International Journal of Biomedical and Health Sciences
Vol. 17, No.4, December 31, 2021
Printed in Nigeria

0794-4748/2021 $10.00 + 0.00

http://journals.niseb.org

IJBHS 17403

Comparison of the impact of lauric acid and coconut oil on sperm quality and testicular function in streptozotocin-induced diabetic male Wistar rats.

M. V. OLUBIYI*[1,2], R. A. MAGAJI[1,6], M. G. MAGAJI[3,7], M. U. KAWU[4,8], M. Y. FATIHU[5,9] and E. A. ALEX[2]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Kogi State University, Anyigba, Kogi State, Nigeria
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria
[3]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria
[4]Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
[5]Dept of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
[6]Email: rabiumagaji@yahoo.co.uk, Phone: +23408023558721
[7]Email: magmas1@yahoo.com, Phone: +2348034685849
[8]Email: mukawu@abu.edu.ng, Phone: +2348037016456
[9]Email: myfatihu@abu.edu.ng, Phone: +2348037862954
[10]Email: alexenoch23@gmail.com, Phone: +2348066171886

*Corresponding Author**.** Email address**:** olubiyi.vm@ksu.edu.ng, Phone number**:** +2348033443287

(Received February 18, 2021; Accepted September 8, 2021)

ABSTRACT: Sperm quality is impaired in diabetic conditions. Coconut oil (CO) possesses anti-diabetic properties and ameliorative effects on testicular dysfunction. Lauric acid (LA) being the most abundant constituent of CO is hypothesized to be responsible for its physiologic actions. This study investigated some testicular and sperm parameters in diabetic male wistar rats treated with lauric acid and coconut oil. Thirty animals were divided into six groups of five each. Group I: Control; Group II: Diabetic untreated; Group III: Diabetic treated with LA (90 mg/Kg). Group IV: Diabetic treated with LA (180 mg/Kg); Group V: Diabetic treated with LA (360 mg/Kg). Group VI: Diabetic treated with CO (1.42 ml/Kg). Compared to Group I, there was a significant decline ($p < 0.05$) in gonadosomatic index, serum testosterone level, sperm quality and testicular structure in Group II. Compared to Group II; the gonadosomatic index, sperm quality were significantly higher ($p < 0.05$) in Group VI. Compared to Group II; Group V and Group III had significantly higher ($p < 0.05$) percentages of normal and progressively motile sperm cells respectively. Testicular histoarchitecture was improved in Groups 5 and 6. Sperm quality was largely improved by coconut oil but not by lauric acid. This contradicts the assumption that lauric acid may be largely credited for this physiologic action of coconut oil.

Keywords: Diabetes, Testis, Sperm, Lauric acid

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia, caused by deficient insulin production by the pancreas or inefficient action by of the insulin produced (1). Diabetes mellitus is one of the major global health challenges affecting about 422 million adults globally and is estimated to double by 2025 with greater threats to developing and underdeveloped countries (2)

Due to the chronic hyperglycaemia, diabetes is associated with a number of complications across the body systems such as retinopathy, neuropathy, nephropathy and cardiovascular dysfunction (3). Diabetes mellitus has also been shown to have a negative impact on sexual and reproductive function. Testicular dysfunction, reduced testosterone level (4), impaired sperm motility, viability, morphology and concentration (5) have been reported to be associated with diabetes, thus causing a reduction in the fertility rate among diabetics. The prevalence of diabetes was shown to be on the rise even among the younger population (6); and diabetes was also associated with the rise in the prevalence of infertility even among young men in their active reproductive age (7).

Sperm cells are produced in the seminiferous tubules of the testes before being stored in the epididymis. Also, testosterone is mainly produced in the interstitial (leydig's) cells of the testis (8). Testosterone facilitates spermatogenesis and also plays a great role in the regulation of male sexual and reproductive functions (9). Thus, testicular damage seen in diabetes (10) leads to the reduction in the production of sperm cells and testosterone, consequently leading to further impairment in sexual function. Semen analysis is a common clinical practice in evaluating fertility in men. Insufficient concentration of sperm cells in the semen or impairment in the structure or function of the sperm cells usually indicates infertility (11).

Considering the public health importance of diabetes mellitus and its role in reproductive dysfunction, it is pertinent to explore ameliorative and safe options for the management of diabetes and its associated testicular complications. Over the years, long before the advent of orthodox medicine, plant products have been used as a therapy for various ailments. Plant products are more accessible, affordable and have fewer side effects compared to orthodox drugs. Therefore, a number of scientific research in the direction of these therapeutic plant products in a bid to elucidate their mechanism of action. Also the active components of these plant products and their physiological roles are also investigated in a bid to create standard drug formation (12)

Attention is being drawn to dietary fatty acids and their impact on sexual function, however, there has been a controversy on the role of dietary saturated fatty acids on reproductive function (13). Lauric acid is the major fatty acid in coconut oil constituting about 50% of the total fatty acid composition. Considering lauric acid makes up a large portion of the constituents of coconut oil it is hypothesized to contribute greatly to the physiologic effects of coconut oil (14). Coconut oil, a common plant product locally consumed has been renowned throughout history for its medicinal and nutritional value. It has been proven to reduce oxidative stress, improve lipid profile and possess antidiabetic properties (15) and improve testicular function and semen quality (16).

Materials and Methods

Animals

Thirty (30) male rats weighing 150-200g were used. The animals were kept in the animal house of the department of Human Physiology, ABU, Zaria. The animals were maintained under standard conditions of temperature, humidity and light. They were fed with standard chow diet and water *ad libitum* (17). Ethical approval was obtained from the Ahmadu Bello University Animal care and use committee with the approval number, ABUCAUC/2017/019

Treatments

The animals (N=30) were divided into 6 groups of 5 rats each (n=5) and treated as follows:
Group 1 (NC): Distilled water 1ml/kg
Group 2 (DM-UT): Diabetic untreated
Group 3 (DM + LA 90): Diabetic treated with lauric acid at 90 mg/kg orally
Group 4 (DM + LA 180): Diabetic treated with lauric acid at 180 mg/kg orally (18)
Group 5 (DM + LA 360): Diabetic treated with lauric acid at 360 mg/kg orally (18)
Group 6: (DM + CO): Diabetic treated with coconut oil 1.42ml/kg (19)

Induction of Diabetes and treatment

Diabetes was induced by the administration of a single intraperitoneal injection of streptozotocin (65 mg/kg) in 0.1M citric acid buffer; pH 4.5 (20). The blood glucose level of the rats was checked 3 days after. Animals with fasting blood glucose level greater than 200 mg/dl were marked as diabetic and placed in the diabetic groups (groups 2, 3, 4, 5 and 6). Lauric acid was suspended in distilled water using Tween 80. Coconut oil prepared by wet extraction method according to Nevin and Rajamohan (21) was used. All treatments were administered through oral gavage for 4 weeks. Then the animals were sacrificed by cervical dislocation, after which the blood, semen and testes were obtained for further analysis.

Measurement of Blood Glucose Level

Blood glucose level was measured by Accu-Chek glucose test strips and glucometer (Roche, USA). The glucometer works based on glucose-oxidase principle (22). After the test strips have been inserted into the glucometer, and a drop of blood from the pricked tail is dropped on the test strip. The glucometer then displays the blood glucose levels.

