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Sperm cells and reproductive hormones abnormalities associated with *Terminalia catappa* (Indian almond) leaf extract administration in streptozotocin-induced diabetic male Wistar rats

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ABSTRACT

Incidences of toxicity of some therapeutic substances on other parts of the body while producing her usefulness are not strange in the management of disease conditions. This study investigated changes in sperm indices and reproductive hormones in diabetic rats treated with aqueous leaf extract of *Terminalia catappa*. Thirty male Wistar rats were divided randomly into five groups of 6 rats per group. Group 1 (control) received 5ml/kg body weight of distilled water orally. Aqueous leaf extract of *Terminalia catappa* at 130mg/ kg of body weight was administered orally to Group 2, while Group 3, the diabetic untreated group, orally received distilled water, 5ml/ kg body weight. Groups 4 and 5 were diabetic rats treated respectively with 130mg/Kg body weight of aqueous leaf extract of *Terminalia catappa* and subcutaneous administration of insulin, 0.75U/Kg body weight. Diabetes induction was by the use of streptozotocin, 65 mg/Kg body weight. Results showed a significant ($p < 0.05$) decrease in sperm motility (78.33+4.60%, 36.67+5.58%, and 16.67+1.05%) and sperm concentration (77.33+2.82%, 46.83+2.55% and 30.92+3.27%) in control, diabetic and diabetic aqueous extract treated groups respectively. The testosterone level also decreased significantly ($p<0,05$) while estrogen, follicle-stimulating hormone, and luteinizing hormone increased significantly ($p < 0.05$) in the *Terminalia catappa* leaves extract treated group. Aqueous extract of *Terminalia catappa* leaves may harm the male reproductive functions, as abnormal alteration occurs in sperm indices and reproductive hormones, while being used for its beneficial anti-diabetic, and anti-inflammatory properties in diabetes mellitus.

Keywords: Sperm analysis, Terminalia catappa, insulin, reproductive hormones, diabetes mellitus.

1. INTRODUCTION

As a significant metabolic condition, diabetes mellitus has a detrimental impact on human health in several ways. It continues to impact multiple organs, including the male reproductive system negatively. Numerous pathophysiological factors, including hyperglycemia, dyslipidemia, chronic inflammation, mitochondrial dysfunction, and endoplasmic reticulum stress, are involved in the onset and progression of diabetes with hyperglycemia and dyslipidemia as regular manifestation due to insulin deficiency of insensitivity (Defeudis et al., 2022). When these symptoms persist because they are not properly managed, they can develop into neuropathy, microangiopathy, and major vascular disease. These conditions damage many organs and tissues, causing dysfunction in those organs and perhaps even failure in the entire body. Research indicates that males with diabetes are more likely than non-diabetic men to experience infertility (Bener et al., 2009).

Men's reproductive health is a crucial aspect of their general health and wellness, although men are frequently disregarded when talking about reproductive health. Several publications that have surfaced in the last few decades have expressed concern about the emergence of reproductive issues in humans and animals (Sengupta, 2014). Additionally, there is a global crisis in male reproductive health, as evidenced by the worldwide decline in sperm counts and the rise in anomalies of the male reproductive system, including germ cell tumors, cryptorchidism, puberty onset, and others (Dejonge and Barratt, 2019). Numerous studies have demonstrated a relationship between the general health of male infertility and the most commonly linked significant health disorders, such as diabetes mellitus illness and cardiovascular disease.

Male infertility of childbearing age may become a more widespread issue due to the rising frequency of diabetes mellitus in recent years and the trend of diabetes at a young age (Al-Saeed et al., 2016). An increasing amount of clinical and epidemiological data suggests that the rise in male infertility and other reproductive issues is trending in a way that is quite similar to that of diabetes in the same age group. The World Health Organization Boivin et al., (2007) has classified infertility as a public health concern, impacting around 10% of couples who are of reproductive age (Lotti and Maggi, 2018). Diabetes mellitus and severe ejaculatory and erectile dysfunction are commonly classified as pre-testicular causes of infertility among the several causes of infertility (Lotti et al., 2020).

Oxidative stress affects male fertility and reproductive health, together with aberrant zinc metabolism, and insulin resistance syndrome impact, all of which are outcomes of diabetes-induced metabolic disease. Until recently, the majority of interventions for diabetes mellitus focused on treating the disease's underlying causes and returning patients to normal health, much attention was not given to the conditions that affected reproductive function. The basis for the reproductive abnormalities seen in people with diabetes mellitus is oxidative stress in the reproductive tissues (Mallidis et al., 2011). Prolonged elevation of blood glucose levels can have detrimental effects on blood vessels and neurons, leading to issues such as erectile dysfunction. Male infertility is the inability to conceive a fertile female in a year of regular, unprotected sexual activity (WHO Infertility).

Numerous natural products are utilized to treat diabetic mellitus, and several of their active constituents have shown considerable amelioration (Vivo-Barrachina et al., 2022; Ben et al., 2023). The potential side effects that using these natural antidiabetic medications may have on the reproductive system have not received as much attention. Leaf extract of *Terminalia catappa* has shown several beneficial health effects in studies, such as having antidiabetic Ahmed et al., (2005), Ben et al., (2023), antioxidative Ben et al., (2021), anti-inflammatory Ben et al., (2019), antimetastatic Yeh et al., (2012), and immunomodulatory De-Araujo et al., (2024), impacts.

In as much as the use of natural products, as interventions in managing several disease conditions has increased, it is important to also monitor any possible indirect impact on other non-target organs or systems during treatment. Therapeutic regimens for ill health can also expose one of the adverse effects on male fertility as the Incidence of toxicity of some therapeutic substances on other parts of the body while producing its usefulness is not strange in the management of disease conditions. This study seeks to determine the adverse impact of *Terminalia catappa* on the male reproductive system; when used as an anti-diabetic agent in the treatment of diabetes mellitus in male Wistar rats.

