



Synergistic Effects of Vitamin D and Exercise on Diabetes-induced Gonadotoxicity in Male Wistar Rats: Role of Xanthine Oxidase/Uric Acid and Nrf2/NfκB Signaling

Adeyemi Fatai Odetayo¹ · Halimat Amin Abdulrahim² · Olaoluwa Tolulope Fabiyi^{3,4} · Taiye Abdulmujeeb Adewole³ · Bright Elijah Ajiboye³ · Adavize Noah Omeiza⁵ · Luqman Aribidesi Olayaki³

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Abstract

Type 2 Diabetes mellitus (T2DM) is one of the oldest known chronic diseases, characterized by elevated fasting blood sugar (FBS). T2DM is a metabolic disorder that can distort the activities of multiple physiological systems, including the reproductive system. Although different drugs have been designed for managing this disorder, these drugs have been reported to have negative side effects. Hence, this study was designed to explore the possible synergistic effect of vitamin D and exercise on T2DM-induced testicular dysfunction. Thirty-six male Wistar rats were randomized into six (6) groups: control, diabetes untreated, diabetes treated with 1000 IU/kg of vitamin D, diabetes treated with 5 min/day of physical exercise, diabetes treated with vitamin D and exercise, diabetes treated with 180 mg/kg of metformin. T2DM induction led to a significant increase in FBS, lactate, and lactate dehydrogenase, and was reversed by vitamin D supplementation and exercise. Also, vitamin D and exercise synergistically blunted T2DM-induced oxido-inflammatory response evidenced by a significant decrease in testicular malondialdehyde, interleukin 1β, interleukin 6, and tumor necrosis factor alpha, and an increase in superoxide dismutase, catalase, glutathione peroxidase, and interleukin 10. These events were associated with a decrease in T2DM-induced increase in XO, UA, and Nf-κb and an increase in T2DM-induced decrease in Nrf2. Also, vitamin D and EX reversed the observed impairment in sperm quality and testicular histology following T2DM-induction. This study revealed the synergistic effect of vitamin D and exercise on T2DM-induced testicular dysfunction.

✉ Adeyemi Fatai Odetayo
adeyemiodetayo@gmail.com

¹ Department of Physiology, Federal University of Health Sciences, Ila-Orangun, Nigeria

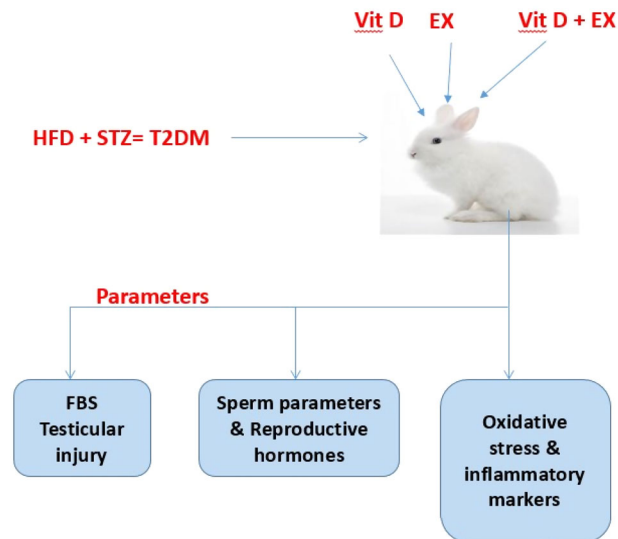
² Department of Medical Biochemistry, University of Ilorin, Ilorin, Nigeria

³ Department of Physiology, University of Ilorin, Ilorin, Nigeria

⁴ Department of Sports and Rehabilitation, Nazarbayev University, Astana, Kazakhstan

⁵ Department of Pharmacology, University of Medical Sciences, Ondo, Nigeria

Graphical abstract



Keywords Diabetes mellitus · Diet therapy · Glucose dysmetabolism · Lifestyle modification · Testicular dysfunction

Introduction

Diabetes mellitus (DM) is one of the most common metabolic disorders and has been identified as a disease of the 21st century [1]. Although there are different types of DM, type 2 DM (T2DM) is the commonest type; it accounts for approximately 90% of DM cases [2]. Lifestyle has been implicated in the development and progression of T2DM, which could even be worsened by the lack of disease awareness [3]. Since over 80% of the people suffering from T2DM are overweight, a sedentary lifestyle was believed to be the most dangerous pandemic of the 21st century before the COVID-19 pandemic [4]. The mounting rates of obesity, sedentary lifestyle, and environmental factors have been speculated to increase T2DM prevalence by 200% within the next 20 years [5].