Organ and Body weight

The body weight of the animals was taken before the beginning of treatment and at week 4. At the end of the experiment, the testes were harvested and weighed. The gonadosomatic index was then evaluated by the formula:

$$\frac{\text{Testes weight (g)}}{\text{Body weight (g)}} \times 100$$

Determination of Serum testosterone level

Estimation of serum testosterone was done by Enzyme-linked immunosorbant assay (ELISA) using rat testosterone ELISA kits (Wuhan Fine Biotech Co., Ltd). Briefly, 50 µl of the standard or sample was added into each well. Then, 50 ul of Biotin-labelled antibody was added and then incubated for 45 minutes at 37^{o}C after which it was washed and aspirated. The 100µl of SABC working solution was then added, incubated for 30 minutes at 37^{o}C, washed and aspirated. Thereafter, 90 µl TMB substrate was then added to the solution, incubated for 15-20 minutes at 37^{o}C. Then 50µl stop solution was added after which absorbance at 450 nm was recorded (23).

Semen analysis

Sperm fluid was obtained from the caudal epididymis of the left testes as follows by mincing the epididymis in a petri dish containing warm normal saline. Sperm count was determined by diluting the supernatant of the macerated epididymis in the ratio 1 to 100 with a solution containing 5 g NaHCO3, and 1 ml formalin (35%) per 100 ml distilled water. About 10 µl was dropped in the improved Neubaur haemocytometer and the placed under a microscope (x200). The number of sperm cells seen in five 16-celled squares in focus was then counted. The sperm concentration was then calculated by multiplying the total counted sperm cells by 5 and expressed as $S \times 10^6$/ml where S is the total sperm count (24).

Sperm motility was determined by diluting the supernatant described above with tris buffer solution to 0.5mL and dropped on a slide, which was then placed under a light microscope with magnification of

x400. The Sperm cells were observed under the microscope. Sperm cells which showed movement were classified as motile, while those who were stationary were classified as non-motile. Motile sperm cells, which exhibited forward movement or movement in large circles, were classified as progressively motile (25)

To assess the sperm morphology, from the original dilution for sperm motility, the sperm fluid was diluted with 10% neutral buffered formalin in the ratio 1:20 and smeared on histological slides. Staining of the slides was done with Giemsa's dye and examined under a microscope. In each rat, 500 sperm cells were evaluated for structural abnormalities. The percentages sperm cells with normal and abnormal structures were then evaluated (26).

Live and dead sperms (Sperm viability) were distinguished by the method of Siegel (27). A drop of Eosin Y was added to a drop of semen and allowed to settle for a 1-2 minutes after which this combination was smeared on a microscopic slide and viewed under a microscope. The dead sperm cells stained red while the live sperm cells were unstained.

Histology of the testes

The testes were extracted from the animals and then a section of the medial portion was fixed in a phosphate buffered formalin solution (10%), after which it was be stained with hematoxylin and eosin (H and E) to highlight the general histological structure as described by Traish *et al* (28)

Chemicals and Drugs

Lauric acid with purity 99% was obtained from AK Scientific Inc. (USA). Streptozotocin with purity ≥ 98% were obtained from Santa Cruz Biotechnology (USA).

Statistical analysis

Results were presented as means ± S.E.M. Data and analyzed using ANOVA and Tukey post-hoc tests with the aid of the *Graph pad 6*. Values with $P< 0.05$ were considered significant.

Results

Gonado-somatic index as shown in Figure 1, is significantly higher ($p < 0.05$) in NC (0.79 ± 0.03) and DM + CO (0.75 ± 0.02) rats respectively compared to the other groups: DM-UT (0.27 ± 0.05); DM + LA 90 (0.34 ± 0.03); DM + LA 180 (0.27 ± 0.02); DM + LA 360 (0.41 ± 0.03), respectively.

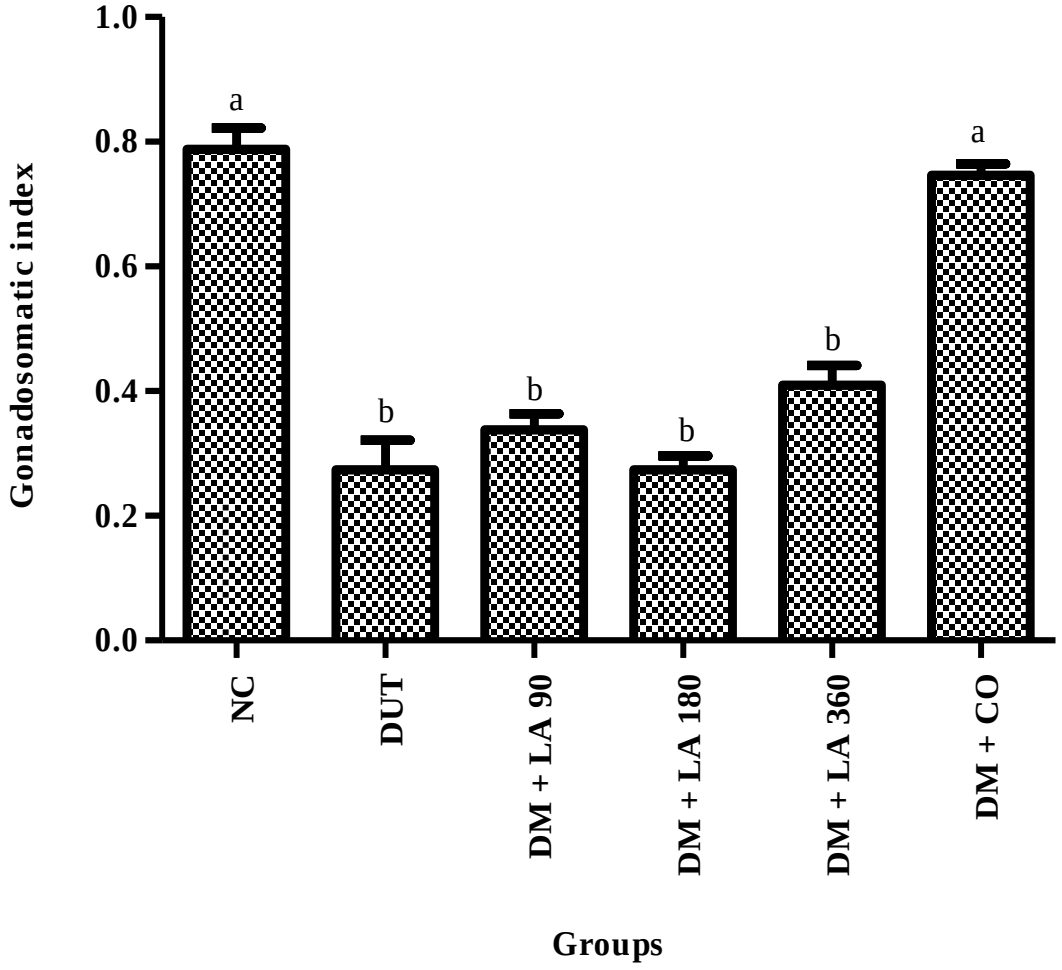

Figure 1: Gonadosomatic Index in Diabetic Male Wistar Rats Treated with Lauric Acid and Coconut Oil

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil. Different superscripts represent significant difference ($p < 0.05$).