2. MATERIALS AND METHODS

Leaf Collection and Identification

Terminalia catappa is a commonly available plant with various tree parts of medicinal value, but the part of interest for this study was the leaves. It involved the plucking of fresh leaves of *Terminalia catappa* from the trees situated within the premises of the University of

Uyo town campus. To ascertain the identity of the *Terminalia catappa* leaf, the botanist at the Department of Botany and Ecological Studies University of Uyo, authenticated the leaf, and gave a UUPH/22(a) herbarium number.

Preparing an aqueous extract

The foliage was cleaned and allowed to air dry for an entire night at room temperature. Five liters of deionized water was used to soak five thousand grams of the ground-up clean leaves for 18 hours. A semi-solid paste containing 204.18 g of the extract was obtained after evaporation, indicating a percentage yield of 4.08%. The mixture was filtered using a muslin cloth and evaporated to dryness using a thermostatic water bath at 45 0C. The extract was kept refrigerated for use.

Preparation of Experimental Animal

The Department of Physiology Animal House, University of Uyo, provided the experimental animals (Wistar rats). The average weight of the adult Wistar rats used in this investigation was 150 g. To allow the animals to acclimate adequately, the animals were housed for two weeks in sturdy wooden cages with wire meshwork at the animal house. The animals fed on standard pellets from Guinea Feeds, PLC Nigeria, and were allowed unlimited access to water to maintain their health.

Induction of diabetes mellitus

Streptozotocin (STZ, Sigma-Aldrich) induced diabetes by basic protocol 2 (Furman, 2021; Donovan and Brown, 2006). Before injection, streptozotocin (STZ) was dissolved in a pH 4.5 citrate buffer (citric acid and sodium citrate, enzyme grade from Fisher). The STZ was injected intraperitoneally at 65 mg per kilogram of body weight (Donovan and Brown, 2006). For the first 24 hours, the animals consumed 10% (w/v) sucrose water (from Sigma) to prevent severe hypoglycemia. The rats had unrestricted access to water and standard rat food. The animals starved of food the night before to evaluate their blood glucose levels during the fast (Furman, 2021).

After 48 hours, the risk of the animal developing diabetes was assessed by taking a blood sample from the tip of their tails. To measure the blood glucose level, a One Touch glucometer (One Touch Ultra, Life Scan Inc., U.S.A.) was used, and a blood sample was dropped into a glucose strip. Rats with normal blood glucose ranges of 80–120 mg/dL were used in this 14-day investigation, and blood glucose levels of 200 mg/dL or higher were termed diabetic (Borgohain et al., 2012).

Experimental Design

For this study, the animals used were distributed randomly into eight (8) groups of six (n=6) rats per group, as stated below:

Group 1: The control group was administered distilled water orally at a dose of 5 ml/kg body weight.

Group 2: Aqueous extract of *Terminalia catappa* at a dose of 130 mg/kg body weight administered orally to normal rats

Group 3: Diabetic rats were given only distilled water orally at a dose of 5ml/Kg body weight.

Group 4: Diabetic rats given 130mg/Kg body weight of *Terminalia catappa* leaf extract via oral administration

Group 5: Diabetic rats given exogenous Insulin at a dose of 0.75U/Kg body weight by subcutaneous administration.

Spermatological Studies

Assessment of Sperm Motility

The percentage of motile spermatozoa is evaluated after the preparation of sperm suspension, 5 uL of epididymal fluid exposed to 1000uL physiological saline was dropped in a glass slide, covered and viewed under the light microscope with x400 magnification. Motility estimation is executed at room temperature, and the microscopic field is systematically scanned. Motility was recorded in percentage and classified as Motile, Non-motile, Actively motile (Progressive), and sluggish (non -progressive) (Azu et al., 2014).

Sperm cell concentration

Determination of sperm concentration was by diluting 50uL of epididymal spermatozoa in 950uL. The mixed solution is pipette into both chambers of the hemocytometer and placed on the microscope. The hemocytometer was viewed, counted, and recorded using x400 magnification. Several squares were counted, and the average number of cells/ concentrations is calculated thus:

$$\text{Average number of cells} = \frac{\text{Sum of cells in each square}}{\text{Number of squares}}$$

Sperm Morphology

5uL of epididymal fluid collected by micropipette and put on the glass slide with a 22 x 22 cover slip. The glass slide was viewed under the microscope with x400 magnification, to determine the morphology. The microscopic field was systematically scanned, and each sperm was assessed and recorded in percentage as Normal Bent and Curved (Azu et al., 2014).

Hormonal Assay

The sex hormone levels were determined by the use of the standard protocols of enzyme-linked immunosorbent assay (ELISA) kits (Roche, Switzerland) for the estimation of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the estrogen hormonal levels.

Statistical analysis

GraphPad Prism 5.0 software was used to analyze the research data collected during the study. Data was analysed by a one-way analysis of variance (ANOVA) and a post hoc Turkey test. The results were shown as mean ± standard error of the mean (SEM), and values with $p<0.05$ were considered significant in the analysis.

3. RESULTS

Motile Sperm

Figure 1 shows the results of the percentage motile sperm as mean values of 78.33 ± 4.59 % for the control group and 80.00 ± 4.83 % for the non-diabetic group administered with extract. The diabetic untreated group had a value of 36.67 ± 5.57, and the diabetic group + extract had 16.67 ± 1.05. The diabetic group treated with insulin had a value of 36.67 ± 4.59 as the mean value.