T2DM is a very complex disorder that can cause metabolic, physiological, or biochemical derailment. A sustained increase in blood sugar (hyperglycemia) is believed to persistently stimulate chronic complications such as neuropathy, retinopathy, nephropathy, and testicular dysfunction [2]. These long-term complications could be a result of T2DM-induced redox imbalance and inflammatory response, which might be associated with impaired xanthine oxidase/uric acid and nuclear factor erythroid 2–related factor 2 (Nrf2)/ Nuclear factor kappa-light-chain-enhancer of activated B cells (NfκB) signaling.

The XO/UA pathway is an inducer of redox imbalance [6, 7]. XO is an enzyme responsible for catalyzing the conversion of hypoxanthine to xanthine and later to UA. During this process, oxygen molecules are used as

electron acceptors, which can lead to the generation of superoxide anions and other reactive oxygen species (ROS). Also, Nrf2 and NfκB are majorly responsible for maintaining redox imbalance and inflammatory response [8]. However, the excessive generation of superoxide anion and ROS can suppress endogenous enzymatic activities, thereby destroying the body's cellular components [9].

Nrf2 is the primary regulator of endogenous antioxidants responsible for maintaining cellular homeostasis in response to redox imbalance [10]. The possible suppression of Nrf2 due to excessive superoxide anion and ROS generation might upregulate NfκB activities, thereby increasing inflammation [7]. Also, the increase in NfκB can further suppress Nrf2 activities, leading to oxidative stress. Hence, Nrf2 and NfκB are essential players in the crosstalk between oxidative stress and inflammation [8]. Hence, it is plausible that the oxido-inflammatory response following T2DM could be associated with T2DM-induced XO/UA signaling upregulation and the consequence modulation of the Nrf2/NfκB pathway.

Over the last decade, numerous pharmacological agents have been introduced for T2DM management. However, most of these synthetic drugs have serious side effects such as weight gain, anemia, urinary tract infection (UTI), and gonadotoxicity [2]. Hence, managing T2DM without any complications is a serious challenge. Hence, our study was designed to explore other treatments with minimal side effects. One of the evidence-based management recommendations for T2DM is lifestyle modification, such as diet and exercise [11].

Regular aerobic exercise has been shown to improve male sexual function and life quality [12]. Physical exercise improves cardiovascular health and endothelial function by increasing nitric oxide (NO) production and endothelial progenitor cell growth, responsible for maintaining normal sexual function [13]. Additionally, physical exercise has been proven to improve glycemic control, maintain body weight, and lower blood pressure in T2DM patients, risk factors for sexual dysfunction [13]. Furthermore, physical exercise improves testosterone production by activating the hypothalamic-pituitary-gonadal (HPG) axis [14]. Finally, physical exercise prevents oxidative stress and inflammatory response [15], which are markers of testicular injury.

Vitamin D is one of the fat-soluble vitamins with hormone-like activities. It is majorly responsible for maintaining calcium and bone metabolism, although its biological role extends to inflammation, oxidative stress, cell differentiation, and immune regulation [16]. Additionally, vitamin D has been considered as a non-enzymatic compound [17]. Findings from previous studies have shown that vitamin D supplementation significantly improved the total antioxidant capacity while decreasing circulatory malondialdehyde (MDA) [18, 19]. Hence, we hypothesized that vitamin D supplementation alone or in combination with physical exercise will ameliorate T2DM-induced testicular injury via oxidative stress-dependent pathways.

Methodology

Chemicals and Drugs

Metformin was purchased from Osworth Nigeria Limited, Lagos, Nigeria, while Vitamin D was obtained from Principle Healthcare International Limited, United Kingdom. Streptozotocin (STZ) was purchased from Sigma-Aldrich in St. Louis, Missouri, USA.

Phosphate buffer was prepared in the laboratory by dissolving monobasic potassium phosphate (KH_2PO_4) and dibasic sodium phosphate (Na_2HPO_4) in distilled water; the volume was adjusted until the proper concentration of the buffer was achieved.

Ethical Consideration

The animals were carefully handled as stated by the National Institute of Health (NIH), and ARRIVE guidelines for reporting experimental findings were strictly followed. The experimental research protocol was designed according to the National Research Council's guidelines for the Care and Use of Laboratory Animals,

and ethical approval was obtained from the University of Ilorin's ethical review committee (approval number UERC/ASN/2023/2582) before the commencement of the research work.

