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Exogenous Testosterone and Vitamin E Ameliorates Diabetes-induced Necrosis of the Pancreas, Lipid and Testicular Disorders in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Authors EOA and AOO designed the study, wrote the protocol and author EOA wrote the first draft of the manuscript. Authors EOA and AOO managed the literature searches, analyses of the study, performed the spectroscopy analysis and author AOO managed the experimental process. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To investigate the effect of combined administration of testosterone and vitamin E on diabetes-induced testicular dysfunctions and dyslipidemia in male Wistar rats.
Place and Duration of Study: Applied and Environmental Physiology Unit, Physiology Department, University of Ibadan, Nigeria, September, 2013 to January, 2014.
Study Design and Methodology: Fifty male Wistar rats (150-200 g) were randomly divided into ten groups of 5 animals: normal control (NC), normal-vit.E treated (NVE), normal-testosterone treated (NT), normal-vit.E+testosterone treated (NVET), diabetic-untreated (DU), diabetic-vit.E treated (DVE), diabetic-testosterone treated (DT), diabetic-vit.E + testosterone treated (DVET), diabetic-insulin treated (DI), and diabetic-glibenclamide treated (DG). Diabetes was induced with a single intraperitoneal injection of 120 mg/kg alloxan monohydrate. Rats with sustained blood

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glucose of ≥ 250 mg/dl were selected as diabetic. Blood glucose was measured using glucose/oxidase principle. Animals were treated for 14 days and by day 15, blood was collected from the retro-orbital plexus for serum lipid analysis. Testes, epididymis and pancreas were excised and caudal epididymal fluid was analyzed for spermatogenic indices while testes and pancreas were processed for histological evaluation. Data were expressed as mean±SEM and statistical analysis performed using Student's unpaired *t* test, one-way analysis of variance (ANOVA). Level of significance is *P* = .05.

Results: The DVET showed significant reduction in serum TC (103.27±12.76), TG (76.25±9.79), VLDLc (15.25±1.96) and LDLc (69.70±14.74) compared with DU (188.67±9.97), (166.75±5.16), (33.55±1.12) and (141.16±11.06) respectively while significant increase was observed in DVET sperm count (59.60±10.31), sperm motility (74.00±2.19) and HDLc (18.31±2.22) compared with DU (25.20±3.99), (66.00±2.19) and (9.70±1.49) respectively.

Conclusion: Combined administration of vit. E and testosterone ameliorates diabetes–induced dyslipidaemia, beta cells necrosis, reduced sperm count and motility in male Wistar rats.

Keywords: Diabetes mellitus; vitamin E; testosterone; dyslipidemia; testicular dysfunctions.

ABBREVIATIONS

Vitamin E (vit. E); Total Cholesterol (TC); Tryglycerides (TG); Low Density Lipoprotein Cholesterol (LDLc); Very Low Density Lipoprotein Cholesterol (VLDLc); High Density Lipoprotein Cholesterol (HDLc).

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from defect in insulin secretion, insulin action or both [1,2]. The defects in insulin availability have been reported to affect fertility in the male [3] and also cause disruption of the lipid profile [4]. La Vigneral et al. [5] reported that the absence or reduced stimulatory effect of insulin on the Leydig cell results in reduced testosterone production, atonia of seminal vesicles, bladder and urethra and increased oxidative stress. Reduced serum testosterone level [6] and increased oxidative stress [7] in diabetes have been reported to be closely linked to male infertility [8]. This study therefore investigated the combined effect of testosterone supplement and free radical scavenger (vitamin E) on the deleterious effect of diabetes on male reproduction.

2. MATERIALS AND METHODS

2.1 Animal Care

Fifty healthy and normoglycaemic male Wistar rats having fasting blood glucose level of 70-80mg/dl and of average weight 172.5±25.00 g were used for this study. The animals were purchased from the animal house of the University of Ibadan, Ibadan. They were kept at normal room temperature and photo-periodicity of 12L: 12D in the animal house of the Faculty of Basic Medical Sciences and fed on rat pellets obtained from Vital Feeds Mill, Orogun, Ibadan. Water was made available *ad libitum.*

2.2 Drugs and Reagents

Alloxan (Sigma Co., USA), Eosin-Nigrosin (Sigma Co., USA), Testosterone propionate (Laborate Pharmaceuticals India LTD), Vitamin E (Sinopharm Xingsha Pharmaceuticals Co., Ltd., China) Insulin Penfil (Penfill^{R}Novo Nordisk, Denmark), Glibenclamide (Fourrts Laboratories Pvt. Ltd., Kelambakkam).

2.3 Induction of Diabetes

Diabetes was induced after an overnight fast by a single intra-peritoneal injection of alloxan monohydrate (120 mg/kg) [9]. Drop of blood from the dorsal vein of rats were used to determine the blood glucose levels at 12 hours, 24 hours, 36 hours, and 48 hours after the alloxan administration and throughout the study using the method of Majekodunmi et al. [10]. Rats with constant blood glucose level of 250 mg/dl or higher were selected into the diabetic group [11].

2.4 Experimental Procedure

Normal saline, vitamin E and glibenclamide were administered orally (P.O.)

Testosterone and Insulin were administered intramuscularly (IM)

*Testosterone (vials) was administered intramuscularly for three consecutive days and then every three days [12]. *Vitamin E was administered orally every three days [13].

2.5 Animal Grouping

Fifty animals were divided into 10 groups of 5 rats each, viz;

Normal control rats (NC) received 1 ml/kg normal saline,

Normal rats treated with vitamin E (NVE) received 500 mg/kg [13],

Normal rats treated with testosterone (NT) received 20 mg/kg [12],

Normal rats treated with vitamin E and testosterone received 500 mg/kg and 20 mg/kg (NVET) respectively,

Diabetic untreated rats (DU) received 1 ml/kg normal saline,

Diabetic rats treated vitamin E (DVE) received 500 mg/kg,

Diabetic rats treated with testosterone (DT) received 20 mg/kg,

Diabetic rats treated with vitamin E and testosterone received 500 mg/kg and 20 mg/kg (DVET) respectively,

Diabetic rats treated with insulin (DI) received 1 IU/kg [11],

Diabetic rats treated with glibenclamide (DG) received 0.5 mg/kg [14].

Animals were treated for 14 days.

2.6 Preparation of Serum

About 4.0 ml of blood was withdrawn from the retro-orbital plexus of each rat into plain bottles and allowed to clot to obtain serum which was later centrifuged at 3500 rpm for 20 minutes. Clear serum was aspirated with a micropipette into new plane bottles and stored in refrigerator at 4°C for lipid profile analysis.