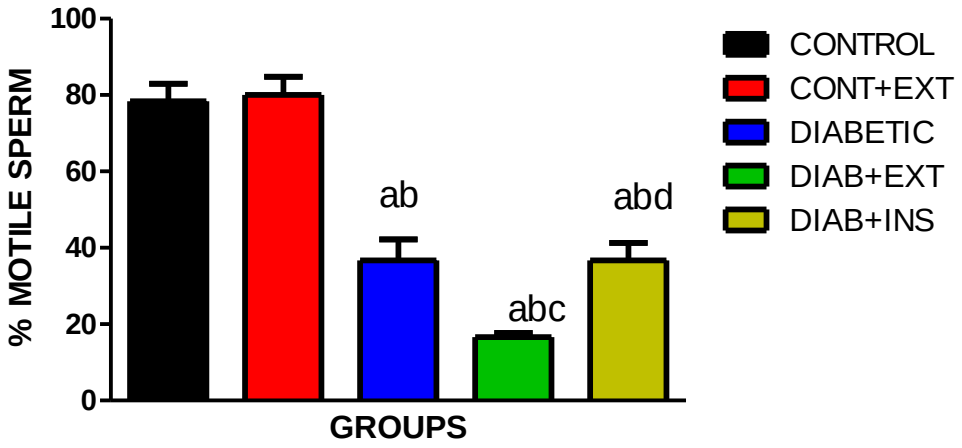

Figure 1 Sperm motility in non-diabetic and diabetic groups. Values are in mean ± SEM, $p<0.05$. a= test vs control, b= test vs control + extract, c= test vs diabetic group, d= test vs diabetic + extract.

Non-Motile Sperm

The mean values for the percentage of non-motile sperm for the control group and non-diabetic groups administered the extract were 24.17 ± 5.06 % and 20.00 ±1.82 %, respectively. The mean value for the diabetic group was 63.33 ± 5.5 %, the group with diabetic rats given the extract had a value of 83.33 ± 1.05 & and the diabetic group given insulin was 63.33 ± 4.59 %, as shown in (Figure 2).

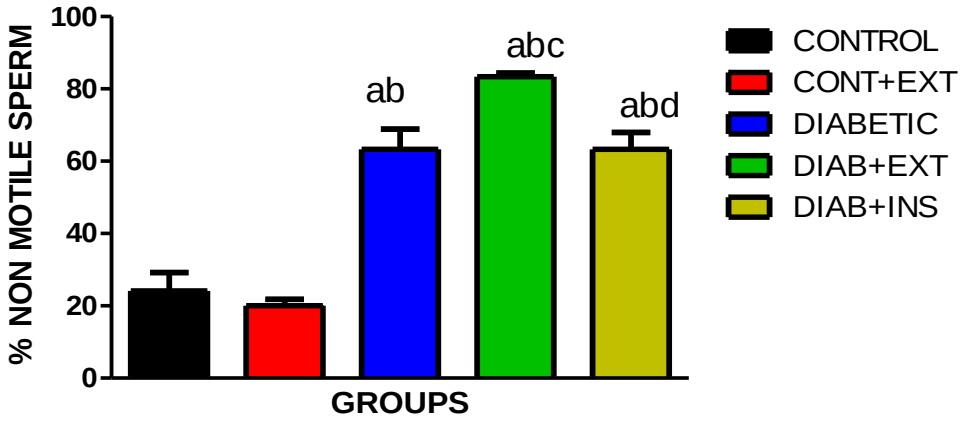

Figure 2 Non-motile sperm in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control + extract, c= test vs diabetic group, d= test vs diabetic + extract.

Progressive Motile Sperm

Figure 3 shows the % progressively motile sperm for the diabetic group and diabetic + Extract as mean values of 47.67 ± 0.91 % and 20.00 ± 1.82 %, respectively, which were significantly lower (p<0.05) than the control and Control + Extract groups, whose values were 86.67 ± 2.78 % and 76.67 ± 5.57 % respectively. The diabetic + Insulin group value was 23.33 ± 2.10 %. The Values for the diabetic + Extract and Diabetic + Insulin groups were significantly lower (p<0.05) compared to the diabetic group.

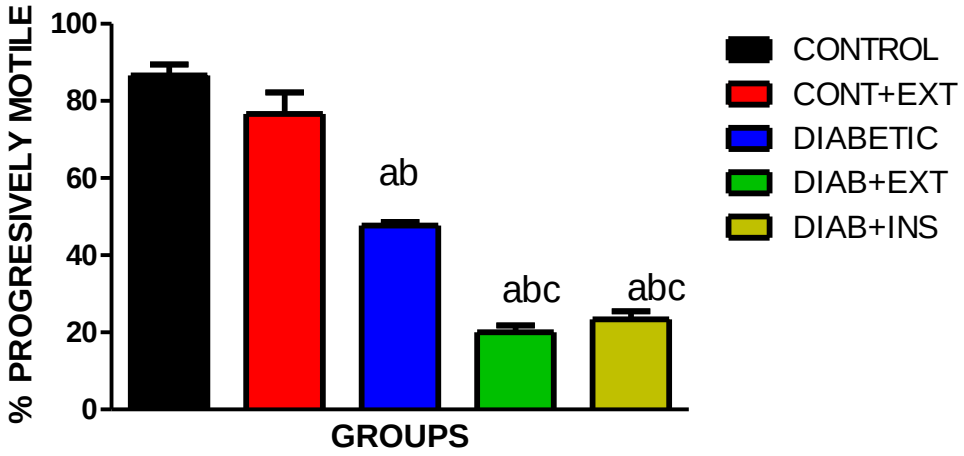

Figure 3 Progressively motile sperm in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control + extract, c= test vs diabetic group.