Figure 2 shows that serum testosterone in all treatment groups were significantly lower ($p < 0.05$) compared to the control rats: NC (0.92 ± 0.07 ng/ml) vs DM-UT (0.31 ± 0.02 ng/ml); DM + LA 90 (0.48 ± 0.07 ng/ml); DM + LA 180 (0.40 ± 0.06 ng/ml); DM + LA 360 (0.27 ± 0.03 ng/ml); DM + CO (0.29 ± 0.01 ng/ml); respectively.

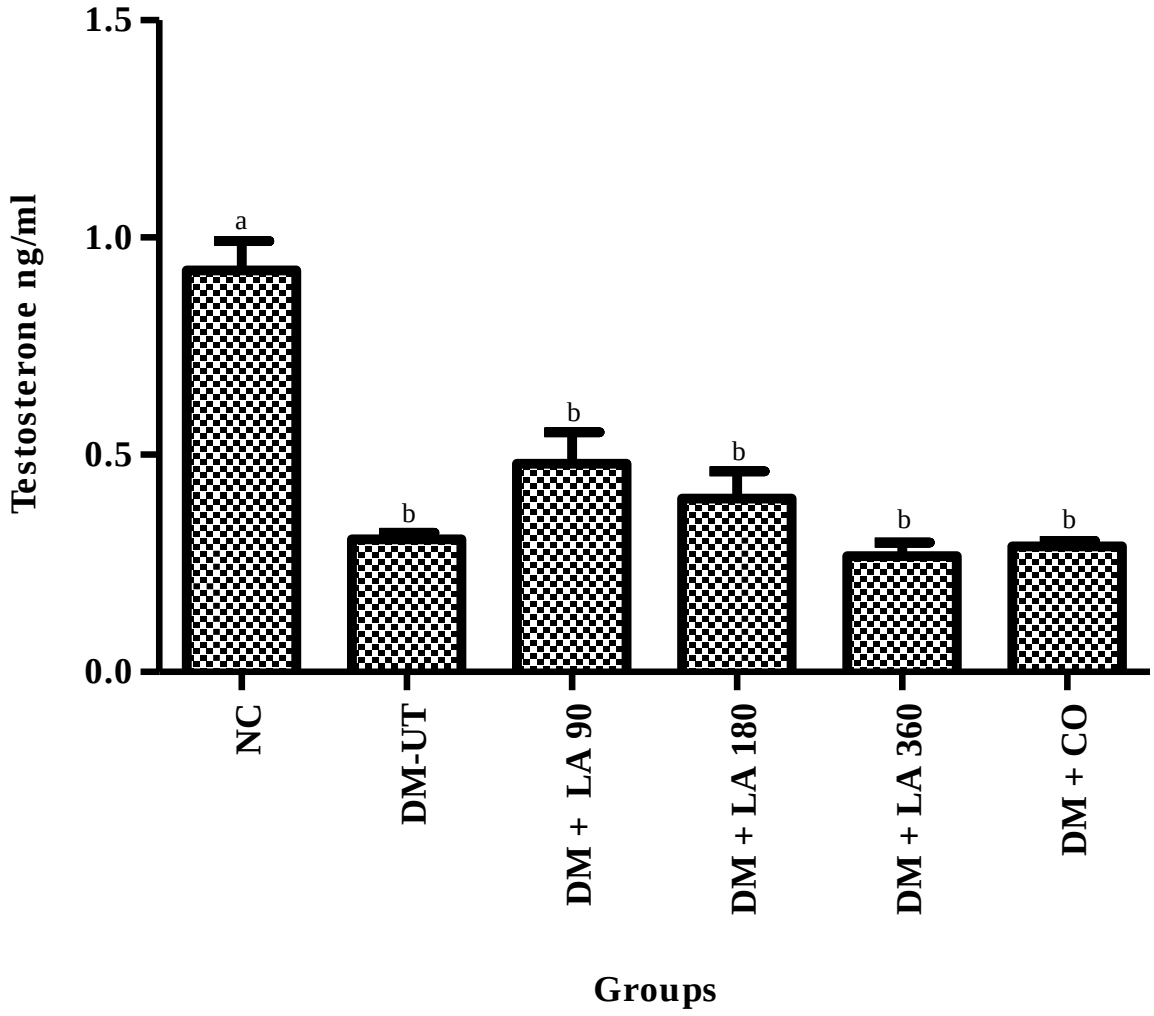

Figure 2: Serum Testosterone Level in Diabetic Male Wistar Rats Treated with Lauric Acid and Coconut Oil

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; Different superscripts represent significant difference ($p < 0.05$)

Figure 3 shows that the sperm concentration (10^6/ml) in NC (51.05 ± 1.85) and DM + CO (40.40 ± 1.99) rats respectively, were significantly higher ($p < 0.05$) compared to other groups: DM-UT (6.91 ± 0.86); DM + LA 90 (9.00 ± 1.48); DM + LA 180 (4.30 ± 0.89); and DM + LA 360 (6.80 ± 0.67); respectively.