2.7 Lipid Profile Analysis

Total cholesterol (TC) and Triglycerides (TG) were estimated using the method of Rifai et al. [15], High Density Lipoprotein Cholesterol (HDLc) was analyzed using the method of Friedewald et al. [16]. Very Low Density Lipoprotein Cholesterol (VLDLc) and Low Density Lipoprotein Cholesterol (LDLc) were calculated from the values of TG, TC and HDLc by Friedelwald's equation [16].

2.8 Sperm Analysis

Animals were sacrificed by cervical dislocation, opened up at the abdomino-pelvic region and the caudal epididymis was excised and immersed in 5 ml formal-saline. The testes and pancreas were also excised for histological studies. The volume of fluid displaced by the caudal epididymis was taken as its volume. The caudal epididymis was homogenized in 5ml formal-saline to a suspension, and the spermatozoa were examined under the light microscope at a magnification of ×100. The sperm count was determined by evaluating five different fields on the improved Neubauer counting chamber (1/10 mm, LABART, Germany) [17], using the formula given below;

SPERM COUNT = COUNT IN SQUARES × DILUTION FACTOR × 50 × 1000 (million/ml).

Sperm progressive motility was determined by the method of Oyeyemi et al. [18].

Eosin-Nigrosin stain (Sigma Co., USA) was used to identify dead sperm cells while live sperm cells were unstained. An hundred cells per slide were counted to obtain the percentage live/dead ratio [17] in determining the sperm viability.

Smear produced for sperm viability was retrieved and the slide was observed under the microscope with x100 objective under immersion oil; making a count of 400 spermatozoa and the spermatozoa abnormalities were classified as described by Badkoobeh et al. [17].

2.9 Histological Preparation

The testes and pancreas of the animals were excised, fixed and stained with H and E and processed for histological evaluation [19].

2.10 Statistical Analysis

Data are expressed as means±SEM. Statistical analysis was performed using Student's unpaired *t* test, one-way analysis of variance (ANOVA). *P* = .05 was considered statistically significant.

3. RESULTS

3.1 Blood Glucose (mg/dl)

There was no significant difference in the blood glucose level of normal rats treated with vit. E (106.80±3.92), normal rats treated with testosterone (106.80±2.10) and normal rats treated with vit E + testosterone (110.20±1.80) when compared with normal control group (103.20±3.09) (p = .05). The diabetic group treated with vit. E (299.80±22.20), testosterone (255.80±44.57), vit. E + testosterone (309.80±24.77), insulin (139.80±2.56) and glibenclamide (189.60±6.77) showed no significant difference compared to diabetic untreated (383.60±22.20) (p = .05). Table 1.

3.2 Lipid Profile

3.2.1 Total cholesterol (mg/dl)

The diabetic groups treated with vit. E (126.67±5.11), testosterone (101.50±15.22), vit. E + testosterone (103.27±12.76), insulin (123.01±8.73) and glibenclamide (126.69±9.40) showed significant reduction (p = .05) in total cholesterol compared with diabetic untreated (188.67±9.97). “Fig. 1”.

3.2.2 Triglycerides (mg/dl)

The diabetic groups treated with vit. E (83.00±7.67), testosterone (75.00±8.98), vit. E + testosterone (76.25±9.79), insulin (88.00±6.33) and glibenclamide (97.90±1.93) showed significant reduction (p = .05) in triglycerides compared with diabetic untreated (166.75±5.16). “Fig. 2”

3.2.3 Low density lipoprotein cholesterol (mg/dl)

The diabetic groups treated with vit. E (97.78±6.49), testosterone (75.62±15.53), vit. E + testosterone (69.70±14.74), insulin (89.66±9.58) and glibenclamide (93.58±9.22) showed significant reduction (p = .05) in LDLc compared with diabetic untreated (141.16±11.06). “Fig. 3”

3.2.4 High density lipoprotein cholesterol (mg/dl)

Diabetic treated with vit. E + testosterone (18.31±2.22) and insulin (15.75±0.44) showed significant increase (p = .05) in HDLc compared with diabetic untreated (9.70±1.49), although, diabetic treated with vit. E (12.29±2.63) and testosterone (10.78±1.25) showed no significant difference in HDLc compared with diabetic untreated (9.70±1.49). “Fig. 4”.

3.2.5 Very low density lipoprotein cholesterol (mg/dl)

The diabetic groups treated with vit. E (16.60±1.54), testosterone (15.00±1.80), vit. E + testosterone (15.25±1.96), insulin (17.60±1.27) and glibenclamide (19.58±0.39) showed significant reduction (p = .05) in VLDLc compared with diabetic untreated (33.55±1.12). “Fig. 5”.

3.3 Spermatogenic Indices

3.3.1 Sperm count (x 10^6/mm^2)

The diabetic groups treated with vit. E (40.80±4.55), testosterone (60.80±5.51), vit. E + testosterone (59.60±10.31), insulin (47.60±6.25) and glibenclamide (32.60±7.68) showed significant increase (p = .05) in sperm count compared with diabetic untreated (25.20±3.99). “Fig. 6”

3.3.2 Sperm motility (%)

The diabetic groups treated with testosterone (78.00±1.79) and vit. E + testosterone (74.00±2.19) showed significant increase (p = .05) in sperm motility compared with diabetic untreated (66.00±2.19) except diabetic treated with glibenclamide (26.00±5.36) which showed significant reduction. Normal groups treated with vit. E (78.00±3.34), testosterone (82.00±1.79) and vit. E + testosterone (80.00±2.82) showed significant reduction (p = .05) in their sperm motility compared with normal control (90.00±2.45). “Fig. 7”

Table 1. Blood glucose level (mg/dl) in vitamin E and testosterone treated normal and diabetic rats

