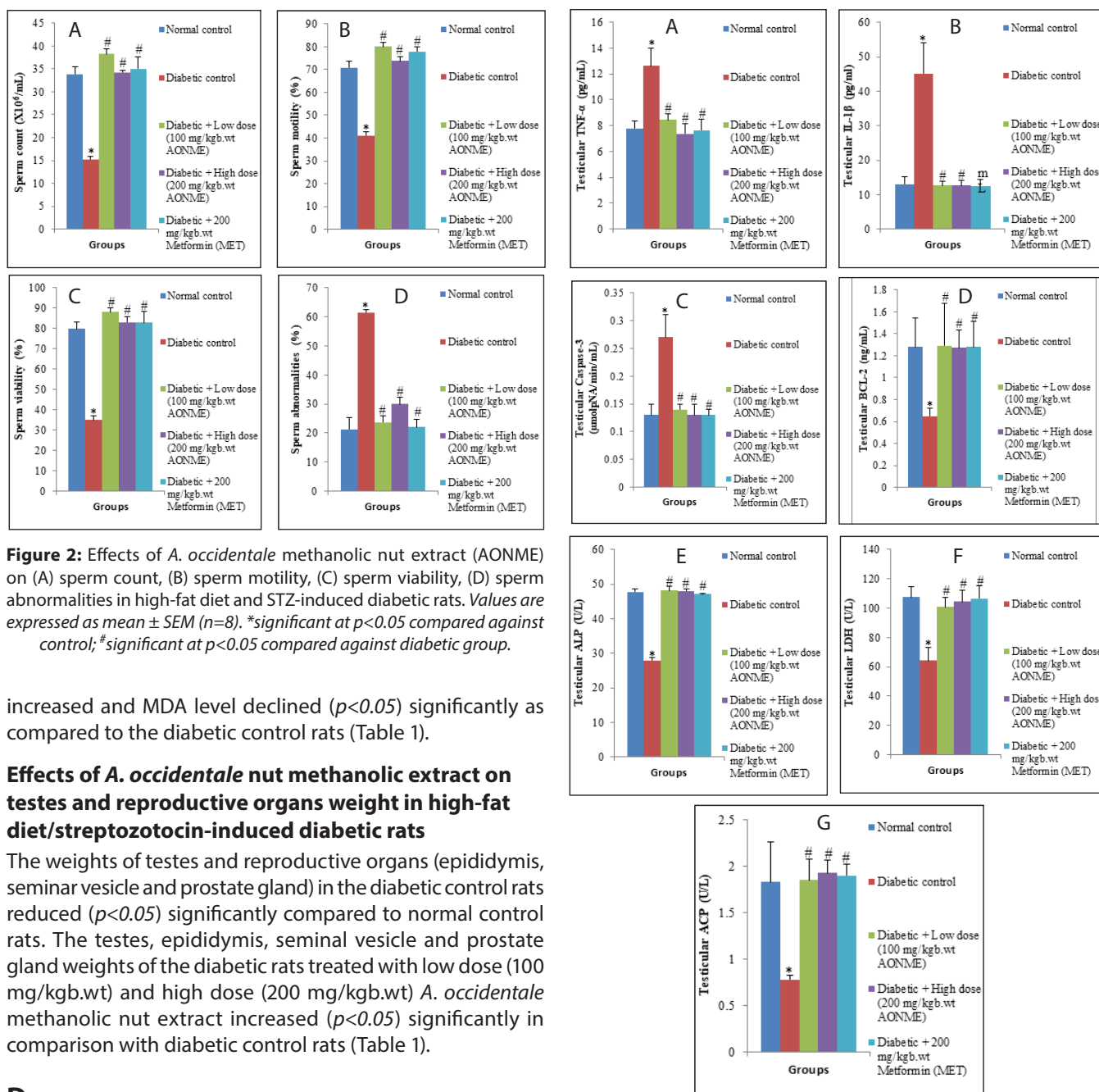


Figure 2: Effects of *A. occidentale* methanolic nut extract (AONME) on (A) sperm count, (B) sperm motility, (C) sperm viability, (D) sperm abnormalities in high-fat diet and STZ-induced diabetic rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared against control; #significant at p<0.05 compared against diabetic group.

increased and MDA level declined (p<0.05) significantly as compared to the diabetic control rats (Table 1).