Animal and Treatment

Thirty-six (36) male Wistar rats with an average weight of 120–150 g were procured from the Biochemistry Department of the University of Ilorin. The rats were kept in plastic cages under standard laboratory conditions and allowed to acclimate for one week. After acclimatization, the animals were randomized into six (6) groups ($n = 6$ rats/group): the control (treated with distilled water), diabetes untreated (DM-U) group, diabetes treated with 1000 IU/kg of vitamin D (DM+ Vitamin D), diabetes treated with 5 min/day of physical exercise (DM + EX), diabetes treated with vitamin D and exercise (DM+ vitamin D + EX), diabetes treated with 180 mg/kg of metformin dissolved in distilled water (DM+ metformin). All treatments were via the oral route and lasted for 28 days. The dosage of vitamin D used in this study is the same as the recommendation of the Expert Group on Vitamins and Minerals [20]. It is also similar to the doses of Krishnamurti et al. (2023) and Gázquez et al. [21], while the treatment period is similar with the study of [22]. Similarly, the dosage of metformin is based on our previous study [23].

Exercise Protocol

Animals were trained by allowing them to run on the modified exercise wheel at 20 m/min for 5 min/day for 2 weeks before the commencement of the experiment (i.e., diabetes induction), and exercise was maintained for animals in the exercise groups for 28 days after T2DM induction. The exercise intensity is within the reported intensity for moderate exercise [24], while the speed of 20 m/min used in this study is similar to that of [25].

T2DM Induction

After feeding with a high-fat diet (HFD) for five weeks, the animals were fasted overnight and then administered a single dose of STZ (35 mg/kg b.w. intraperitoneally) [23]. STZ was dissolved in chilled citrate buffer (pH - 4.5). Seventy-two (72) hours after the induction of diabetes, the rats' blood glucose was checked using a Fine test glucometer, and the rats with a blood glucose concentration of more than 250 mg/dl were considered diabetic. The animals in the control group also received 0.5 mL of citrate buffer through a similar route.

Sample Collection

Overnight fasted rats were sacrificed via IP administration of 40 mg/kg of ketamine and 4 mg/kg of xylazine [26]. Blood samples were collected via cardiac puncture, emptied into plain bottles, and centrifuged at 5000 rpm for 15 min to obtain serum. Also, the left testes were harvested and preserved for tissue homogenate using a phosphate buffer solution while the right testes were removed and preserved in bouin solution for histology.

Biochemical Analysis

Fasting blood sugar and serum insulin

Fasting blood glucose level was determined by glucose oxidase method using a digital glucometer (On Call®Plus ACON Laboratories, Inc. San Diego, CA, USA) while serum insulin was determined using an ELISA method (RayBio, GA, USA).

Testicular injury markers

Testicular lactate dehydrogenase (LDH) and lactate (Aggape Diagnostic, Switzerland) were assayed by a spectrophotometric method using ELISA kits.

Reproductive hormones

The serum level of gonadotropin-releasing hormone (GnRH) and testosterone (Elabsceince, US), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (Bio-Inteco, US) were estimated using the ELISA method.

Semen analysis

Each caudal epididymis was carefully cut into small pieces in a clean petri dish and sperm count, motility, and abnormal sperm morphology were determined as previously described [26, 27].

Oxidative stress markers

Testicular glutathione peroxidase (GPx) (Fortress Diagnostic Kit, Switzerland) was assayed using a colorimetry method. Testicular malondialdehyde (MDA) was determined as previously documented [27, 28]. Testicular superoxide dismutase (SOD), Nrf2, catalase (CAT), and glutathione (GSH) were determined using an ELISA method (Elabsceince, US).

Inflammatory markers

Testicular tumor necrotic factor- α (TNF- α), interleukin 6 (IL-6), interleukin 1 beta (IL-1 β), interleukin 10 (IL-10), and NF- κ B (Elabsceince, USA) were assayed using ELISA kits.

Xanthine oxidase and uric acid

Testicular XO activities were determined as previously described [29], while UA concentration was determined using colorimetric methods (Precision, UK) using a spectrophotometer.

Histology

Testicular histology was carried out as previously established [27, 30]. “The testis was preserved in bouin solution, dehydrated with ethanol series, cleared with toluene, and blocked in paraffin wax and Hematoxylin and Eosin (H&E) stain was applied to the five μ m thick paraffin sections of the testis”

Results

As shown in Fig. 1, there was a significant increase in FBS and a decrease in insulin following diabetic induction compared with the control. These observed differences were reversed in animals treated with vitamin D and exercise only, although a better reduction was observed in animals treated with vitamin D. Also, animals treated with the combination of vitamin D and exercise exhibited a better ameliorative response than their counterparts treated with either vitamin D or exercise.