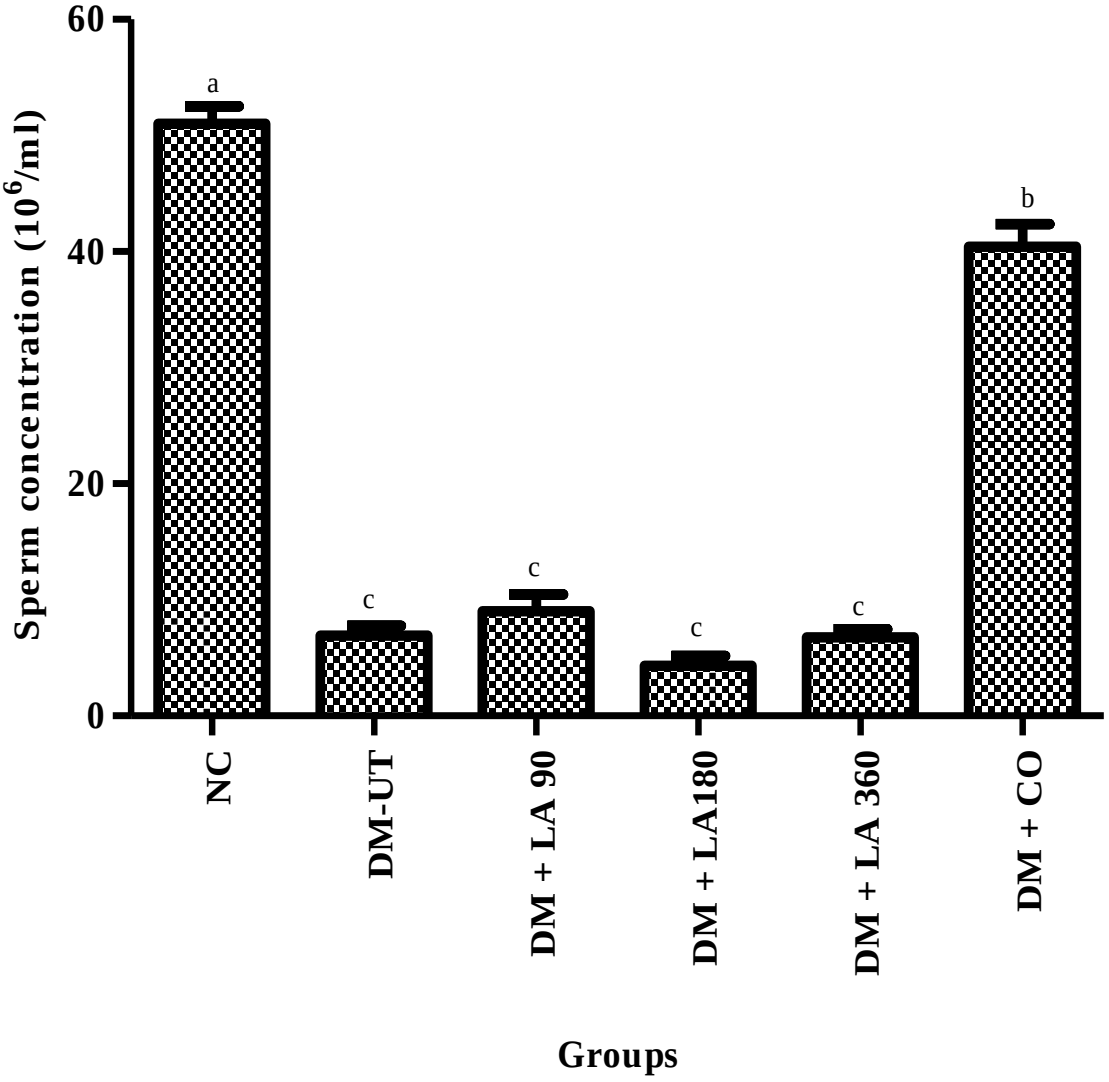

Figure 3: Sperm Concentration in Diabetic Male Rats Treated with Lauric Acid and Coconut Oil
NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; Different superscripts represent significant difference ($p < 0.05$)

As shown in Figure 4; the percentage of motile sperm cells in NC (71.2 ± 3.12) and DM + CO (78.60 ± 2.4) rats respectively, were significantly higher ($p < 0.05$) compared to the other treatment groups: DM-UT (25.40 ± 2.31); DM + LA 90 (24.60 ± 2.21); DM + LA 180 (8.80 ± 1.24); DM + LA 360 (12.20 ± 1.16); respectively. The percentage of motile sperm cells in: DM + LA 180 (8.80 ± 1.24); DM + LA 360 (12.20 ± 1.16) rats respectively, were significantly lower ($p < 0.05$) compared to DM-UT (25.40 ± 2.31); and DM + LA 90 (24.60 ± 2.21); respectively. The percentage of progressively motile sperm cells in all the treatment groups were significantly lower ($p < 0.05$) compared to the control: NC (42.60 ± 1.54) vs DM-UT (0.00 ± 0.00); DM + LA 90 (9.20 ± 1.43); DM + LA 180 (0.00 ± 0.00); DM + LA 360 (0.00 ± 0.00); DM + CO (33.80 ± 1.93) respectively.

The percentage of progressively motile sperm cells in: DM-UT (0.00 ± 0.00); DM + LA 180 (0.00 ± 0.00); DM + LA 360 (0.00 ± 0.00) rats respectively were significantly lower ($p < 0.05$) compared to DM + LA 90 (9.20 ± 1.43) and DM + CO (33.80 ± 1.93) rats respectively. In addition, the percentage of progressively motile sperm cells in DM + CO (33.80 ± 1.93) rats was significantly higher ($p < 0.05$) compared to DM + LA 90 (9.20 ± 1.43).

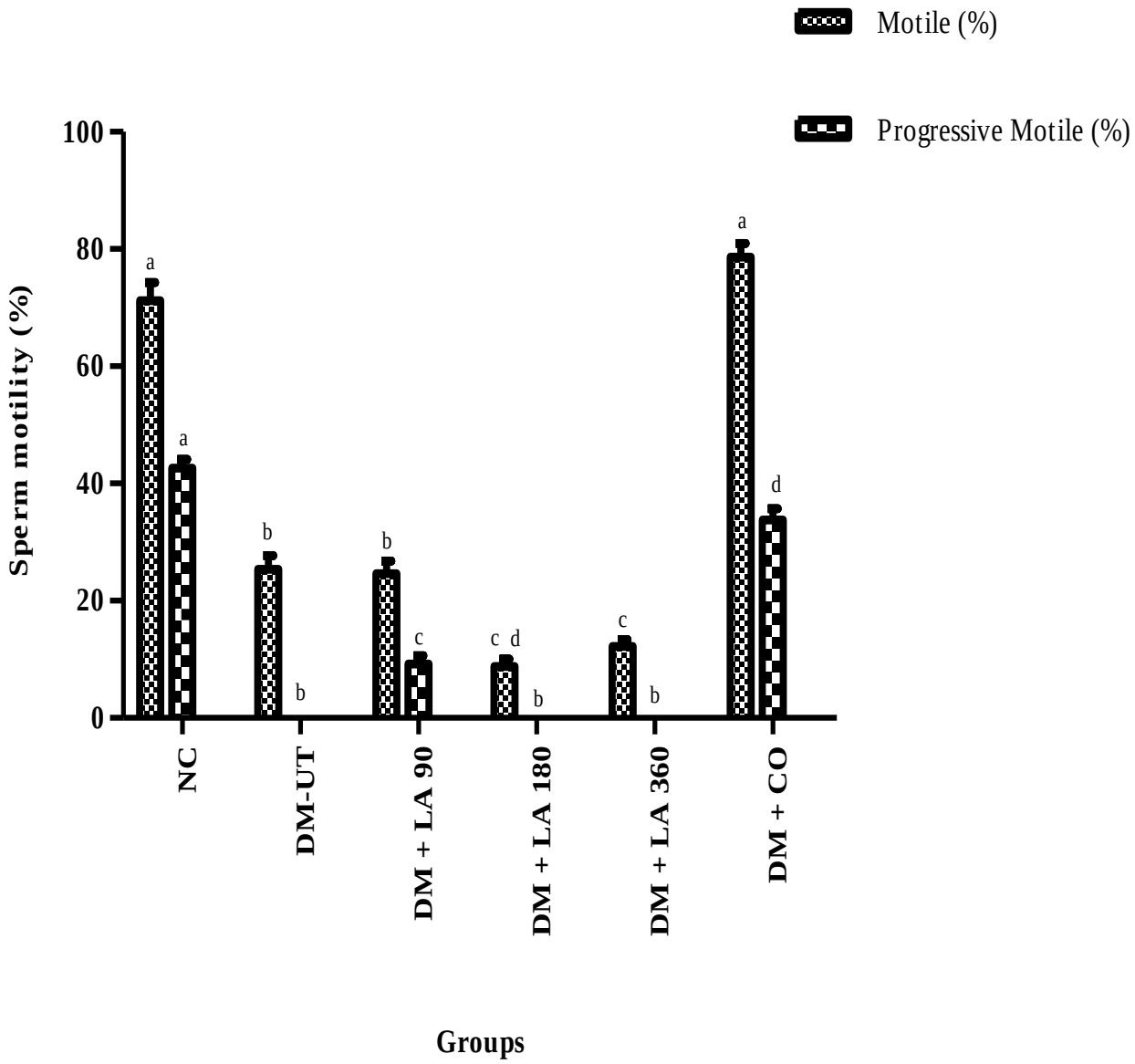

Figure 4: Sperm Motility in Diabetic Male Rats Treated with Lauric Acid and Coconut Oil
NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; Different superscripts represent significant difference ($p < 0.05$)