Effects of *A. occidentale* nut methanolic extract on testes and reproductive organs weight in high-fat diet/streptozotocin-induced diabetic rats

The weights of testes and reproductive organs (epididymis, seminal vesicle and prostate gland) in the diabetic control rats reduced (p<0.05) significantly compared to normal control rats. The testes, epididymis, seminal vesicle and prostate gland weights of the diabetic rats treated with low dose (100 mg/kg bw) and high dose (200 mg/kg bw) *A. occidentale* methanolic nut extract increased (p<0.05) significantly in comparison with diabetic control rats (Table 1).

DISCUSSION

Diabetes mellitus is a chronic metabolic disease that causes dysfunction of many organs and systems.²² There have been extensive research on the consequence of diabetes mellitus on the reproductive system.^{23,24} So far, novel therapeutic approach for diabetes-induced-reproductive impairment remains inconclusive.

In the current study, diabetic-induced rats exhibited typical diabetes symptoms such as hyperglycemia, polydipsia, and body weight reduction accompanied by loss of weight of reproductive organs including testes, epididymis, seminal vesicle and prostate gland, which consistent with findings of Nabil *et al.*²⁵ who also reported a significant reduction in body and reproductive organs weights in

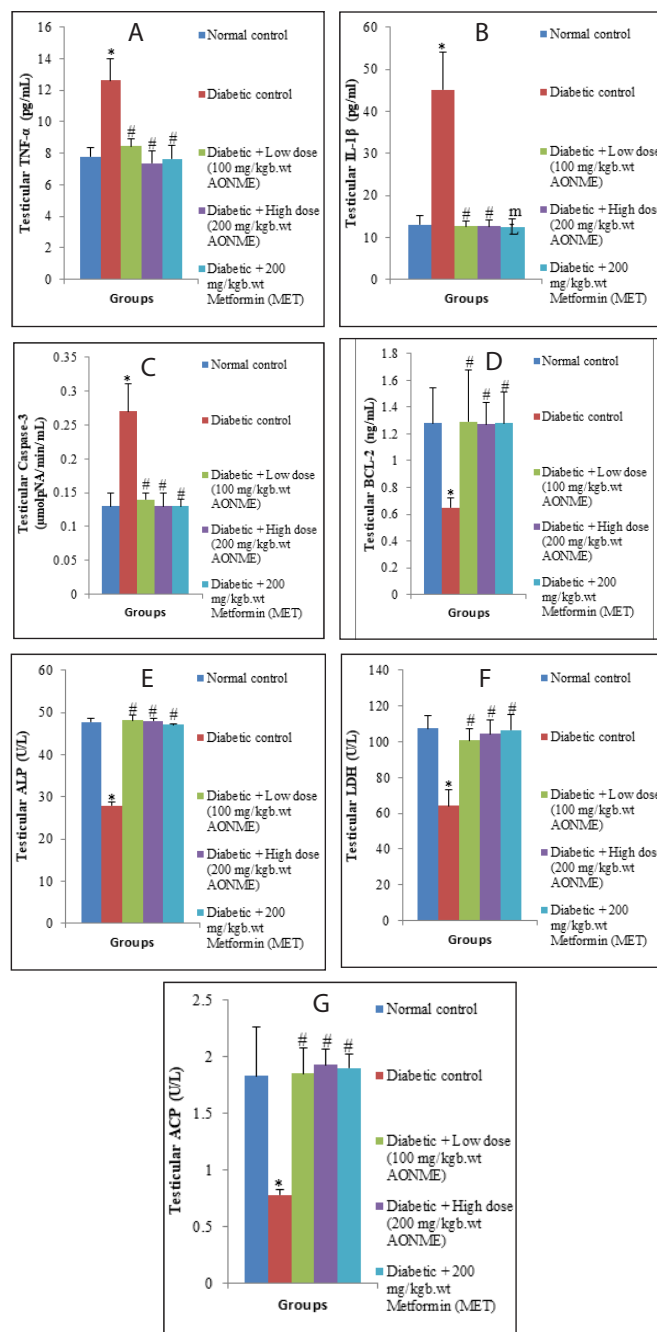


Figure 3: Effects of *A. occidentale* methanolic nut extract (AONME) on (A) testicular inflammatory marker tumor necrosis factor-alpha (TNF-α), (B) testicular anti-inflammatory marker interleukin-1 beta (IL-1β), (C) testicular apoptotic marker caspase-3, (D) testicular anti-apoptotic marker B-cell lymphoma-2 (BCL-2), (E) testicular alkaline phosphatase (ALP), (F) testicular lactate dehydrogenase (LDH), (G) testicular acid phosphatase (ACP) abnormalities in high-fat diet and STZ-induced diabetic rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared against control; #significant at p<0.05 compared against diabetic group.

lead acetate exposed rats. Body weight reduction may be attributed to increment in tissues proteolysis.²⁶ Also, decline in weight of reproductive organs may be consequence of diabetes testicular deterioration and reduction of epididymis weight.²⁷ In supporting the findings of Karawya *et al.*²⁸ on