In addition, the observed increase in LDH and lactate following T2DM-induction compared with the control was significantly decreased in animals treated with vitamin D, exercise, and vitamin D + exercise (Fig. 2). However, animals treated with vitamin D+ exercise exhibited a better ameliorative effect than their counterparts treated with either vitamin D or exercise.

T2DM induction significantly disrupted hormonal balance by decreasing GnRH, LH, FSH, and testosterone's circulatory levels while reducing 5 α reductase activities (Fig. 3). These observed T2DM-induced hormonal imbalances were ameliorated in animals treated with vitamin D, exercise, and vitamin D+ exercise. Although all the treated animals exhibited ameliorative effects, the animals treated with vitamin D+ exercise recovered more from the T2DM-associated hormonal imbalance.

Fig. 1 Effect of vitamin D and exercise on (a) fasting blood sugar (FBS) (b) serum insulin. ^a $P < 0.05$ vs control, ^b $P < 0.05$ vs DM-U; ^c $P < 0.05$ vs DM+ vitamin D; ^d $P < 0.05$ vs DM+ exercise, ^e $P < 0.05$ vs DM+ vitamin D+ exercise. Data were analyzed by one way ANOVA and Tukey's posthoc test. DM-U: Diabetes untreated, DM-diabetes

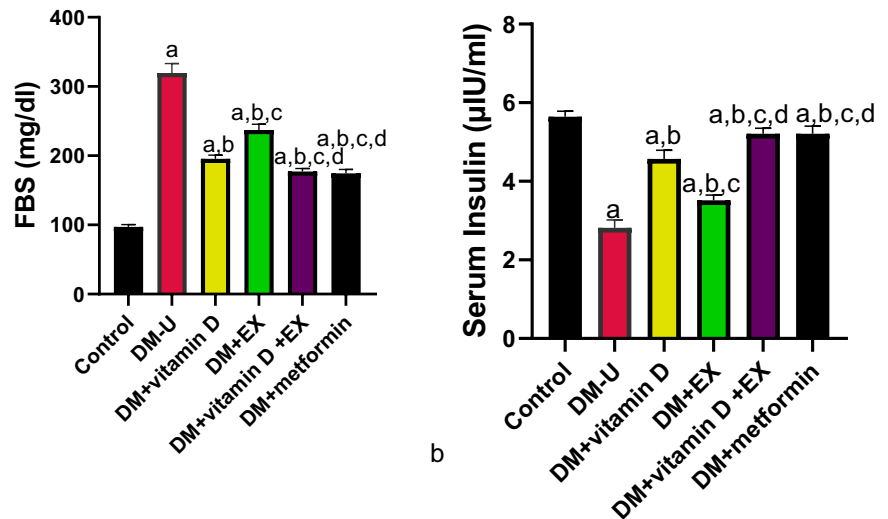
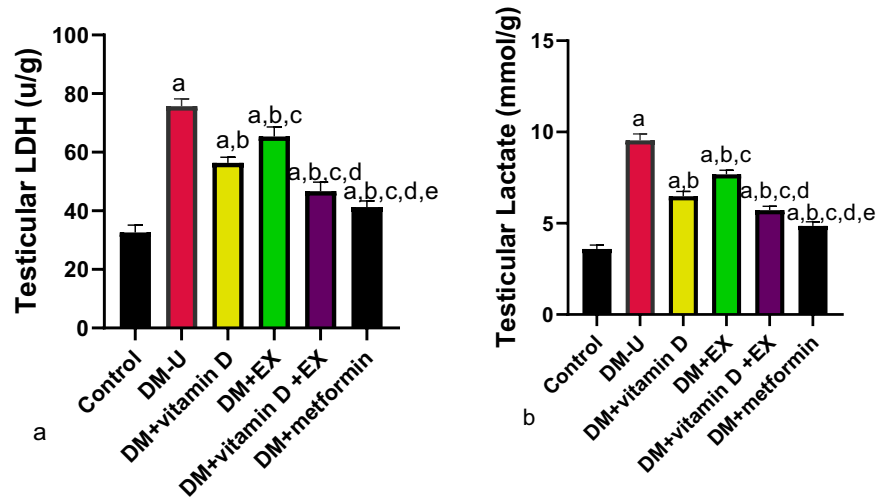


Fig. 2 Effect of vitamin D and exercise on testicular (a) LDH (b) Lactate. ^a $P < 0.05$ vs control, ^b $P < 0.05$ vs DM-U; ^c $P < 0.05$ vs DM+ vitamin D; ^d $P < 0.05$ vs DM+ exercise, ^e $P < 0.05$ vs DM+ vitamin D+ exercise. Data were analyzed by one way ANOVA and Tukey's posthoc test. DM-U Diabetes untreated, DM-diabetes



Also, T2DM induction led to a significant decrease in sperm quality evidenced by a significant increase in abnormal sperm morphology and a decrease in sperm motility and count compared with the control (Fig. 4). Although these observed impairments were reversed in animals treated with either vitamin D or EX, their counterparts treated with their combination exhibited a better recovery.