Treatment groups	Day 0 (Before diabetes induction) (mg/dl)	Day 1 (of treatment after diabetes induction & stabilization) (mg/dl)	Day 4 (mg/dl)	Day 8 (mg/dl)	Day 11 (mg/dl)	Day15 (mg/dl)	Change value (Day 15 minus Day 1) (mg/dl)	% change (Day 15 minus day 1) (mg/dl)
NC	69.04±2.10	98.80±4.20	100.02±2.10	102.04±2.80	101.18±1.11	103.20±3.09*	4.40±1.11	4.26
NVE	48.50±2.45	107.00±4.50	100.00±2.10	83.05±2.41	99.14±1.42	106.80±3.92*	0.20±0.58	0.19
NT	46.44±2.33	107.80±4.00	99.91±1.00	109.33±1.87	103.32±2.83	106.80±2.10*	1.00±1.90	0.94
NVET	65.32±3.89	109.00±2.46	104.12±2.14	105.17±2.89	106.37±2.14	110.20±1.80*	1.20±0.66	1.09
DU	58.23±2.22	467.00±95.73	460.14±42.85	433.25±47.89	400.00±40.82	383.60±22.20[a]	83.40±73.53	21.74
DVE	60.42±3.11	380.00±41.63	380.61±50.00	350.80±62.80	320.20±52.83	299.80±49.59[a]	80.20±7.96	26.75
DT	55.01±1.28	450.40±37.50	401.10±20.82	371.00±32.48	346.00±42.11	255.80±44.57[a]	194.60±7.07	76.08
DVET	60.01±3.81	472.40±40.04	450.11±58.48	415.20±68.21	455.00±33.33	309.80±24.77[a]	162.60±15.27	52.49
DI	46.00±2.82	395.20±31.33	330.39±43.24	296.33±44.42	203.17±21.02	139.80±2.56[a]	255.40±28.77	64.63
DG	48.40±3.06	414.00±32.49	400.14±23.89	391.00±21.42	251.33±26.83	189.60±6.77[a]	224.40±25.72	54.20

*Values are mean±SEM; n = 5. a Significantly different from normal control *Significantly different from diabetic untreated (p = .05)*

KEYS: NC; Normal Control, NVE; Normal Vitamin E Treated, NT; Normal Testosterone Treated, NVET; Normal Vitamin E + Testosterone Treated, DU; Diabetic Untreated, DVE; Diabetic Vitamin E Treated, DT; Diabetic Testosterone Treated, DVET; Diabetic Vitamin E + Testosterone Treated, DI; Diabetic Insulin Treated, and DG; Diabetic Glibenclamide Treated

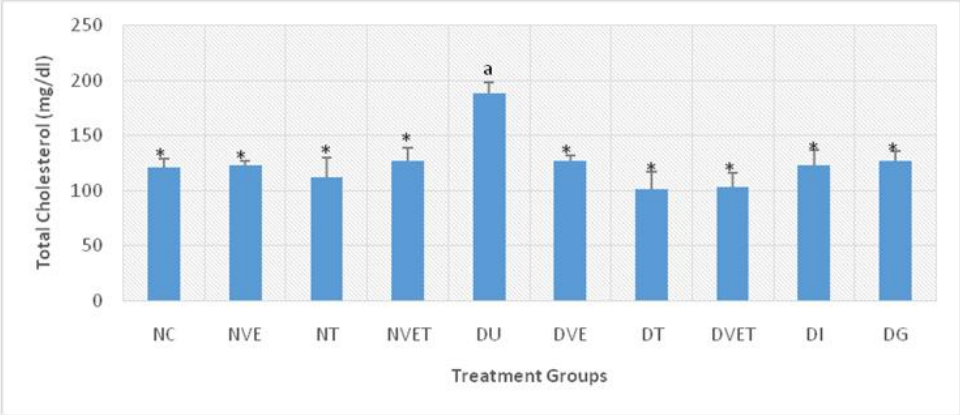

Fig. 1. Serum total cholesterol in Vit. E and testosterone treated normal and diabetic rats; *Values are mean±SEM; n = 5*

*[a] Significantly different from NC; * Significantly different from DU (P = .05)*

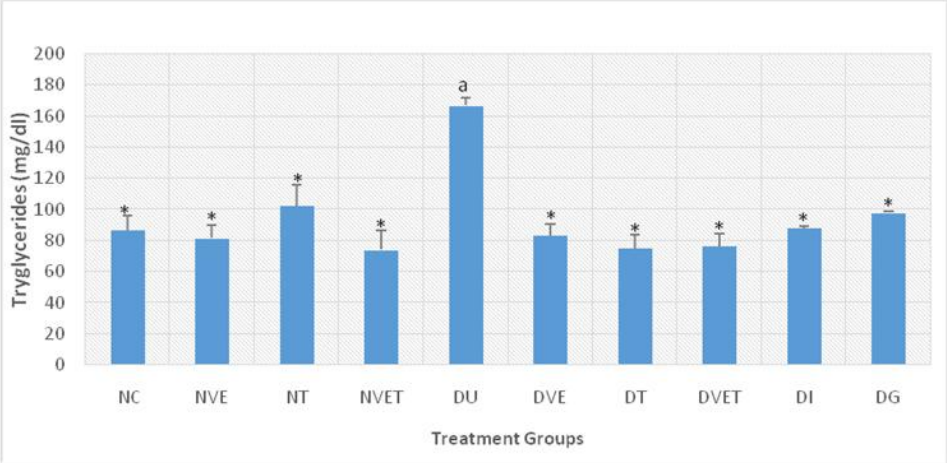

Fig. 2. Serum triglycerides in Vit. E and testosterone treated normal and diabetic rats; *values are mean±SEM; n = 5*

*[a] Significantly different from NC ; * Significantly different from DU (P = .05)*

3.3.3 Sperm viability (% live spermatozoa)

There was no significant difference (p = .05) in the sperm viability of the diabetic groups treated with vit. E (90.60±3.08), testosterone (96.20±0.66), vit. E + testosterone (96.80±0.66), insulin (93.60±1.99) and glibenclamide (90.40±1.54) compared with diabetic untreated (89.00±3.28) and normal control (96.20± 0.66).

3.4 Sperm Morphology

3.4.1 Head abnormalities (per 400 spermatozoa)

There was no significant difference (p = .05) in the sperm head abnormalities of the diabetic groups treated with vit. E (3.80±0.52), testosterone (3.80±0.59), vit. E + testosterone (3.60±0.78), insulin (4.00±0.56) and glibenclamide (4.40±0.61) compared with diabetic untreated (3.60±0.54) and normal control (4.00±0.49).

3.4.2 Sperm middle piece abnormalities (per 400 spermatozoa)

The sperm middle piece abnormalities of diabetic untreated rats (17.40±0.46) and diabetic treated with vit. E (19.20±0.77), testosterone (20.60±2.30), vit. E + testosterone (18.80±0.71) and insulin (18.00±1.52) were significantly different from normal control (12.80±1.07) except

diabetic treated with glibenclamide (21.00±0.80) which showed significant increase compared with diabetic untreated (17.40±0.46). However, diabetic untreated rats showed significant increase (17.40±0.46) in sperm middle piece abnormalities compared with normal control (12.80±1.07).

3.4.3 Sperm tail abnormalities (per 400 spermatozoa)

The diabetic rats treated with glibenclamide (30.00±0.89) showed significant increase in sperm tail abnormalities compared with diabetic untreated (24.80±1.11). However, there was no significant difference in sperm tail abnormalities between normal control (20.60±1.22) and diabetic untreated (24.80±1.11).