Figure 5 shows the percentage sperm cells with normal morphology in all the groups were significantly lower ($p < 0.05$) compared to the control: NC (78.80 ± 3.22) vs DM-UT (43.20 ± 1.63); DM + LA 90

(53.40 ± 2.54); DM + LA 180 (33.80 ± 1.72); DM + LA 360 (61.00 ± 3.56); DM + CO (61.60 ± 1.03) respectively. The percentage of normal sperm cells in: DM-UT (43.20 ± 1.63); and DM + LA 180 (33.80 ± 1.72) rats respectively, were significantly lower ($p < 0.05$) than DM + LA 90 (53.40 ± 2.54); DM + LA 360 (61.00 ± 3.56); and DM + CO (61.60 ± 1.03) rats respectively.

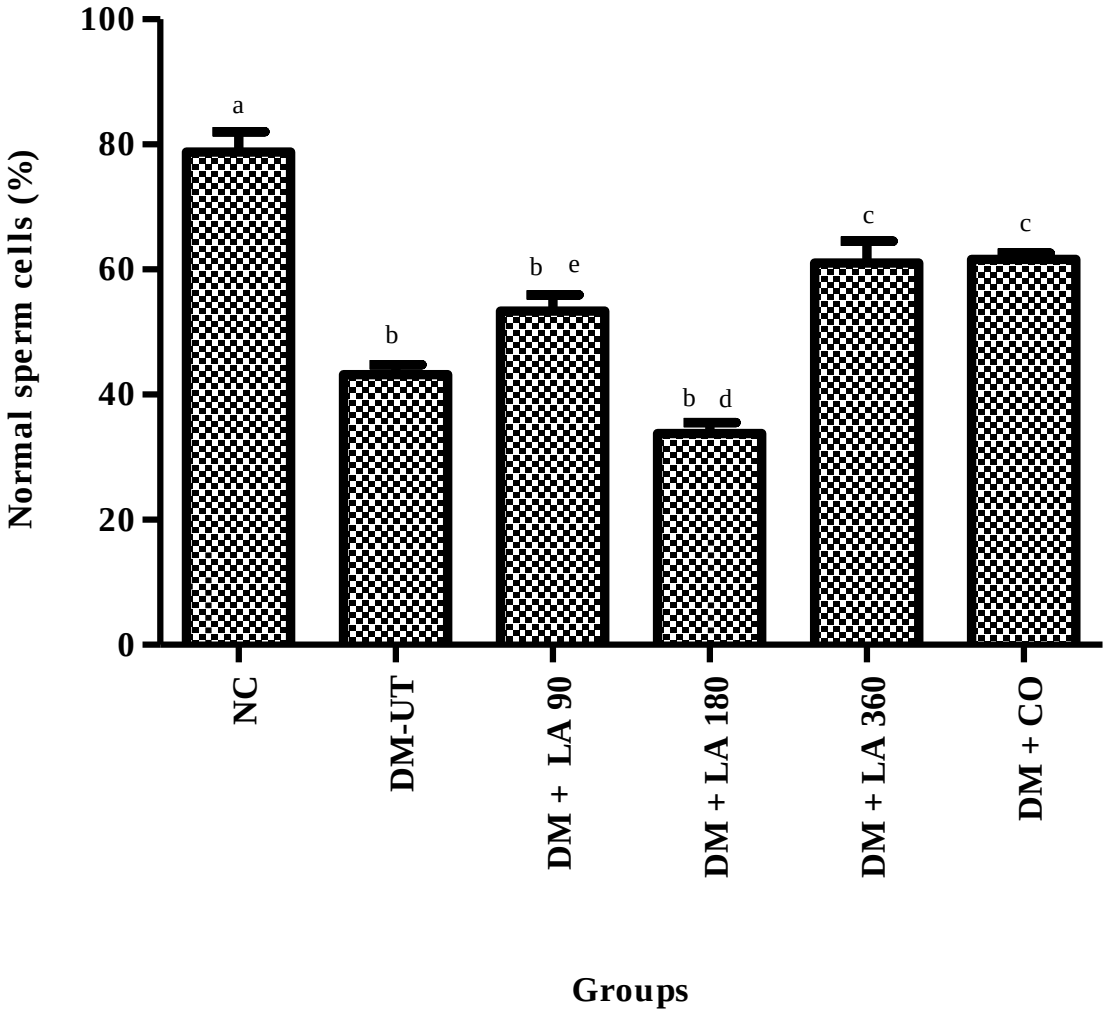

Figure 5: Percentage of Sperm Cells with Normal Morphology in Diabetic Male Wistar Rats Treated with Lauric Acid and Coconut Oil

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; Different superscripts represent significant difference ($p < 0.05$)

Figure 6 shows that the percentage of live sperm cells in NC (69.60 ± 2.98) and DM + CO (58.00 ± 2.47) rats respectively, were significantly higher ($p < 0.05$) compared to the other treatment groups: DM-UT (22.20 ± 2.52); DM + LA 90 (25.00 ± 3.69); DM + LA 180 (15.00 ± 3.69); DM + LA 360 (21.60 ± 2.34) respectively.