As shown in Figs. 5 and 6, T2DM significantly increased testicular MDA, TnF- α , IL-1 β , and IL-6 while decreasing testicular GPx, SOD, catalase, and IL-10 compared with the animals in the control group. These observed alterations were ameliorated by vitamin D, exercise, and vitamin D+ exercise treatment. However, animals treated with vitamin D+ exercise exhibited a better ameliorative effect.

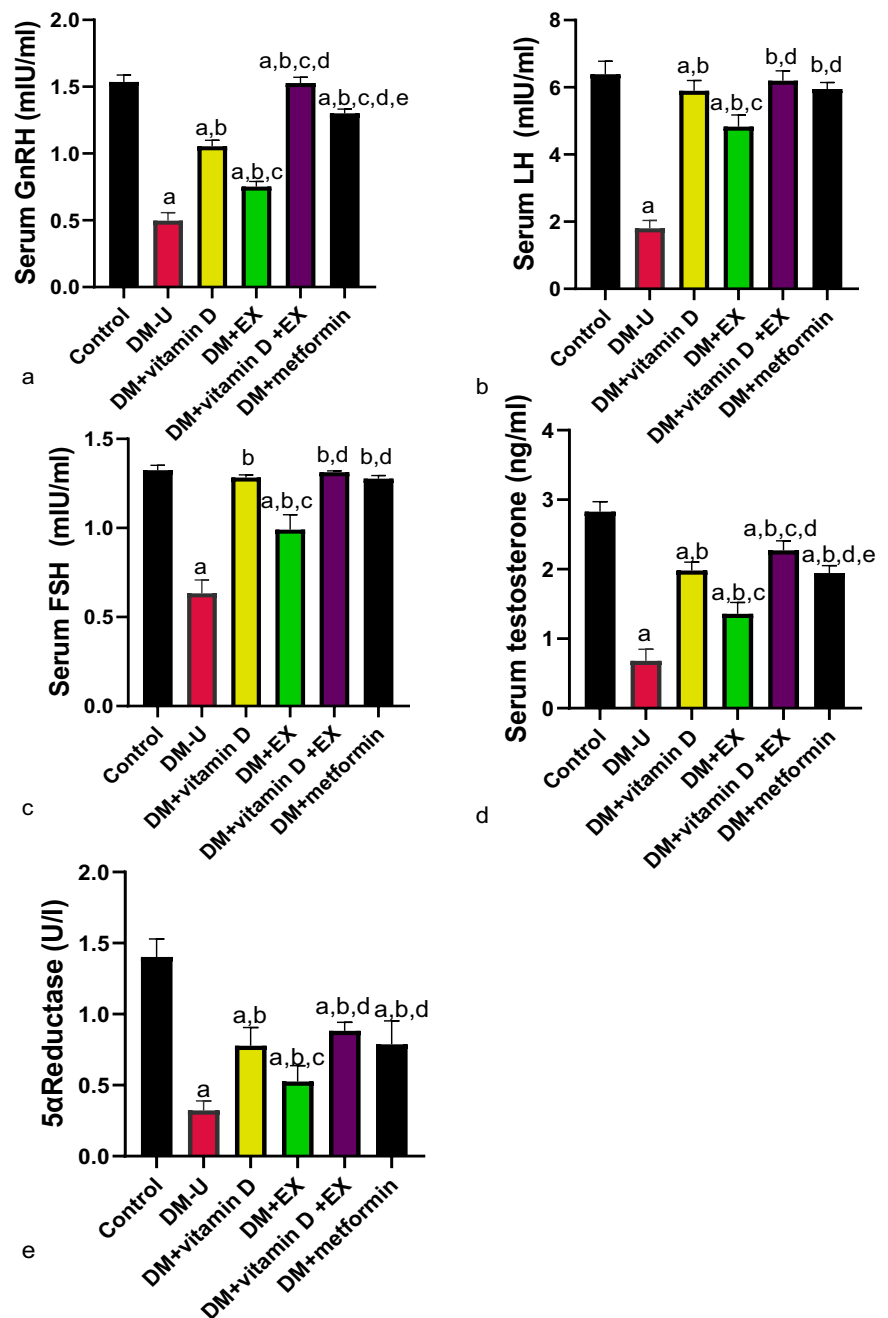
Furthermore, T2DM induction significantly increased testicular XO and UA compared with the control (Fig. 7).