increased testicular weight following administration of *C. esculentus*, the methanolic nut extract of *A. occidentale* supplement at low (100 mg/kgb.wt) and high (200 mg/kgb.wt) alleviated the reduced body and reproductive organs weights of streptozotocin-induced diabetic rats. TST and other androgen possess anabolic effects by increasing protein synthesis, which increases muscle mass. Increase in volume and weight of the testis and epididymis is one of androgens function via stimulation of protein synthesis.²⁹ Elevated body and reproductive organ weights observed may result from increased testosterone concentration after administration of the extract.

Generally, TST, FSH and LH are fundamental biomarkers reproductive hormones in diagnosis and prognosis of male reproductive fertility function. Reproductive functions are regulated by hypothalamus-pituitary-gonadal axis. It has been previously reported that the production of TST by Leydig cells is augmented by LH secreted from anterior pituitary gland. In addition, LH stimulates FSH to bind with sertoli cells for spermatogenesis process stimulation.³⁰ Consistent with findings of many researchers who reported remarkable reduction in TST, FSH and LH concentrations in male diabetic rats and diabetic patients,³¹⁻³⁵ current study also observed markedly diminution in TST, FSH and LH concentrations in diabetic rats. The observed reduction in TST, FSH and LH concentrations in the diabetic rats imply diabetes deleterious effect on biosynthesis and secretion of FSH and LH from anterior pituitary thus, disturb the Leydig and Sertoli cells functions and consequently in testicular steroidogenesis and spermatogenesis impairment. Low FSH, LH, and TST concentrations has been linked with insulin deficiency which hinder gonadotropins secretion thereby reduce testicular TST releases³⁶ which contrary the current finding as diabetic rats exhibited hyperinsulinemia and this suggest a state of insulin resistance, a marker of type-2-diabetes mellitus. The methanolic extract of *A. occidentale* nut administered to diabetic rats at low (100 mg/kgb.wt) and high (200 mg/kgb.wt) doses considerably elevated the TST, FSH and LH hormones concentrations, which are consistent with finding of Ebong *et al.*³⁷ who reported considerable increase in TST, FSH and LH hormones in diabetic rats administered with 80% *Moringa oleifera* leaves ethanolic extract. The improvement in TST, FSH and LH hormones observed after treated the diabetic rats with *A. occidentale* nut extract in the current study could be due to restorative effects of bioactive compounds in the extract on pancreas for synthesis and secretion of insulin that lead to resumption of pituitary as well as functions of Leydig and sertoli cells in the testes.