Non-Progressive motile Sperm

The % non-progressive motile sperm value for the control group and Control + Extract was 13.33 ± 1.05 % and 23.33 ± 5.57 % respectively. The mean values for the diabetic group and diabetic + Extract, were 52.33 ± 0.91 % and 80.00 ± 0.85 %, respectively while the diabetic + Insulin group value was 76.67 ± 5.57 %. The mean values of the % non-progressive motile sperm for the diabetic, diabetic + Extract, and diabetic + Insulin groups were significantly higher (p<0.05) compared to the control group, and control + Extract group. The values for the diabetic + extract, and diabetic + insulin group, were significantly higher (p<0.05) compared with the diabetic group, as shown in (Figure 4).

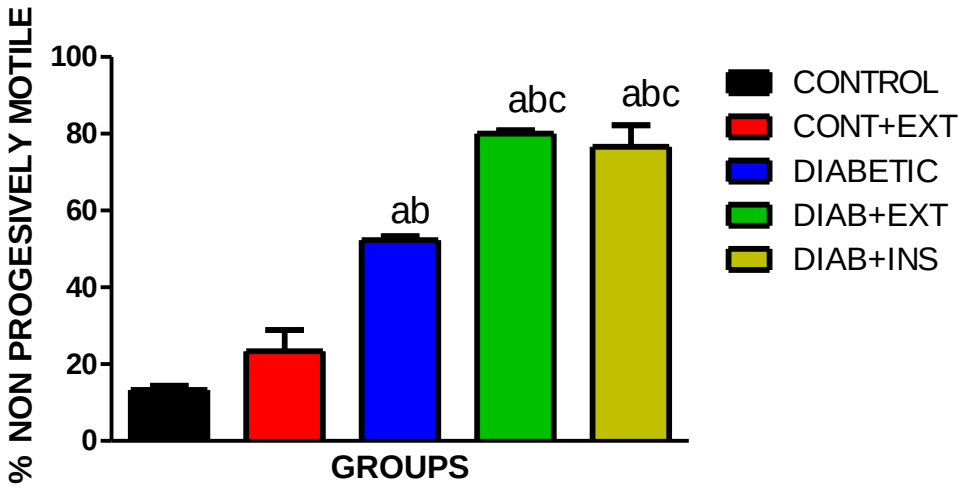

Figure 4 Non-progressive motile sperm in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control + extract, c= test vs diabetic group.

Sperm Concentration

Figure 5 shows the mean value of the control group as 77.33 ± 2.82 %, while the value for the Control + Extract group was 82.00 ± 0.91 %. The values for the diabetic group and the Diabetic + Extract were 46.83 ± 2.55 % and 30.92 ± 3.26 %, respectively. The diabetic + Insulin group value was 42.67 ± 3.54 %. The Values for the diabetic group, diabetic + Extract, and the diabetic + Insulin group were significantly lower (p<0.05) compared to the control group and as well as when compared to the Control + Extract group. There was a significant decrease (p<0.05) in the values for the diabetic + Extract group compared to the diabetic group. The diabetic group + Insulin was also significantly increased (p<0.05) compared to the diabetic + Extract group.

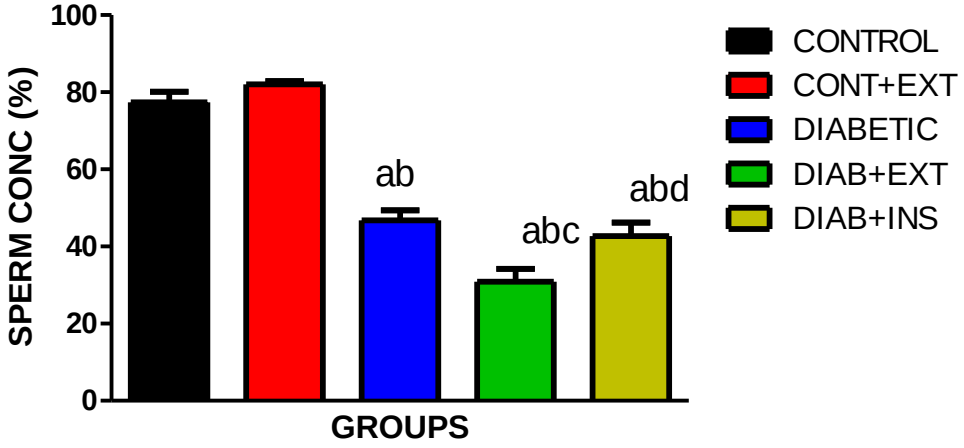

Figure 5 Sperm concentration in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control + extract, c= test vs diabetic group, d= test vs diabetic + extract.

Sperm morphology

The Sperm morphology mean values were 66.67 ± 2.10% for the control group and 71.67 ±1.05% for the control + Extract group. The Diabetic group and diabetic + extract group had mean values of 63.33 ± 2.78% and 80.00 ± 1.82%, respectively while the sperm morphology value for the diabetic + Insulin group was 71.67 ± 5.57%. The sperm morphology value for the diabetic group was significantly (p<0.05) lower compared to the control group, as shown in (Figure 6).