3.5 Histology of the Pancreas

3.5.1 Normal Control (NC)

The blood vessels are severely congested, exocrine (acinar) and endocrine (islets) portions appear normal “Fig. 8”.

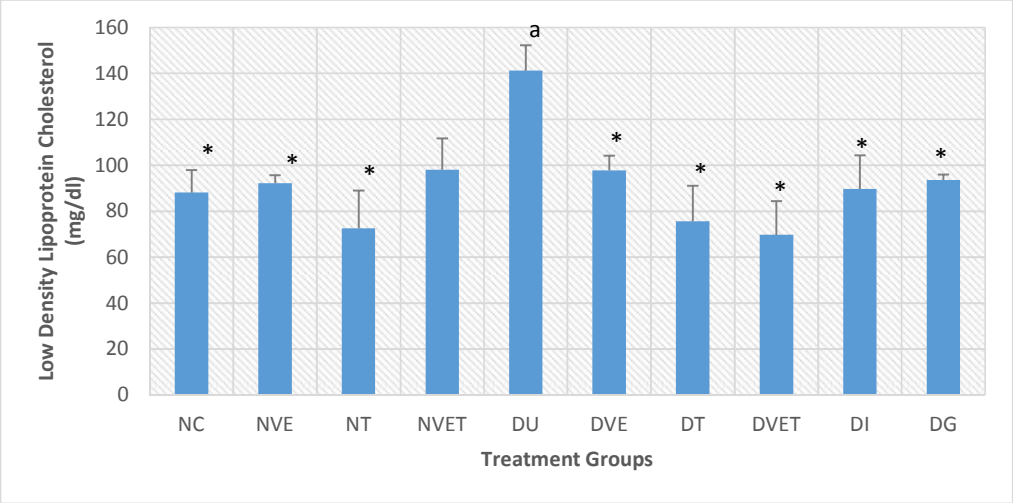

Fig. 3. Serum low density lipoprotein cholesterol in Vit. E and testosterone treated normal and diabetic rats; *Values are mean±SEM;*

*n= 5. [a] Significantly different from NC; * Significantly different from DU (P = .05)*

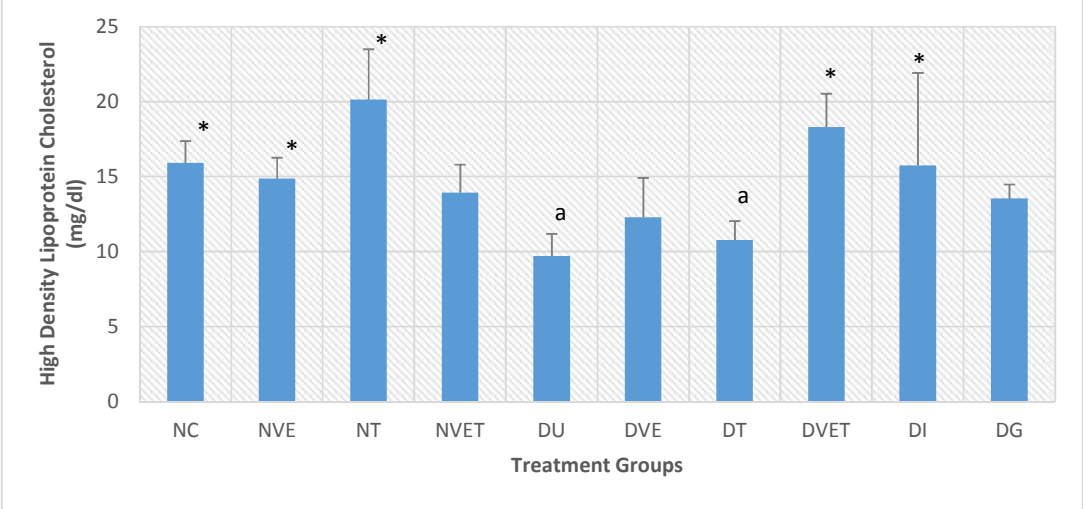

Fig. 4. Serum high density lipoprotein cholesterol in Vit. E and testosterone treated normal and diabetic rats; *Values are mean±SEM;*

*n= 5. [a] Significantly different from NC ; * Significantly different from DU (P = .05)*

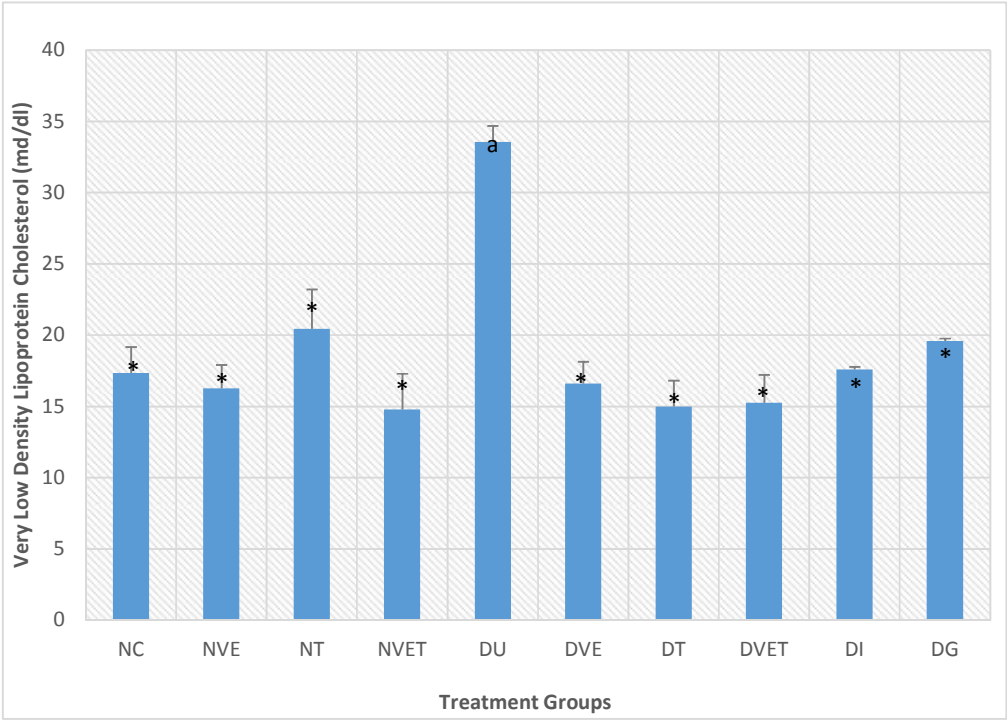

Fig. 5. Serum very low density lipoprotein cholesterol in Vit. E and testosterone treated normal and diabetic rats; *Values are mean±SEM;*
n = 5. [a] *Significantly different from NC; * Significantly different from DU (P = .05)*