However, these observed alterations were abrogated in animals treated with vitamin D, exercise, and vitamin D+ exercise. Although animals treated with vitamin D+ exercise exhibited a better ameliorative effect than their counterparts treated with vitamin D or exercise.

Additionally, there was a significant decrease in testicular Nrf2 and an increase in Nf- κ B compared with the control group (Fig. 8). These observed alterations were reversed in animals treated with vitamin D, exercise, and vitamin D+ exercise, although vitamin D+ exercise treatment was observed to be more effective than either vitamin D or exercise.

As shown in Fig. 9, the histology of animals in the control group consist of seminiferous tubules (ST) lined with series of spermatogenic cells; spermatogonia (SG), primary spermatocytes PS) and spermatids (S). Tubules are surrounded by basement membrane. The animals in the untreated group exhibited severe histopathological

Fig. 3 Effect of vitamin D and exercise on serum (a) GnRH (b) LH (c) FSH (d) testosterone (e) 5 α reductase. ^a P < 0.05 vs control, ^b P < 0.05 vs DM-U; ^c P < 0.05 vs DM+ vitamin D; ^d P < 0.05 vs DM+ exercise, ^e P < 0.05 vs DM+ vitamin D+ exercise. Data were analyzed by one way ANOVA and Tukey's posthoc test. DM-U Diabetes untreated, DM-diabetes, GnRH gonadotropin releasing hormone, LH luteinizing hormone, FSH follicle stimulating hormone

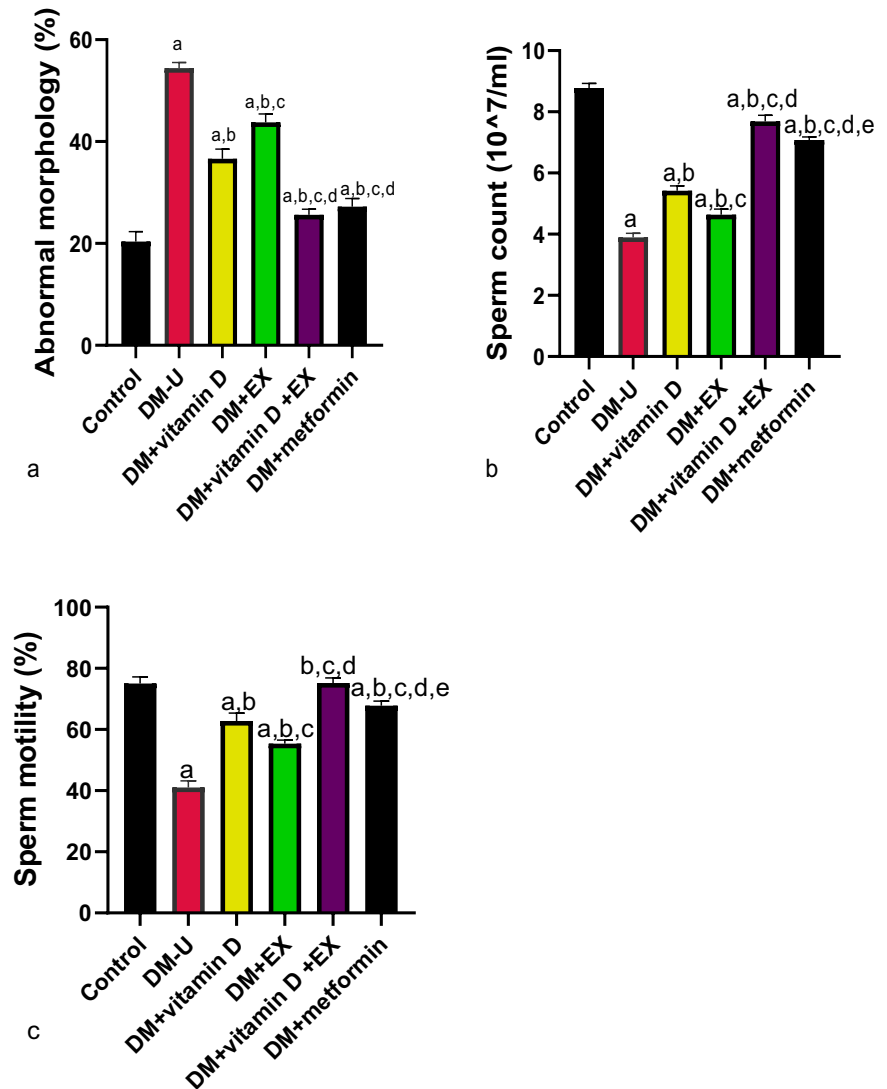


changes of the ST and scanty Interstitial space (IS). Also, animals in the DM + vitamin D and DM + EX groups exhibited mild pathology; vacuolated and degeneration of germinal epithelium and thinning of the basement membrane. Additionally, animals treated with combination therapy with vitamin D and EX shows nearly preserved architecture of the seminiferous tubule with atrophy. Finally, alteration of seminiferous tubules (ST) and degeneration of spermatogenic cells was observed in the testicular histology of animals treated with metformin.