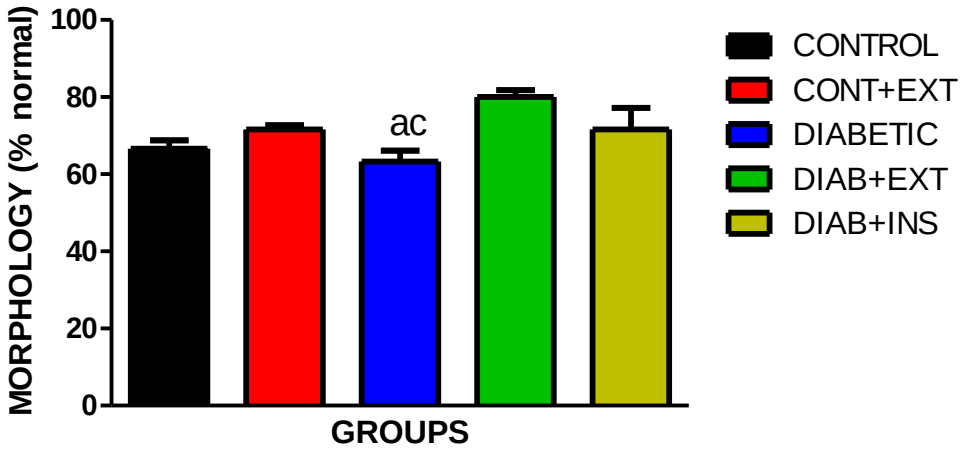

Figure 6 Sperm morphology in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, c= test vs diabetic group.

Bent neck Sperm

Figure 7 shows values of the bent neck sperm as 5.00 ± 0.25 % and 6.66 ± 1.05 %, for the control group, and control extract respectively. The value for the diabetic group was 5.0 ± 0.25 %, and the diabetic + extract group was 5.00 ± 0.51 %. The diabetic + insulin group had a mean value of 6.66 ± 0.84 %. There was just marginal variation in the mean value among groups.

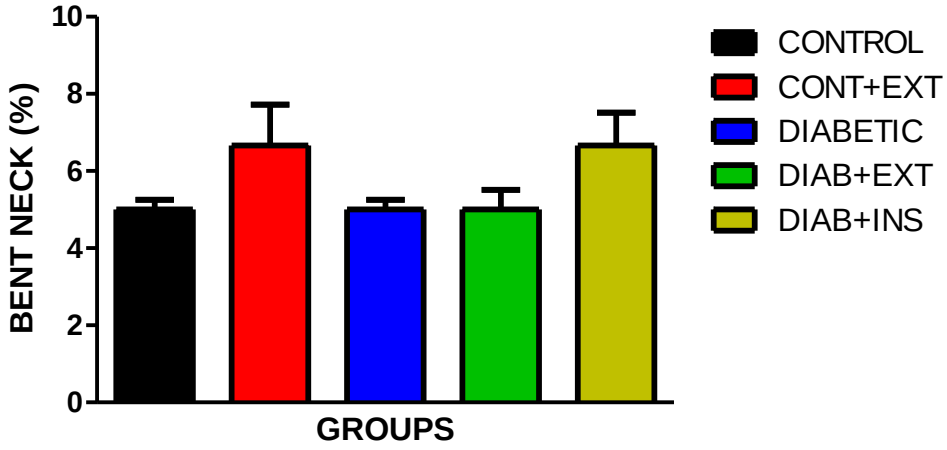

Figure 7 Sperm with bent neck in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05.

Curved Sperm

The curved sperm value for the control group was 28.33 ± 2.10 %, and the control + extract group was 21.67 ± 1.05 %. The diabetic group and diabetic + extract group had values of 31.67 ± 2.78 % and 15.00 ± 1.82 % respectively. The last experimental group (diabetic + insulin) had a curved sperm mean value of 21.67 ± 4.59 %. The value for the diabetic group was significantly higher (p<0.05) compared to the control group and also when compared to the control + extract group, as shown in (Figure 8).

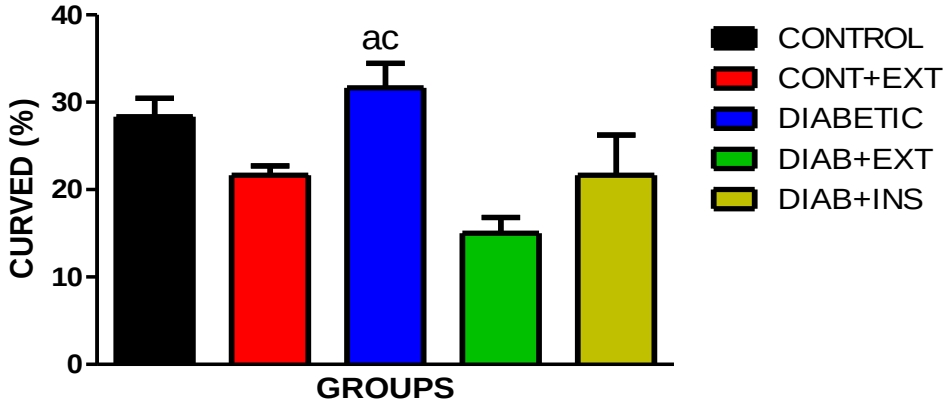

Figure 8 Sperm with curves in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, c= test vs diabetic group.

Testosterone Level

Figure 9 shows the mean testosterone values as 7.33 ± 0.42 % for the control group and 3.93 ± 0.05 % for the control + extract group. The diabetic group and the diabetic + extract group had mean values of 2.36 ± 0.22 % and 1.53 ± 0.15 %, respectively. The diabetic + insulin group had a value of 4.26 ± 0.39 %. The values for control + extract, diabetic, diabetic + extract, and diabetic + insulin groups were significantly lower (p<0.05) compared to the control. The diabetic + extract group value was significantly lower (p<0.05) compared to the diabetic group, and the testosterone level in the diabetic + insulin group was significantly increased in the diabetic + insulin group (p<0.05) compared to the diabetic group.