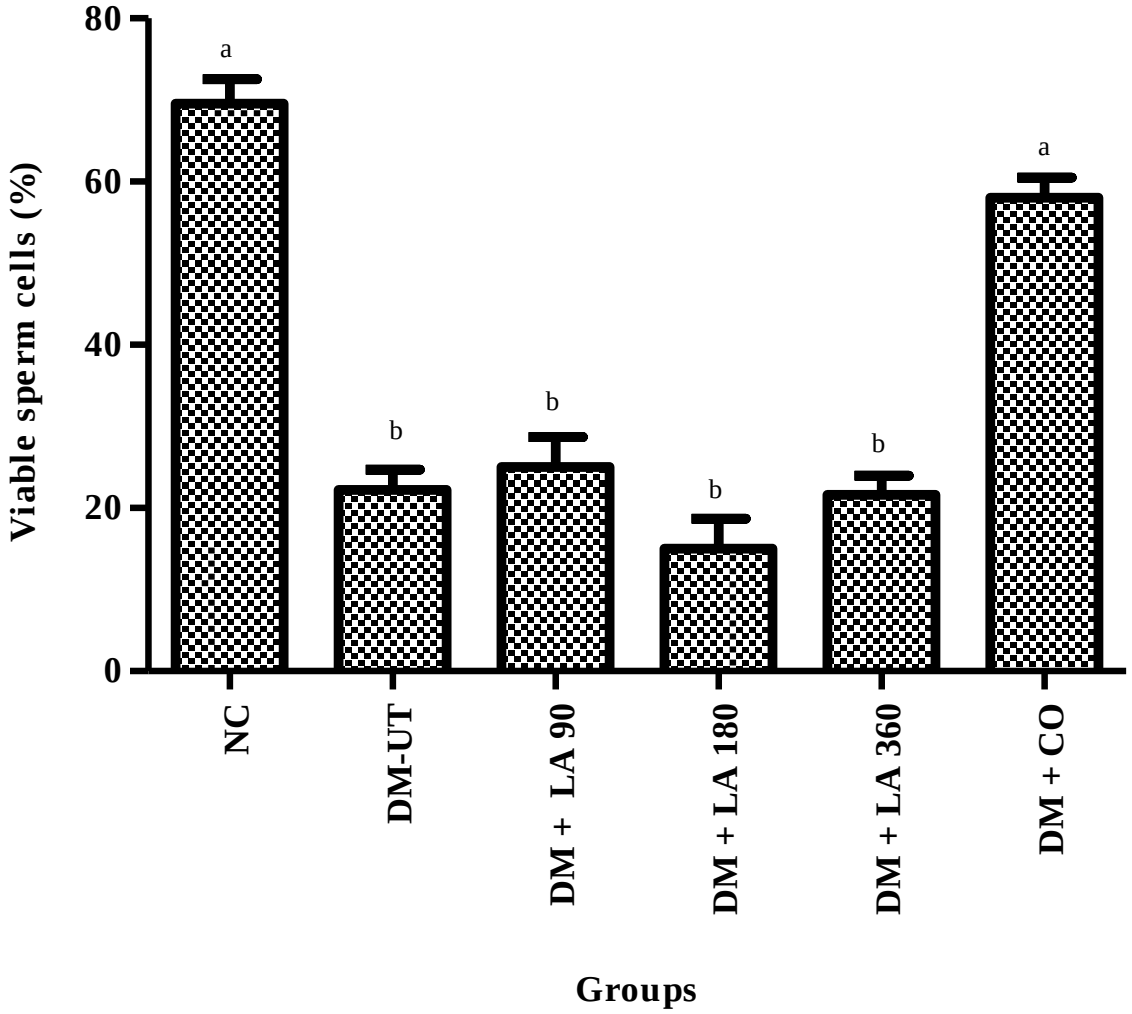

Figure 6: Sperm Viability in Diabetic Male Wistar Rats Treated with Lauric Acid and Coconut Oil
NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Silldenafil; Different superscripts represent significant difference ($p < 0.05$)

Figure 7 shows the histological section of the testes in the various treatment groups. In the NC rats, the H & E staining revealed normal testicular structure with the seminiferous tubules, spermatids and surrounding interstitium highlighted. However, in the DM-UT rats the testicular tissue showed degeneration and distortion with a reduction in size of the seminiferous tubules and very sparse spermatids compared to the normal control. In DM + LA90 rats, there was testicular degeneration with great reduction in the size of the seminiferous tubules and reduced number of spermatids. Vacuolization was observed within the seminiferous tubules and degenerated interstitial tissues. In DM + LA 180 rats, the testicular morphology showed normal sized seminiferous tubules with some vacuolization. Few spermatids were also seen to be present. In DM + LA360 rats, the testicular morphology showed very little to no sign of degeneration or lesion and spermatids were seen to be present. In DM + CO rats, the testicular tissue showed no lesion. There was also abundant presence of spermatids.

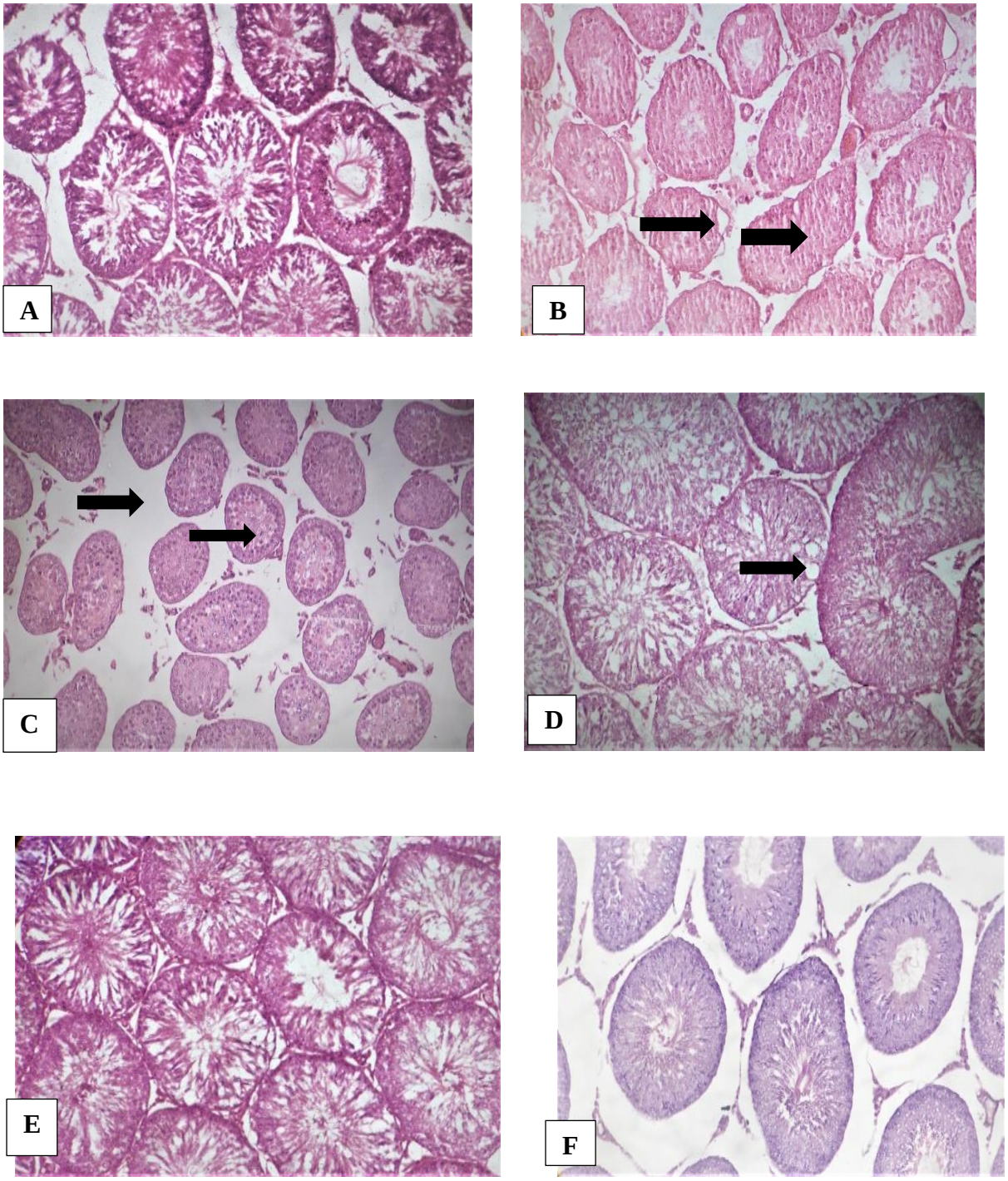

Figure 7: Histological section (H & E) of the testes of diabetic male rats treated with lauric acid and coconut oil (× 200). A – Normal control; B – Diabetic untreated; C – Diabetic treated with Lauric acid (90 mg/kg); D - Diabetic treated with Lauric acid (180 mg/kg); E - Diabetic treated with Lauric acid (360 mg/kg); F – Diabetic treated with coconut oil; Arrows indicates abnormal morphological alterations in the seminiferous tubular tissues.