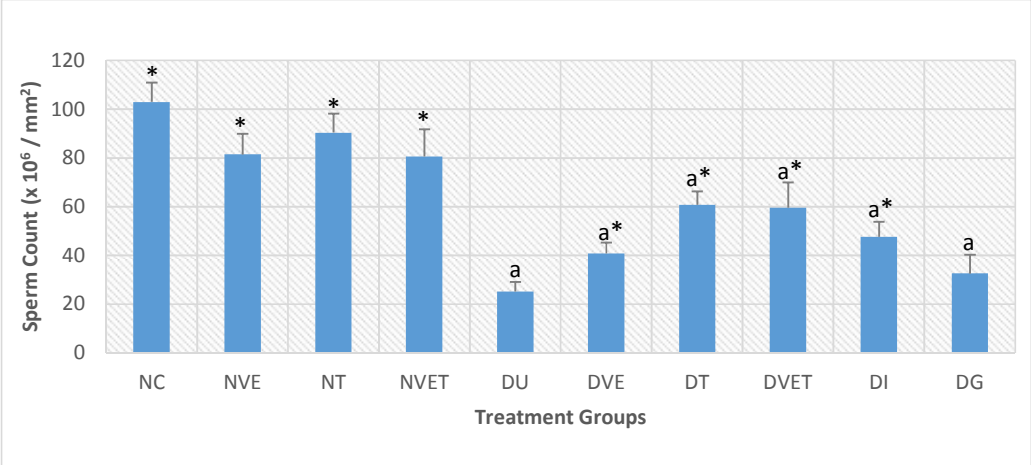

Fig. 6. Sperm count of the Vit. E and testosterone treated normal and diabetic rats; *Values are mean±SEM; n = 5,*
[a] *Significantly different from NC; * Significantly different from DU (P = .05)*

3.5.2 Diabetic Untreated (DU)

No vascular congestion seen, pancreatic islets are shrunken, there was no visible lesion with acinar portions “Fig. 8”.

3.5.3 Diabetic Vit. E treated (DVE)

The blood vessels are markedly congested, islets appear small, shrunken and with irregular outlines “Fig. 8”.

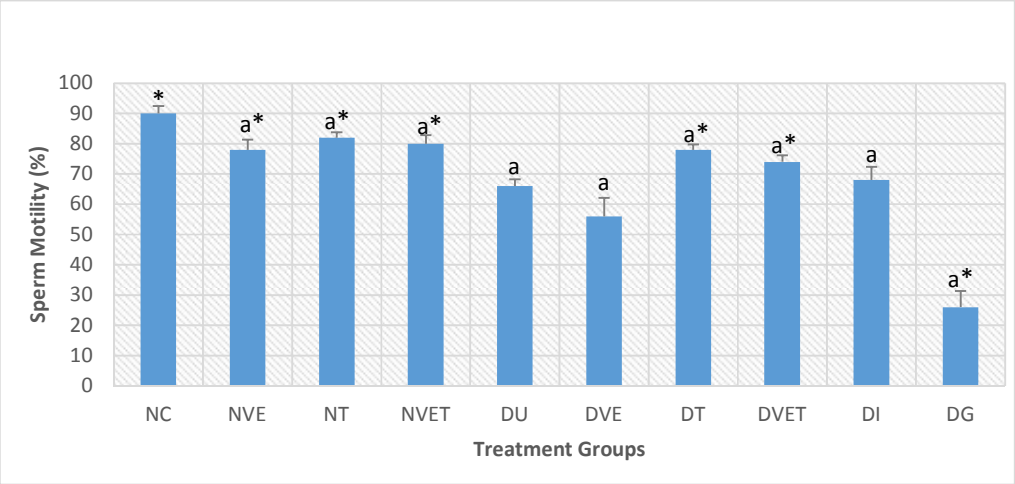

Fig. 7. Sperm motility of the Vit. E and testosterone treated normal and diabetic rats; *values are mean±SEM; n = 5,*

*[a] Significantly different from NC; * Significantly different from DU (P = .05)*

3.5.4 Diabetic Testosterone treated (DT)

The blood vessels are markedly congested especially within the islets; exocrine portions appear normal; mild cytoplasmic vacuolation of cells of the islets "Fig. 8".

3.5.5 Diabetic Vit. E + Testosterone treated (DVET)

There is marked vacuolar change of the cells of the pancreatic acinar "Fig. 8".

3.6 Histology of the Testicles

3.6.1 Normal Control (NC)

Normal control group showed marked congestion of the blood vessels, numerous seminiferous tubules with normal regular outlines as well as adequate numbers of spermatogenic series (spermatogonia, spermatocytes and spermatids) "Fig. 9".

3.6.2 Diabetic Untreated (DU)

Diabetic untreated group showed a few seminiferous tubules with irregular outlines and depleted spermatogenic epithelium, devoid of late-spermatids with presence of abundant residual bodies "Fig. 9".

3.6.3 Diabetic Vit. E treated (DVE)

Diabetic vit. E treated group showed numerous closely-packed seminiferous tubules with regular outlines and abundant spermatogenic epithelium, early and late spermatids are abundant with presence of little amounts of residual bodies "Fig. 9".

3.6.4 Diabetic Testosterone treated (DT)

There are numerous closely-packed seminiferous tubules with regular outlines and moderately depleted spermatogenic epithelium. There are abundant late spermatids, abundant residual bodies, markedly congested blood vessels and accumulation of eosinophilic fluid around the seminiferous tubules "Fig. 10".

3.6.5 Diabetic Vit. E + Testosterone treated (DVET)

There are numerous closely-packed seminiferous tubules with regular outlines and abundant spermatogenic epithelium. Also present are abundant early and late spermatids, little amounts of residual bodies and mildly congested blood vessels "Fig. 10".

4. DISCUSSION

The significant increase in the blood glucose due to alloxan injection in the diabetic untreated rats (Table 1) agrees with the report of Szkudelski [9] in diabetes induction. The hyperglycemia and dyslipidemia observed in the diabetic untreated (Table 1, Figs. 2, 3 & 5) [11] could be due to reduced glucose uptake and utilization in muscles [20] typically observed in diabetes and reduced lipoprotein lipase activity in the liver [21].