In chronic hyperglycemia, sperm count and motility are considered as major parameters in assessing sperm dysfunction.³⁸ Altered sperm count, motility, and viability have been widely observed in male diabetic rats. Further, humans with diabetes have reduced spermatogenesis and testosterone levels.³⁹ In agreement with the findings of Soliman *et al.*⁴⁰ that recorded reduced sperm count, motility,

viability and increased sperm abnormalities in diabetic rats, findings from this study also revealed reduced sperm count, motility, and viability in diabetic rats with increased sperm abnormalities percentage. Overproduction of reactive oxygen species (ROS) and generation of lipid peroxidation is one of mechanism involves in sperm characteristic disruption in diabetes condition. High ROS distort sperm cell's membrane and lesser the available energy thus, may obstruct sperm motility and viability.⁴¹ Also, diminish sperm motility and fertilization potential has been associated with perturb sperm membrane by high lipid peroxidation.⁴² *A. occidentale* methanolic nut extract administered to diabetic rats low (100 mg/kgb.wt) and high (200 mg/kgb.wt) doses ameliorates the sperm count, motility, and viability and attenuate the percentage sperm abnormalities which similar with the findings of Nermin⁴³ on improve sperm characteristics in diabetic rats treated with ginger and taurine extracts. As reported by Hodek *et al.*,⁴⁴ antioxidant in bioactive molecules neutralize free radicals or prohibit the enzymes involve in their formation. This protects cells including testes cells against reactive oxygen species damages and enlarges their size and their secretory function.⁴⁵ The observed enhancement in sperm count, motility and viability may be due to the antioxidant properties of the extract to protect sperm cells against hyperglycemia induced oxidative stress attack.

In diabetes mellitus, persistent hyperglycemia implicate in the pathogenesis of testicular oxidative stress that lead to reproductive complications and infertility.^{46,47} Oxidative stress causes production of excessive reactive oxygen species and depletion in antioxidant defense system.⁴⁸ Extreme levels of reactive oxygen species induced testicular germ cells apoptosis which resulted in testes degeneration.⁴⁹ In consonance with finding of Jangir *et al.*,⁵⁰ who reported lessen antioxidant superoxide dismutase (SOD), catalase (CAT) and non-enzymatic antioxidant reduced glutathione (GSH) and high malondialdehyde (MDA) in testes of diabetic rats, diminished antioxidant defense system SOD, CAT, GSH and elevated oxidative stress marker MDA levels were observed in testes of diabetic rats of the current study. The observed decline in antioxidant defense system could be resulted from their inactivation by excessive free radicals overwhelm or glycation product formation.⁵¹ Administration of *A. occidentale* methanolic nut extract to diabetic rats at low (100 mg/kgb.wt) and high (200 mg/kgb.wt) doses markedly increase the testes antioxidant enzymes SOD, CAT and GSH and inhibit testicular oxidative stress as marked by low level of MDA. The findings specify the presence of potent antioxidant bioactive molecule in *A. occidentale* nut with free radical scavenge efficacy in ameliorating male reproductive dysfunction complications which corroborate the finding of Balasundram *et al.*⁵² on antioxidant properties of phenolic compound in plant.

In addition, oxidative stress induced activation of nuclear factor kappa B (NF- κ B) under chronic hyperglycemia

condition. NF- κ B triggers the activation of pro-inflammatory cytokines, which are considered to be vital in an inflammatory process.⁵³ Over-expression of pro-inflammatory cytokines in testes of diabetic rats have been reported,^{54,55} which parallel with this present findings as there was over-expression of testicular pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF- α) and interleukin-1 β (IL-1 β) in the diabetic rats. The diabetic rats administered with low (100 mg/kgb.wt) and high (200 mg/kgb.wt) doses of *A. occidentale* methanolic nut extract had reduced testicular pro-inflammatory cytokines, TNF- α and IL-1 β levels, suggesting its anti-inflammatory effect to protect the progression of diabetes complications mediators. This effect may be attributed to inhibition of testicular oxidative stress development by this extract which similar with the results of Nna *et al.*⁵⁶ on synergetic anti-inflammatory activity of Malaysian propolis and metformin in pancreas of diabetic rats.