Discussion

Semen analysis involves the evaluation of sperm count, sperm motility, sperm morphology and sperm viability among others, in a bid to access male fertility (29). A decline in sperm quantity (count) has been associated with a decline in sperm quality e.g. motility and morphology (30) as also confirmed in this study. Diabetes mellitus has been shown to cause a decline in spermatogenesis, which is responsible for impaired sperm quality. Also, diabetes usually causes reduced synthesis and secretion of testosterone and testicular degeneration. This thus accounts for impaired sperm quality and thus infertility associated with diabetes (31).

The reduction in serum testosterone observed in this study could have been due to damage to the leydig's cell (32), and/or disruption in the process of steroidogenesis (33) which has been reported in diabetic conditions. Lauric acid and Coconut oil failed to rescue the decline in testosterone level in this study and this may point to the inability to restore leydig's cell function. Also, lauric acid is specifically reported to cause a reduction in testosterone level which enables it to prevent androgenetic alopecia (34). In addition, has been shown to cause a reduction in low-density lipoproteins (LDL) (35), which is one of the sources of cholesterol, that serves as a precursor for the production of testosterone (36). Thus, these may account for the failure of lauric acid to rescue the decline in testosterone level. Considering the pivotal role of testosterone in general male reproductive function, especially in spermatogenesis and, this may explain the decline in sperm quality observed (37).

The reduced testes-body weight ratio, which is also referred to as the gonado-somatic index (GSI) shown in this study, suggests a reduction in testicular tissue and content. This generally indicates or leads to compromised testicular structure and function as shown by Atta *et al* (38). The histological analysis of the testes revealed an abnormal structure in the diabetic rats, confirming the report that chronic hyperglycaemia causes damage to the testes. Although this study showed that that treatment of diabetic rats with lauric acid failed to improve the GSI, the highest dose of lauric acid showed great improvement in the histological structure of the testes portending a repair of testicular damage and improvement of testicular function. This may account for the slight improvement of sperm progressive motility and sperm morphology by lauric acid. Failure of lauric acid to largely improve sperm quality despite the improvement of the testicular structure and the presence of spermatids, shows that lauric failed to completely rescue the disruption in spermatogenesis in diabetic conditions. This could be due to the reduction in the level of testosterone, which promotes spermatogenesis (33). It could also possibly be due to failure of lauric acid to mitigate the diabetes induced damage to the epididymis, where the final stages of maturation of sperm cells take place (32).

Although coconut oil failed to salvage the decrease in testosterone level in this study, it improved testicular and sperm parameters in diabetic rats. This is probably due to its anti-hyperglycaemic and antioxidant properties (15), which may have a protective function on the oxidative impairment of sperm and testicular parameters in diabetic conditions (39). This thus reverses the damage to sperm quality and testicular tissue. This is in line with the reports on the ameliorative effect of coconut oil on reproductive function (40).

Lauric acid generally failed to improve the decline in sperm quality as much as seen with coconut oil treatment. This could imply that the action of lauric acid depends on the synergistic support of other constituents in coconut oil. In addition, the chemical property of lauric acid as a saturated fatty acid may have an adverse effect on the sperm quality. There has been a controversy on the effect of consumption of saturated fatty acids in the diet, as some reports have associated it with a decline in sperm quality and quality (41). The bi-lipid cell membrane of sperm cells has been shown to be affected by dietary fatty acids. Saturated fatty acids are reported to offset the balance between cholesterol and phospholipids in the sperm cell membrane. This eventually compromises the integrity and function of the sperm cell membrane, thus leading to impaired sperm motility, sperm count, sperm morphology and overall sperm viability (42). However, the improvement of sperm progressive motility and morphology of the sperm cells by lauric acid treatment in this study showed a slight improvement in the quality despite the failure of lauric acid to

improve the quantity of sperm cells produced. Coupled with the improvement in testicular structure, it shows there might have probably been an ongoing restoration of testicular function and sperm quality. Thus a longer period of lauric acid treatement or an adjusted dose of lauric acid may improve the reproductive function as much as was seen with coconut oil.

Conclusion

Diabetes mellitus caused a reduction in testes/body weight ratio, sperm quality and serum testosterone level. Lauric acid failed to completely improve sperm parameters in the diabetic rats compared to the coconut oil treatment.

References

1. WHO. Overview Symptoms Treatment [Internet]. 2020. p. 1–10. Available from: https://www.who.int/health-topics/diabetes#tab=tab_1
2. WHO. Global Report on Diabetes [Internet]. Vol. 978, Isbn. 2016. Available from: http://www.who.int/about/licensing/copyright_form/index.html%0Ahttps://apps.who.int/iris/handle/10665/204871%0Ahttp://www.who.int/about/licensing/
3. Punthakee Z, Goldenberg R, Katz P. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Canadian Journal of Diabetes. 2018;42:S10–5.
4. Rato L, Alves MG, Duarte AI, Santos MS, Moreira PI, Cavaco JE, et al. The International Journal of Biochemistry Testosterone deficiency induced by progressive stages of diabetes mellitus impairs glucose metabolism and favors glycogenesis in mature rat Sertoli cells. International Journal of Biochem isty and Cell Biology. 2015;66:1–10. Available from: http://dx.doi.org/10.1016/j.biocel.2015.07.001
5. Zhu J, Dong X, Liang J, Zhang Z, Hu X, Li L. Effects of diabetes mellitus on semen quality in adult men : a systematic review and meta-analysis. International Journal of Clinical and Experimental Medicine. 2017;10(8):11290–303.
6. Mayer-Davis EJ, Beyer J, Bell RA, Dabelea D, D'Agostino Jr R, Imperatore G, et al. Diabetes in African American youth: prevalence, incidence, and clinical characteristics: the SEARCH for Diabetes in Youth Study. Diabetes Care. 2009;32 Suppl 2(Suppl 2):S112–22. Available from: https://pubmed.ncbi.nlm.nih.gov/19246576
7. Lutz W, Leridon H, Aitken RJ, Von Eyben FE. Fertility rates and future population trends: Will Europe's birth rate recover or continue to decline? International Journal of Andrology. 2006;29(1):25–33.
8. Durairajanayagam D, Rengan A. Sperm Biology from Production to Ejaculation. In: Schattman G., Esteves S., Agarwal A. (eds) Unexplained Infertility. 2015; Springer, New York, NY. https://doi.org/10.1007/978-1-4939-2140-9_5
9. Corona G, Mannucci E, Forti G, Maggi M. Following the common association between testosterone deficiency and diabetes mellitus , can testosterone be regarded as a new therapy for diabetes ? International Journal of Andrology. 2009;431–41.
10. Ghanim H, Dhindsa S, Abuaysheh S, Batra M. Diminished androgen and estrogen receptors and aromatase levels in hypogonadal diabetic men : reversal with testosterone. European Journal of Endocrinology. 2018; 178(3):277-283
11. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HWG, Behre HM, et al. World Health Organization reference values for human semen characteristics. Human Reproduction Update. 2009;16(3):231–45. Available from: https://doi.org/10.1093/humupd/dmp048
12. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. Journal of clinical biochemistry and nutrition. 2007;163–173.
13. Esmaeili V, Shahverdi AH, Moghadasian MH, Alizadeh AR. Dietary fatty acids affect semen quality: A review. Andrology. 2015;3(3):450–61.
14. Dayrit FM. The Properties of Lauric Acid and Their Significance in Coconut Oil. Journal of American Oil Chemists' Society. 2015;92(1):1–15.
15. Iranloye B, Oludare G, Olubiyi M. Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. Journal of Diabetes Mellitus. 2013;3(4):221–6.