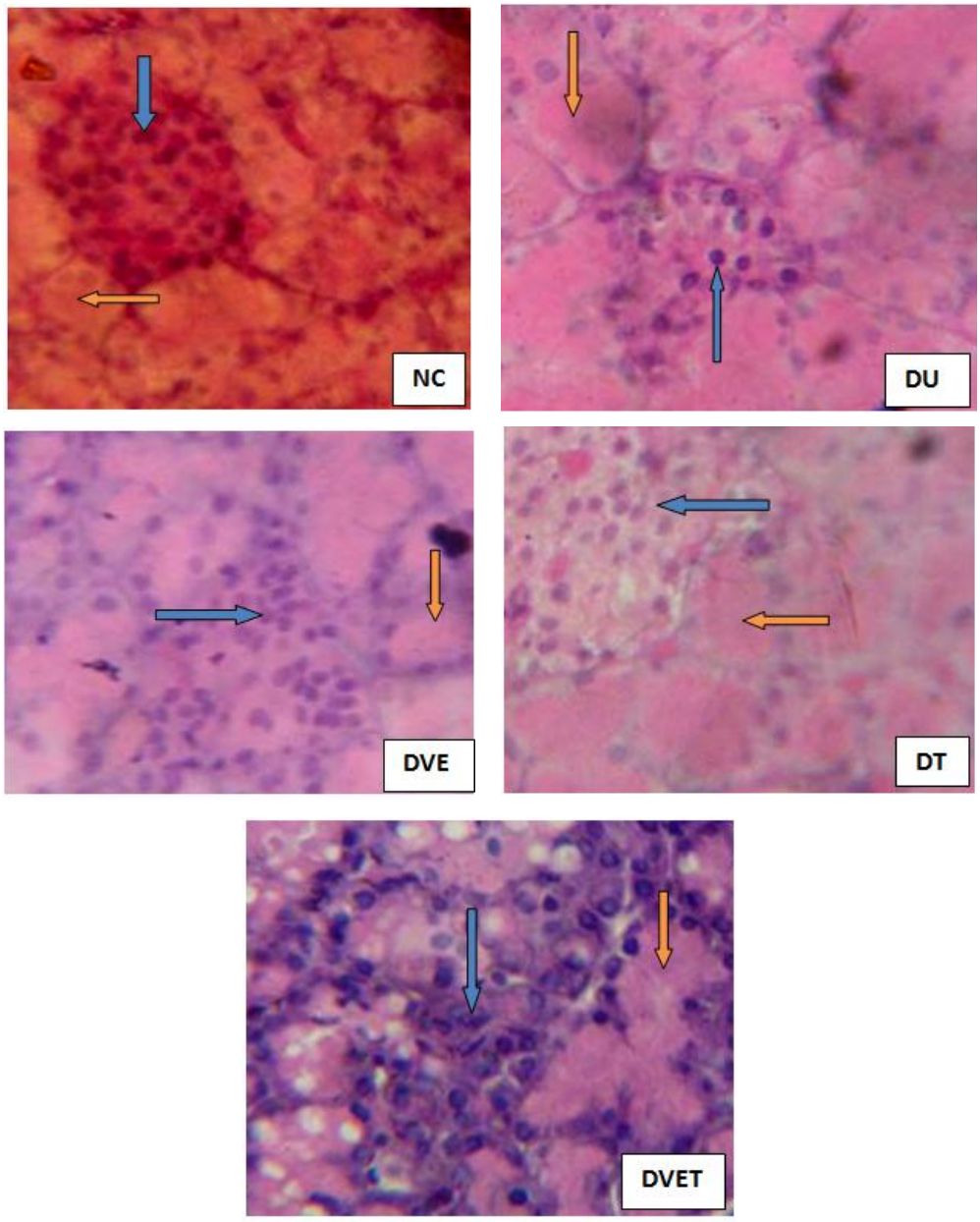

Fig. 8. Pancreatic sections of Vitamin E and testosterone treated normal and diabetic rats. Stain: H and E, magnification X 400;

KEYS: Green arrow indicates Acinar cells; Blue arrow indicates Islet of Langerhans.
NC; Normal Control, DU; Diabetic Untreated, DVE; Diabetic Vitamin E Treated, DT; Diabetic Testosterone Treated, DVET; Diabetic Vitamin E + Testosterone Treated,

The significant reduction in sperm count, sperm motility and significant increase in sperm middle piece abnormalities in the diabetic untreated rats could be due to high oxidative stress resulting from diabetes [22]. Reduced concentration of scavenging enzymes [22,23] has been reported to contribute to oxidative stress and this could also be responsible for the abnormalities observed in the sperm middle piece of the diabetic untreated rats.

The irregular outline of the islet and congested blood vessels which became conspicuous in diabetic group treated with vitamin E could

possibly be the restorative effect of vitamin E compared to diabetic untreated (Fig. 8). The lipid profile in the diabetic vitamin E treated also showed significant reduction in total cholesterol, triglycerides, low density lipoprotein cholesterol, and very low density lipoprotein cholesterol (Figs. 1, 2, 3 & 4) which also confirm possible protective effect of vitamin E. Mendez and Balderaz [24] reported the protective effect of vitamin E on the membrane-bound lipoprotein lipase against lipid peroxidation and this may be the likely mechanism through which irregular outlines of the islets in the diabetic untreated was restored in the diabetic treated with vitamin E (Fig. 8). The significant increase in sperm count observed in the diabetic vitamin E treated (Fig. 6) could be attributed to the antioxidant effect of vitamin E which could have neutralized the free radicals as reported by Trabers and Steven [25] in a similar study.

Bal et al. [26] reported reduced testosterone in diabetes. Klentze [27] also reported that testosterone enhances the sensitivity of the peripheral tissues to insulin. In the testosterone treated diabetic group, a glycaemic reduction of 78.08% was observed and this could be a result of improved sensitivity of peripheral tissues to insulin and consequent utilization of blood glucose [27]. In addition, the involvement of peroxisomal PPAR-α receptor in fat metabolism [27,28] could not be excluded; hence, the significant reduction in total cholesterol, triglycerides, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and significant increase in high density lipoprotein