Discussion

This study investigated the effects of vitamin D supplementation and exercise on testicular functions in HFD and STZ-induced diabetic Wistar rats. The findings from this study established the beneficial effects of vitamin D supplementation and aerobic exercise on improving T2DM-induced hyperglycemia, hormonal imbalance, and oxidative-inflammatory response. Hence, this study identified the importance of lifestyle modification and micronutrient supplements for managing T2DM-induced gonadotoxicity.

Fig. 4 Effect of vitamin D and exercise on serum (a) abnormal sperm morphology (b) sperm count (c) sperm motility. ^a $P < 0.05$ vs control, ^b $P < 0.05$ vs DM-U; ^c $P < 0.05$ vs DM+vitamin D; ^d $P < 0.05$ vs DM+exercise, ^e $P < 0.05$ vs DM+vitamin D+ exercise. Data were analyzed by one way ANOVA and Tukey's posthoc test. DM-U Diabetes untreated, DM-diabetes, EX Exercise

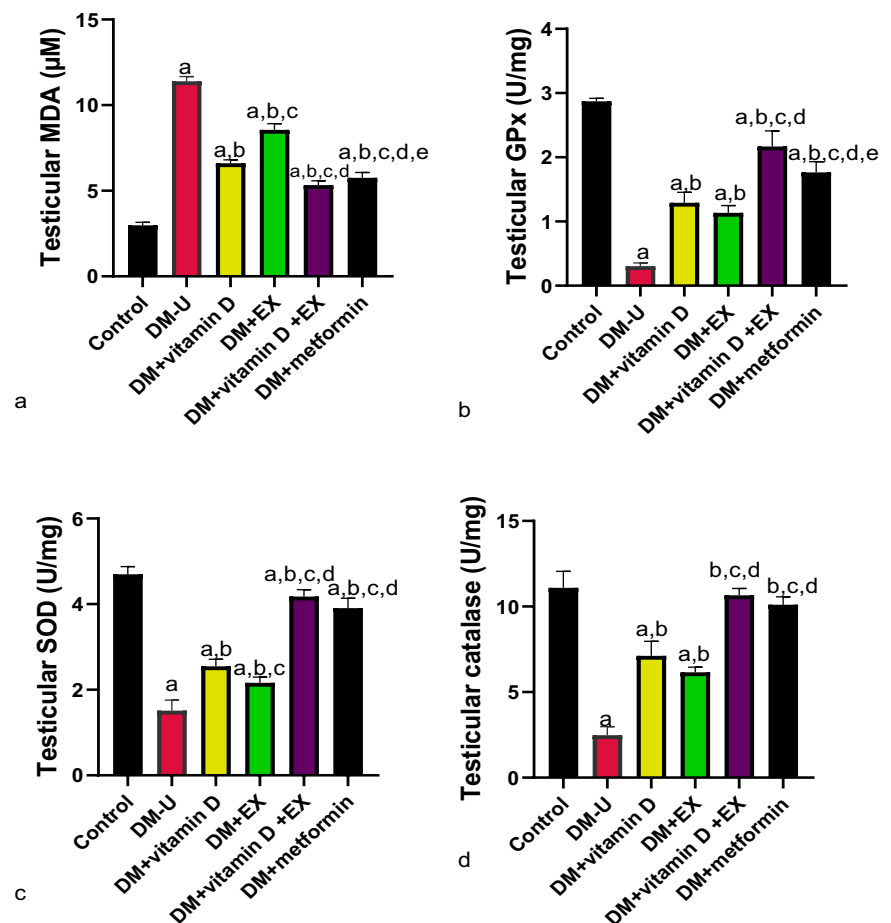


In tandem with previous findings [23, 31], all the animals in the diabetes group exhibited a significant increase in FBS, a hallmark of diabetes. However, this study revealed that the combination of vitamin D supplementation with exercise led to a significant reduction in FBS in diabetic rats. Importantly, these observed decreases were more pronounced in animals treated with vitamin D and exercise than their counterparts treated with vitamin D or exercise. This finding suggests that physical exercise is crucial to achieving the maximal beneficial effects of vitamin D on glycemic control. Vitamin D supplementation may improve FBS in diabetic rats by improving T2DM-induced insulin resistance (IR). Several studies have shown that vitamin D deficiency increases IR and insulin production impairment [32, 33]. Hence, vitamin D is suggested to improve insulin sensitivity and enhance beta cell activities through multiple pathways [32].