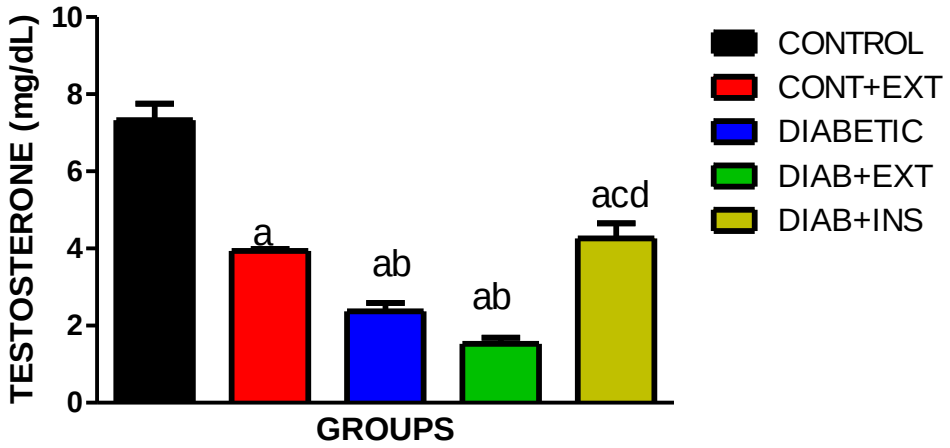

Figure 9 Testosterone levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control + extract, c= test vs diabetic group, d= test vs diabetic + extract.

Estrogen Level

The control and control + extract groups had mean values of 69.67 ± 4.93 % and 85.33 ± 0.55 % respectively. The diabetic group had a value of 103.3 ± 6.39 %, while the diabetic + extract and the diabetic + insulin groups had 121.00 ±7.41 % and 120.7 ± 5.29 %, respectively. There was a significant increase (p<0.05) in the diabetic group, diabetic + extract group, and the diabetic + insulin group compared to the control group. This is shown in (Figure 10).

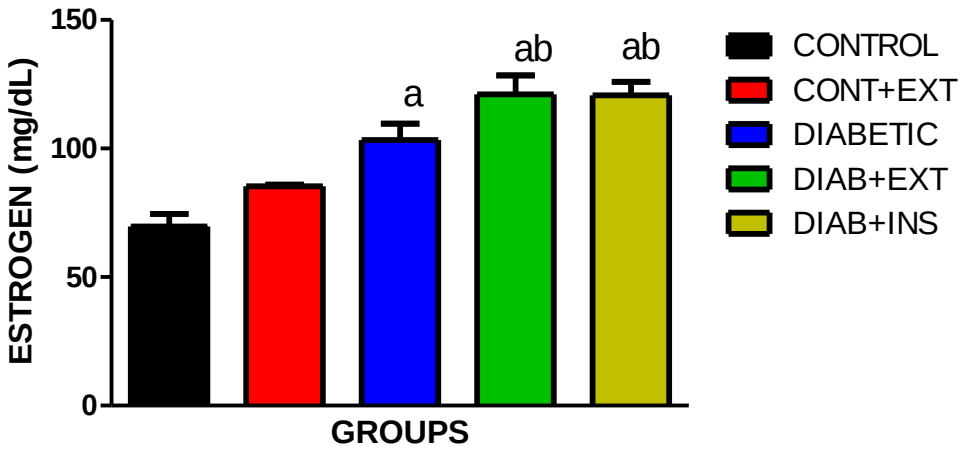

Figure 10 Estrogen levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control + extract, c= test vs diabetic group, d= test vs diabetic + extract.

Follicle-Stimulating Hormone Level

The Mean value for the follicle-stimulating hormone for the control group was 0.23 ± 0.04 mg/dl, and the control + extract group was 0.36 ± 0.01 mg/dl. The diabetic group and diabetic + extract group were 0.27 ± 0.01 mg/dl and 0.48 ± 0.04 mg/dl, respectively. The last experimental group (diabetic + insulin) had a mean value of 0.17 ± 0.00 mg/dl. There was a significant increase (p<0.05) in the diabetic + extract group compared to the control, control + extract, and the diabetic group, as shown in (Figure 11).

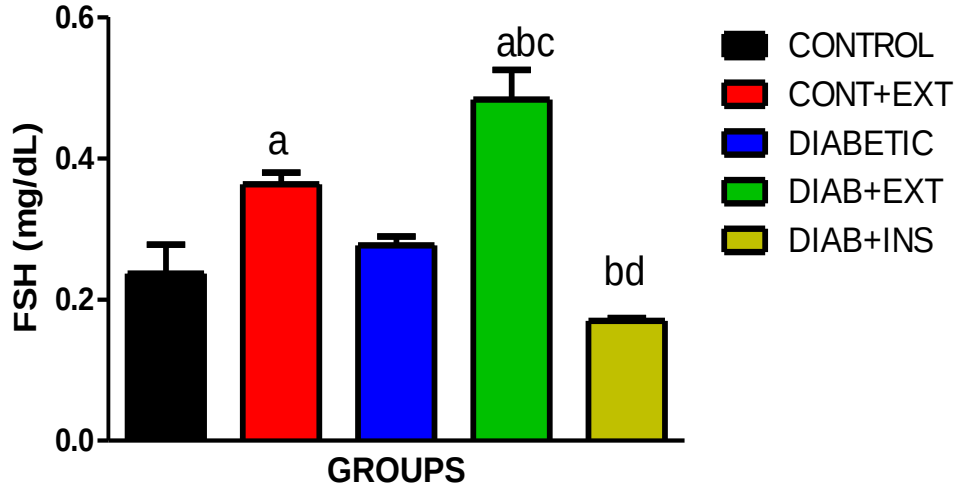

Figure 11 Follicle Stimulating Hormone in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control + extract, c= test vs diabetic group, d= test vs diabetic + extract.