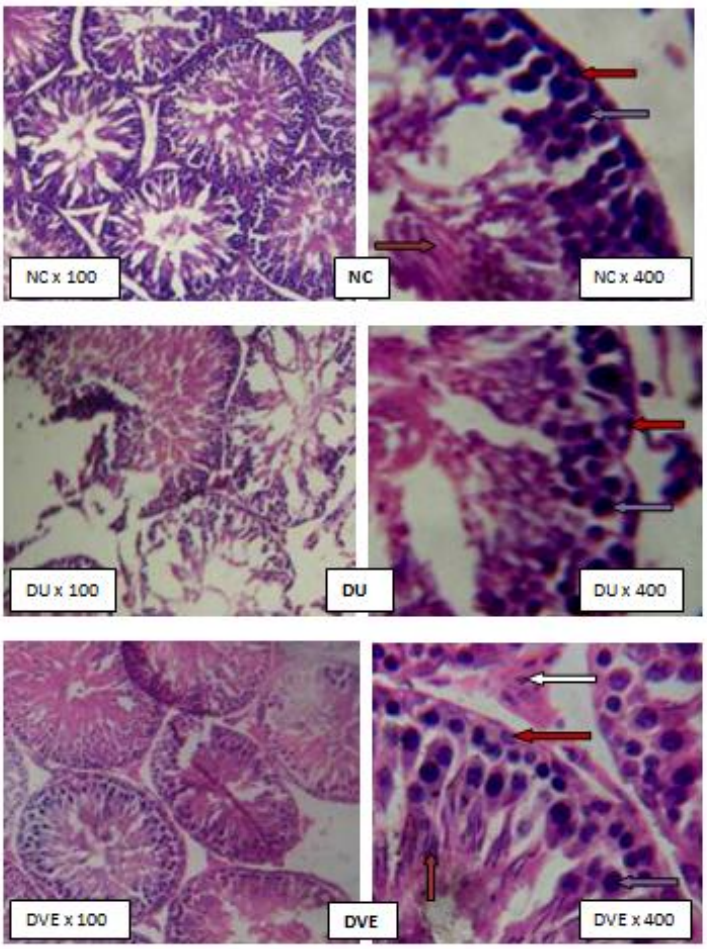

Fig. 9. Sections of seminiferous tubules of Vitamin E and testosterone treated normal and diabetic rats. Stain: H and E; Mag: x 100 and x 400 respectively

KEYS: Red arrow indicates Spermatogonia; Yellow arrow indicates Primary spermatocyte; Orange arrow indicate late spermatids; White arrow indicates Leydig cell; Green arrow indicates Eosinophilic fluid. NC; Normal Control, DU; Diabetic Untreated, DVE; Diabetic Vitamin E Treated, DVET;

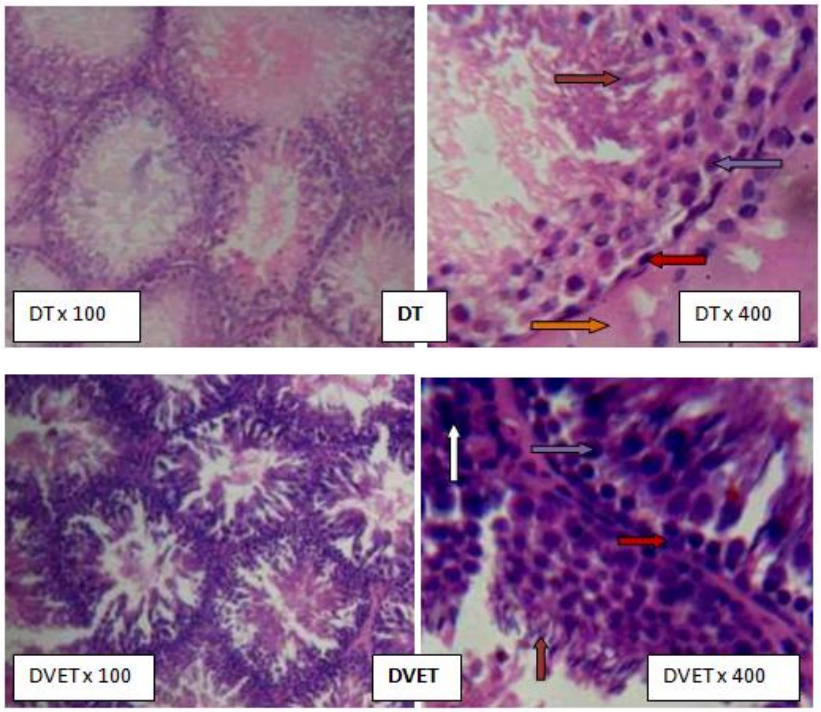

Fig. 10. Continues: Sections of seminiferous tubules of Vitamin E and testosterone treated normal and diabetic rats. Stain: H and E; Mag: x 100 and x 400 respectively

KEYS: Red arrow indicates Spermatogonia; Yellow arrow indicates Primary spermatocyte; Orange arrow indicate late spermatids; White arrow indicates Leydig cell; Green arrow indicates Eosinophilic fluid. DT; Diabetic Testosterone Treated, DVET; Diabetic Vitamin E + Testosterone Treated

cholesterol observed in diabetic group treated with testosterone. Considering the previous works of Bal et al. [26] and La Vigneral et al. [5] in which they reported significant reduction in the serum testosterone level in diabetics, it is most likely that the administered testosterone increased the plasma testosterone level, thereby increasing the stimulatory effect of testosterone not only on the peripheral tissues and peroxisomal PPAR-α receptor but also on the Sertoli cells with resultant significant increase in their sperm count and sperm motility (Figs. 6 & 7) [29,30].

The significant improvement observed in the lipid profile (Figs. 1-3), sperm count and sperm motility of the vitamin E + testosterone treated diabetic rats (Figs. 6 & 7) could be attributed to the combined antioxidant effect of vitamin E and the stimulatory effect of increased plasma testosterone.

Furthermore, the significant reduction in the sperm motility of the normal rats treated with vitamin E (Fig. 7) could be attributed to the oxidative stress effect of high α-tocopherol intake in unstressed individual [31]. Tilbrook and Clark [32] reported oxidative stress decreases axonemial protein phosphorylation and sperm mobilization which subsequently affect membrane fluidity and sperm motility.

The significant reduction in the sperm motility observed in the normal rats treated with testosterone (NT) (Fig. 7), could be attributed to negative feedback effect of the administered testosterone on the hypothalamic – pituitary - gonadal axis resulting in decreased testosterone secretion [21].

However, the significant decrease in sperm motility observed in the normal group treated with vitamin E + testosterone (Fig. 7) could be due to oxidative stress resulting from high concentration of vitamin E in circulation and the negative feedback effect of exogenously administered testosterone. Vitamin E levels in normal animals plus the administered vitamin E constituted high level of vitamin E which most likely resulted in oxidative stress. Again, the exogenous

testosterone added to the normal level of testosterone in the normal animals could have resulted to a high dose of this hormone which could have invariably stimulated negative feedback mechanism through the hypothalamic – pituitary-gonadal axis. This could result in reduced testosterone secretion and plasma concentration. Combination of these effects i.e. the oxidative stress and reduced testosterone level could have resulted in reduced sperm motility which was observed in the study.