16. Ogedengbe OO, Jegede AI, Onanuga IO, Offor U, Naidu ECS, Peter AI, et al. Coconut oil extract mitigates testicular injury following adjuvant treatment with antiretroviral drugs. Toxicological Research. 2016;32(4):317–25.
17. National Research Council (US) Subcommittee on Laboratory Animal Nutrition. Nutrient Requirements of Laboratory Animals: Fourth Revised Edition, 1995. Washington (DC): National Academies Press (US); 1995. Available from: https://www.ncbi.nlm.nih.gov/books/NBK231927/ doi: 10.17226/4758
18. Patil A.A., Veeresh B, Adikrao Y. Combination of lauric acid and myristic acid prevents benign prostatic hyperplasia (BPH) symptoms in animal model. African Journal of Pharmacy and Pharmacology. 2016;10(8):101–6.
19. Shariq B, Zulhabri O, Hamid K, Sundus B, Mehwish H, Sakina R, et al. Evaluation of Anti-Atherosclerotic Activity of Virgin Coconut Oil in Male Wistar Rats against High Lipid and High Carbohydrate Diet Induced Atherosclerosis. UK Journal of Pharmaceutical Biosciences. 2015;3(2):10.
20. Young L De, Yu D, Freeman D, Brock GB. Effect of PDE5 inhibition combined with free oxygen radical scavenger therapy on erectile function in a diabetic animal model. International Journal of Impotence Research. 2003;347–54.
21. Nevin KG, Rajamohan T. Virgin coconut oil supplemented diet increases the antioxidant status in rats. Food Chemistry. 2006;99(2):260–6. Available from: http://www.sciencedirect.com/science/article/pii/S0308814605006412
22. McMillin JM. Blood Glucose. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths. 1990; Chapter 141. Available from: https://www.ncbi.nlm.nih.gov/books/NBK248/
23. McCann D, Kirkish L. Evaluation of Free Testosterone in serum. Journal of Clinical Immunoassay. 1985; 8: 234–236.
24. Yokoi K, Uthus EO, Nielsen FH. Nickel deficiency diminishes sperm quantity and movement in rats. Biological Trace Element Research. 2003;93(1–3):141–54.
25. Sönmez M, Türk G, Yüce A. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology. 2005;63(7):2063–72.
26. Atessahin A, Türk G, Karahan I, Yılmaz S, Ceribasi, Ali O, Bulmus Ö. Lycopene prevents adriamycin-induced testicular. Fertility and sterility; 2006;(85):1216–22.
27. Siegel MS. The male fertility investigation and the role of the andrology laboratory. Journal of Reproductive Medicine. 1993;38(5):317–34.
28. Traish AM, Toselli P, Jeong SJ, Kim NN. Adipocyte accumulation in penile corpus cavernosum of the orchiectomized rabbit: A potential mechanism for veno-occlusive dysfunction in androgen deficiency. Journal of Andrology. 2005;26(2):242–248.
29. Vasan SS. Semen analysis and sperm function tests: How much to test?. Indian Journal of Urology. 2011;27(1):41-48. doi:10.4103/0970-1591.78424
30. Sharpe RM. Sperm counts and fertility in men: a rocky road ahead. Science & Society Series on Sex and Science. EMBO Reports. 2012;13(5):398-403. doi:10.1038/embor.2012.50
31. Jangir RN, Jain GC. Diabetes mellitus induced impairment of male reproductive functions: a review. Current Diabetes Reviews. 2014;10(3):147–57.
32. La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Diabetes mellitus and minireview sperm parameters. Journal of Andrology. 2012;33(2):145–53.
33. Ding GL, Liu Y, Liu ME, Pan JX, Guo MX, Sheng JZ, et al. The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. Asian Journal of Andrology. 2015;17(6):948–53.
34. Mccoy J, Ziering C. Botanical Extracts for the Treatment of Androgenetic Alopecia. International Journal of Life Science and Pharma Research. 2012;2(4):31–8. Available from: www.ijlpr.com/admin/php/uploads/154_pdf.pdf
35. Dayrit FM. Lauric Acid is a Medium-Chain Fatty Acid , Coconut Oil is a Medium-Chain Triglyceride. Philippine Journal of Science. 2014; 143(2);157-166
36 Weinbauer G, Luetjens CM, Simoni M, Nieschlag E. Physiology of Testicular Function. In: Andrology: Male Reproductive Health and Dysfunction. 2010. p. 11–59.
37. Bird BM, Zilioli S. Testosterone BT - Encyclopedia of Evolutionary Psychological Science. In: Shackelford TK, Weekes-Shackelford VA, editors. Cham: Springer International Publishing; 2018. p. 1–4. Available from: https://doi.org/10.1007/978-3-319-16999-6_229-1
38. Atta MS, Almadaly EA, El-Far AH, Saleh RM, Assar DH, Al Jaouni SK, et al. Thymoquinone defeats diabetes-induced testicular damage in rats targeting antioxidant, inflammatory and aromatase expression. International

Journal of Molecular Sciences. 2017;18(5).
39. Omolaoye Temidayo S, Du Plessis Stefan S. Diabetes mellitus and male infertility. Asian Pacific Journal of Reproduction. 2018;7(1):6–14.
40. Dosumu O, Duru F, Osinubi A, Oremosu A, Noronha C. Influence of virgin coconut oil (VCNO) on oxidative stress, serum testosterone and gonadotropic hormones (FSH, LH) in chronic ethanol ingestion. Agriculture and Biological Journal of North America. 2010;1(6):1126–32.
41. Jensen TK, Heitmann BL, Blomberg Jensen M, et al. High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population. American Journal of Clinical Nutrition. 2013;97 (2) : 411-418. doi:10.3945/ajcn.112.042432
42. Dadkhah H, Kazemi A, Nasr-Isfahani M-H, Ehsanpour S. The relationship between the amount of saturated fat intake and semen quality in men. Iranian Journal of Nursing and Midwifery Research. 2017;22(1):46.