Luteinizing Hormone

Figure 12 shows the mean values for the control and the control + extract group were 0.39 ± 0.02 mg/dl and 0.82 ± 0.02 mg/dl, respectively. The diabetic group had a mean value of 0.74 ± 0.03 mg/dl, while the diabetic + extract and diabetic + insulin groups had 0.96 ± 0.06 mg/dl and 0.32 ± 0.01 mg/dl, respectively. The Luteinizing hormone level for the control + extract group, diabetic group, and the diabetic + extract group increased significantly (p<0.05) compared with the control group. The diabetic + extract group value

increased significantly ($p<0.05$) compared to the diabetic group. The diabetic + Insulin group decreased significantly compared to the control + extract group, diabetic group, and the diabetic + extract group.

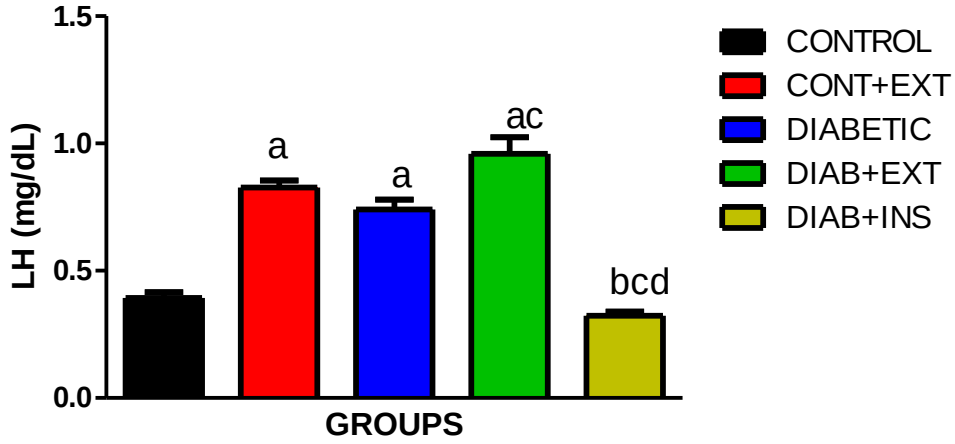

Figure 12 Luteinizing Hormone in nondiabetic and diabetic groups. Values are in mean ± SEM, $p<0.05$. a= test vs control, b= test vs control + extract, c= test vs diabetic group, d= test vs diabetic + extract.

Nitric Oxide level

Figure 13 shows the mean value of the control and control + extract groups as 4.36 ± 0.27 pg/dl and 7.61 ± 0.50 pg/dl. The diabetic group and the diabetic + extract group had mean values of 3.43 ± 0.84 pg/dl and 5.45 ± 0.34 pg/dl. The diabetic + Insulin group had a value of 5.18 ± 0.16 pg/dl. The control + extract group increased significantly ($p<0.05$) compared to the control group. The diabetic group, diabetic + extract group, and the diabetic + insulin group decreased significantly ($P<0.05$) compared to the diabetic + extract.

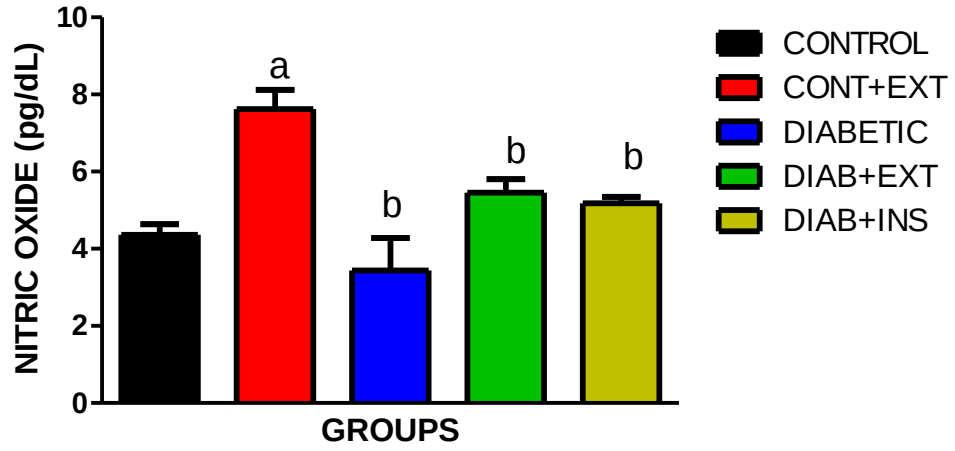

Figure 13 Nitric oxide levels in nondiabetic and diabetic groups. Values are in mean ± SEM, $p<0.05$. a= test vs control, b= test vs control + extract.

4. DISCUSSION

This study investigated changes in sperm indices and reproductive hormones in diabetic rats treated with aqueous leaf extract of *Terminalia catappa*. According to, sperm characteristics are evaluated to help determine the underlying cause of male infertility, and this study did just that. Since there are several reproductive deficits in streptozotocin-induced diabetes mellitus that are comparable to those

seen in human diabetics, this model is ideal for studying reproductive dysfunctions under diabetic circumstances Soudamani et al., (2005) because reduced male fertility and sexual dysfunction have been significantly positively correlated with diabetes (Shalaby and Mounier, 2010).

The sperm motility in the diabetic group treated with extract was significantly lower than the control and the diabetic untreated group; a study of *Uvaria chamae* root extract on sperm motility and viability gave similar outcomes (Ujah et al., 2021). Alkaloid and tannins metabolite decrease sperm motility; possible that the extract had active ingredients that interfered with the enzymatic activity associated with the Na/K ATPase ion pump involved in movement and eventually motility brought about by alteration in the membrane properties (Woo et al., 2000). Abnormal low sperm motility lowers fertility in males significantly (Nallela et al., 2006). Insulin treatment on the diabetic rats showed a significant increase in motility compared to the diabetic untreated group and the diabetic group treated with the extract, showcasing its ROS scavenging ability and promoting motility.