5. CONCLUSION

This study showed that combined administration of vitamin E and testosterone ameliorates diabetes–induced dyslipidaemia, beta cells necrosis, and reduced sperm count and sperm motility in male Wistar rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar PJ, Clark M. Textbook of clinical medicine. Pub: Saunders (London). 2002;1099-1121.
2. Lindberg G, Lindblad U, Melander A. Sulfonylureas for treating type 2 diabetes mellitus. Cochrane Database Systemic Reviews. 2004;3.
3. Arikawe AP, Daramola AO, Odofin AO, Obika LF. Alloxan-induced and insulin resistant diabetes mellitus affect semen parameters and impair spermatogenesis in male rats. Reprod Health. 2006;10;106-113.
4. Je HD, Shin CY, Park HS, Huh IH, Sohn UD. The comparison of vitamin C and vitamin E on the protein oxidation of diabetic rats. J. Auton. Pharm. 2001;21: 231-36.
5. La Vignera SL, Condorelli R, Vicari E, D'Agata R, Calogero AE. Mini review; Diabetes mellitus and sperm parameters. J. Androl. 2012;33(2).
6. Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, et al. Low testosterone levels are common and associated with insulin resistance in men with diabetes. Clin Endo and Met. 2008;93(5):1834-40.
PMID: 18319314
7. Liu RT, Chung MS, Wang PW, Chen CD, Lee JJ, Lee WC, et al. The prevalence and predictors of androgen deficiency in taiwanese men with type 2 diabetes. Urol. 2013;82(1):124-9.
PMID: 23676359.
8. La Vignera S, Vicari E, Condorelli R, D'Agata R, Calogero AE. Ultrasound characterization of the seminal vesicles in infertile patients with type 2 diabetes mellitus. Eur J Radiol. 2011a;80:e64– e67.
9. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50:536-46.
10. Majekodunmi SO, Oyagbemi AA, Umukoro S, Odeku OA. Evaluation of the anti-diabetic properties of *Mucuna pruriens* seed extract. Asian Pac J Trop Med. 2011;4(8):632-636.
11. Ukwenya V, Ashaolu O, Adeyemi D, Obuotor E, Tijani A, Biliaminu A, et al. Evaluation of antioxidant potential of methanolic leaf extract of *Anarcardium occidentale* (Linn) on the testes of STZ-induced diabetic wistar rats. Eur. J. Anat. 2002;17(2):72-81
12. Nemani H, Nappanveettil G. Restoration of libido in WNIN/GR-Ob male rats by administration of high dose testosterone propionate. Advan Stud in Bio. 2012;4(12): 557-571.
13. Seven A, Guzel S, Seymen O, Civelek S, Bolayirh M, Uneu M, et al. Effects of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats; investigation of liver and plasma. Yonsei Med Journal. 2004;45(4):703-710.
14. Biswas M, Kar B, Bhaltacharya S, Swesh Kumar RB, Kumar Ghosh A, Huldar PK. Antihyperglycaemic activity and antioxidant role of *Terminalia arjuna* leaf in streptozotocin induced diabetic rats.

Pharmaceutical Biology. 2011;49(4):335-340.

15. Rifai N, Bachorik PS, Albers JJ. Lipids; Lipoproteins and apo-lipoproteins. In: Tietz textbook of clinical chemistry; 3rd edition; 1993.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of LDL-cholesterol. Clin. Chem. 1972;18(6):499-515.
17. Badkoobeh P, Parmark, Kalantar SM, Hosseini SD, Salabar A. Effect of nano-zinc oxide on doxoribino-induced oxidative stress and sperm disoders in adult male Wistar rats. Iran J. Repro. Med. 2013;11(9): 355-364.
18. Oyeyemi MO, Oluwatoyin O, Ajala OO, Leigh, Adesiji TF. The spermiogram of male Wistar rats treated with aqueous leaf extract of *Venonia amygdalina*. Foliaueterinaria. 2008;2:98-101.
19. Frouzan A, Movahedin M, Mowl SJ. Assessmnet of testes histological changes and sperm parameters in experimentally-induced unilateral and bilateral Cryptorchid mouse model. Iranian J. Rep. Med. 2008;6(3):143-148.
20. Muhammad A, Ralph AD. Pathogenenesis of insulin resistance in skeletal muscle J. Biomed. Biotech. 2010;2010:Article ID 476279:1.
21. Ganong WF. Review of medical physiology. 24th Edition; McGraw-Hill Education, USA; 2012.
22. Agbaje IM, Rogers DA, McVicar CM, McClins N, Atianson AB, Malidis C, Lewis SE. Insulin dependent diabetes mellitus: Implications for male reproductive functions. Human Repro. 2007;22(7): 1871-1877.
23. Amaral S, Oliveira PJ, Ramalho-Santos J. Diabetes and the impairment of reproductive functions: Possible role of mitochondria and reactive oxygen species. Curr Diabetes Rev. 2008;4(1):46-54.
24. Mendez JD, Balderas F. Regulation of hyperglycemia and dyslipidemia by exogenous larginine in diabetic rats. Bio Chimie. 2001;83(5):453-458.
25. Traber MG, Stevens JF. Free radical biology and medicine – Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radical Biology and Medicine. 2011;51(5):1000–13.
PMC 3156342.
PMID 21664268.
26. Bal R, Tu¨rk G, Tuzcu M, Yilmaz O, Ozercan I, Kuloglu T, et al. Protective effects of nanostructures of hydrate C-60-fullerene on reproductive function in streptozotocin -diabetic male rats. Toxicol. 2011;282:69–81.
27. Klentze M. Testosterone, the male hormone connection: Treating diabetes and heart diseases (chapter 7). Klentze Institute of Anti-Aging, Munich, Germany; 2013.
28. Morimoto S, Fernandez-Mejia C, Romero-Navarro G, Morales-Peza N, Diaz-Sanchez V. Testosterone effect on insulin content, messenger ribonucleic acid levels, promoter activity, and secretion in the rat. Endocrinol. 2001;142(4):1442-7.
29. Schlatt S, Weinbauer GF, Arslan M, Nieschlag E. Appearance of alpha-smooth muscle actin in peritubular cells of monkey testes is induced by androgens, modulated by follicle-stimulating hormone, and maintained after hormonal withdrawal. J Androl. 1993;14:340–350.
30. Schlatt S, Arslan M, Weinbauer GF, Behre HM, Nieschlag E. Endocrine control of testicular somatic and pre-meiotic germ cell development in the immature testis of the primate *Macaca mulatta.* Eur J Endocrinol. 1995;133:235–247.
31. Regina B, Frank JK, Tukka TS, Jiri N, Jean Marc Z, Angelo A. Review article; The European perspective on vitamin E; current knowledge and future research. Am J Clin Nutr. 2002;76:703-16.
32. Tilbrook AJ, Clarke IJ. Negative feedback regulation of the secretion and actions of GnRH in male ruminants. J. Reprod Fertil Supply. 1995;49:297-306.

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