The pathogenesis of testicular germ cell apoptosis in diabetes have been linked with consequence of oxidative stress and inflammation.^{57,58} Anti-apoptotic marker B-cell lymphoma-2 (Bcl-2), a negative regulator of cell death prevents cells from undergoing apoptosis induced by inflammatory mediators such as TNF- α .⁵⁹ Also, superfluous of oxidative stress with the mitochondria stimulate the releasing of cytochrome c, causes the activation of caspase-3 and apoptosis.⁶⁰ Extreme apoptosis can aggravate semen quality and sperm production abnormalities, consequently in oligozoospermia and asthenospermia.⁶¹ Diabetes induced down-regulation of testicular anti-apoptotic marker, Bcl-2 and up-regulation of testicular of apoptotic marker, caspase 3.^{62,63} The present findings also observed down-regulation in testicular anti-apoptotic marker, Bcl-2 and up-regulation in testicular apoptotic marker, caspase-3 in the diabetic rats, similar with the findings of Chen *et al.*⁶⁴ Moreover, *A. occidentale* methanolic nut extract administered at low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) suppressed the testicular apoptotic marker, caspase-3 and up-regulated testicular anti-apoptotic marker, Bcl-2 in diabetic rats of current study, proving the anti-apoptotic potential of this extract on hyperglycemia induced testicular apoptosis owing to the possession of anti-oxidative and anti-inflammatory properties which is in accord with report of AL-Megrin *et al.*,⁶⁵ on attenuation of testicular apoptosis with Green *Coffea arabica* extract administration to high-fat/STZ induced diabetic rats.

The enzymes necessary for energy production, biotransformation, and regulation of secretory activity in testes for spermatogenesis process are altered in diabetes condition. Decrease testicular lactate dehydrogenases (LDH), acid phosphatase (ACP), as well as alkaline phosphatase (ALP) have been reported in diabetes [66, 67]. The testicular LDH, ACP and ALP in diabetic rats of the current finding distinctly reduced, which harmonize the finding of Geng *et al.*[68]. The observed reduction in ACP, ALP and LDH have directly linked with damage of seminiferous tubules, Leydig cells, sertoli cells malfunction and loss of spermatogenic cell layers by oxidative

stress attack induced by hyperglycemia of diabetes [69]. The administration of low dose (100 mg/kgb.wt) and high dose (200 mg/kgb.wt) of *A. occidentale* nut methanolic extract to the diabetic rats recovered and normalized the testicular enzymes almost as the control level, affirming the testicular protective efficiency of the extract which correspond with Chao *et al.*[70] who reported similar result in testicular enzymes recovery on loganin administration.

CONCLUSION

From these findings, *A. occidentale* nut alleviates testicular function, via attenuation of testicular oxidative stress, inflammation and apoptosis induced by chronic hyperglycemia. This nut could be use as effective alternative therapy for diabetes-induced reproductive disorder complications.

DECLARATIONS

Authors' Contributions

All authors have made considerable contribution to the work and approved the final version of the manuscript. FO conceived the original idea.

Ethical Approval

All procedures were approved by the Animal care committee of the Ladoke Akintola University of Technology and conducted according to the "Principles of Laboratory Animal Care" and specific national laws where applicable.

Consent for Publication

All authors agreed to publish the article.

Availability of Data and Materials

All data generated and analyzed during this study are included in this article.

COMPETING INTERESTS

No competing interests.

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