The percentage of progressive motile sperm reduced significantly in the diabetic untreated group compared to the control and the finding agrees with other studies (Facondo et al., 2022; Longo et al., 2024). The disruption in mitochondrial function of the spermatozoa can be responsible for the decline in the progressive motility present in diabetes mellitus. The mitochondrial energetic status responsible for ATP production required for the movement of the spermatozoa flagellum is controlled by the mitochondrial membrane potential; therefore, a disruption of mitochondrial function affecting mitochondrial membrane potential is associated with decreased spermatozoa progressive motility (Pellicone et al., 2011). The progressive motile sperm was significantly reduced in the diabetic group treated with extract when compared to the untreated diabetic group, which could be due to modifications in sperm plasma membrane integrity.

The finding agrees with a study that assesses the spermicidal effects of methanol leaf extract of *Euphobia hirta* Linn (Oguejiofor et al., 2021). On the other hand, non-progressive motile sperm was significantly increased in the test groups when compared to the control group, and also a significant increase in the diabetic group treated with extract when compared to the diabetic untreated group; it is suggestive that the extract could have interfered with the maturation of the released sperm cells from the testis during their epididymal passage thereby affecting the required modification with concerning small molecular weight compounds and surface proteins to enable the emergence of the progressive sperm cells (Cooper, 1995). Considering the sperm concentration, the diabetic untreated group was significantly lower compared to the control group. It is in line with previous studies by Roessner et al., (2012), Ma et al., (2020), and these changes could be due to an increase in inflammatory cytokines and oxidative stress, which can impair spermatogenesis and inhibit sperm vitality (Navarro et al., 2008).

Moreover, this lower sperm concentration could also be due to a diabetic-induced higher sperm nuclear DNA fragmentation. In comparing the sperm concentration of the diabetic group treated with extract to the diabetic untreated group, there was a significant decrease in sperm concentration. This finding is similar to results obtained in a study of the anti-fertility activity of *Dactyloctenium aegyptium* in male Wistar rats (Naik et al., 2016). It could be due to the ability of the extract to interfere with spermatogenic processes in the seminiferous tubules, and epididymal function, affecting the activities of the testosterone on the hypothalamic release factor and anterior pituitary secretions of gonadotropins; all this adverse interference may lead to changes in spermatogenesis (Udoh and Udoh, 2005). As part of the reproductive indices affected by the harmful effect of diabetes, the sperm morphology in the diabetic untreated group was significantly lower compared to the control.

This atypical form could be associated with hyperglycemia lipid peroxidation, which damages specific sperm cell cytoskeleton components, leading to aberrant sperm cell movement and structure. This result is consistent with earlier research (La-Vignera et al., 2015). Furthermore, sperm with a curve was significantly higher in percentage in the diabetic untreated group compared with the control group. This abnormality in the shape of the sperm is related to diabetes-induced oxidative stress, which causes peroxidation of membrane lipids of sperm cells, resulting in alteration in transport processes and membrane fluidity (Sanocka and Kurpisz, 2004). The abnormal curved shape of sperm associated with these diabetic rats could be due to abnormal membrane and nuclear changes induced by diabetes (Sureh et al., 2013). To further investigate the reasons for the observed abnormalities in sperm indices, an assessment of reproductive hormones was carried out.

Testosterone levels for the diabetic untreated group were significantly lower when compared to the control group; this finding is similar to previous studies by (Sudha et al., 2000; Tag et al., 2015). Severe chronic hyperglycemia and the resulting metabolic disorder may affect the function of the hypothalamic-pituitary-gonadal (HPG) axis, interfere with the endocrine system, and impair the production of steroids by Leydig cells (Steger and Rabe, 1997). The testosterone level was significantly lowered in the diabetic group

treated with the leaf extract compared to the untreated diabetic group. Lowered testosterone in males may impair spermatogenesis and cause male infertility. Despite the increase in LH and FHS, it is predictive that the extract exerted this low testosterone effect by influencing an excessive rise in the Luteinizing hormone, which in turn alters/ poses an inhibition to the regular LH-triggered release of testosterone from the Leydig cells due to the excessive secretion of LH.

In addition, the estrogen was assessed, and it was found that the level was significantly higher in the diabetic untreated group and the diabetic group treated with extract compared to the control and the diabetic group, respectively. This increase could be due to the extract's ability to convert testosterone to estrogen Carr and Blackwell, (1993), Naik et al., (2016) and estrogen, in turn, converts the sperm cells to oogonia thus reducing the sperm concentration. Besides all the abnormalities observed, the nitric oxide level was significantly higher in non-diabetic and diabetic rats that were given the leaf extract compared with the control and the diabetic group. It could be attributed to the extracted constituent (saponin) being able to induce nitric oxide production through the activation of endothelial nitric oxide (Li et al., 2001).

5. CONCLUSION

In this study, abnormal changes occur in sperm indices and reproductive hormones; therefore, aqueous leaf extract of *Terminalia catappa* may be detrimental to the male reproductive functions while being useful in its anti-diabetic or anti-inflammatory functions in diabetes mellitus. Caution should be taken in its usage for the known therapeutic potential.

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Authors Contribution

This work was carried out in collaboration between all authors. Author Ben EE designed the study, author Jonah CA carried out statistical analysis and author George UA conducted the actual laboratory work. Authors Asuquo AE and Taiwo AA wrote the manuscript and author Suleiman HO managed the literature searches. All authors read and approved the final manuscript.

Ethical Approval

The Faculty Animal Research Ethics Committee (FAREC-FBMS) reviewed and approved the protocol that was adopted for this study, hence the ethical approval number issued was 021PY30417. The Animal ethical guidelines are followed in the study for experimentation.

Informed Consent : Not applicable

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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