Additionally, exercise has been established as a good strategy for alleviating T2DM symptoms [34]. Previous studies have shown that exercise can improve energy metabolism, decrease IR, and maintain glucose homeostasis [35, 36]. Hence, combining vitamin D and exercise might open another therapeutic window for managing T2DM-induced glucose dysmetabolism.

T2DM is a metabolic disorder that can distort the activities of multiple physiological systems, including the reproductive system [37]. Different reports indicate a continuous rise in T2DM prevalence in young patients within the reproductive age range [38, 39]. This increase in T2DM occurrence in young people is a serious concern as it declines fertility rates of more males in their reproductive age. Consistent with previous studies [40, 41], the findings from this research that T2DM led to a significant decrease in circulatory testosterone (hypogonadism) and impaired sperm quality confirmed the gonadotoxic effect of this

Fig. 5 Effect of vitamin D and exercise on testicular (a) malondialdehyde (MDA) (b) glutathione peroxidase (GPx) (c) superoxide dismutase (SOD) (d) catalase. ^a $P < 0.05$ vs control, ^b $P < 0.05$ vs DM-U; ^c $P < 0.05$ vs DM+ vitamin D; ^d $P < 0.05$ vs DM+ exercise, ^e $P < 0.05$ vs DM + vitamin D+ exercise. Data were analyzed by one way ANOVA and Tukey's posthoc test. DM-U Diabetes untreated, DM-diabetes



metabolic disorder. This observed hypogonadism can either be primary (direct testicular injury) or secondary (distortion of the hypothalamic-pituitary-gonadal (HPG) axis).

The male reproductive activities require a cascade of events such as testosterone and spermatogenesis. These processes are essential for maintaining male fertility and are regulated by the HPG axis. In males, the hypothalamus is responsible for secreting GnRH, which stimulates the secretion of LH and FSH from the pituitary gland. While the former stimulates the testis to produce and release testosterone, the latter is responsible for spermatogenesis. The released testosterone then inhibits the release of LH, FSH, and GnRH [42]. In concurrence with previous studies [43, 44], the findings from this study that T2DM distorts the HPG axis suggests a hormonal-dependent pathway (secondary hypogonadism) in T2DM-induced male infertility.

On the other hand, T2DM might reduce circulatory testosterone by directly impairing testicular function. Similar to previous findings [23], this study observed a significant increase in lactate and LDH following T2DM induction, which could indicate testicular injury. Lactate is a marker of energy balance [45], and an increase in testicular lactate could indicate testicular dysfunction. This increase could be

due to the observed increase in testicular LDH, which primarily converts pyruvate to lactate under an anaerobic state. The increase in testicular lactate and LDH could result from the compensatory adaptation of the testis to restore testicular function following T2DM-induced oxidative testicular injury [7]. Interestingly, these events were consistent with our histopathological findings.

Physiologically, there must be a balance between the production and removal of ROS. However, when excessive ROS (pro-oxidants) is produced, it alters the balance between ROS (pro-oxidants) generation and clearance (antioxidants). This leads to a condition known as oxidative stress, which can damage the activities of various organs in the body. In this study, an increase in testicular ROS generation was observed following T2DM, evident by a significant increase in testicular MDA and a reduction in testicular GPx, SOD, and CAT. These significant alterations have been linked with impaired testicular functions [46] and could account for the elevated testicular injury markers that were observed earlier. The observed increase in pro-oxidants following T2DM induction could account for the observed inflammatory response (an increase in TnF- α , IL-6, and IL-1 β and a decrease in IL-10) since oxidative stress

Fig. 6 Effect of vitamin D and exercise on testicular (a) Tumor necrosis factor (TNF- α) (b) Interleukin-10 (IL-10) (c) Interleukin-6 (IL-6) (d) Interleukin-1 beta (IL-1 β). ^a $P < 0.05$ vs control, ^b $P < 0.05$ vs DM-U; ^c $P < 0.05$ vs DM+vitamin D; ^d $P < 0.05$ vs DM+exercise, ^e $P < 0.05$ vs DM+vitamin D+ exercise. Data were analyzed by one way ANOVA and Tukey's posthoc test. DM-U Diabetes untreated, DM-diabetes

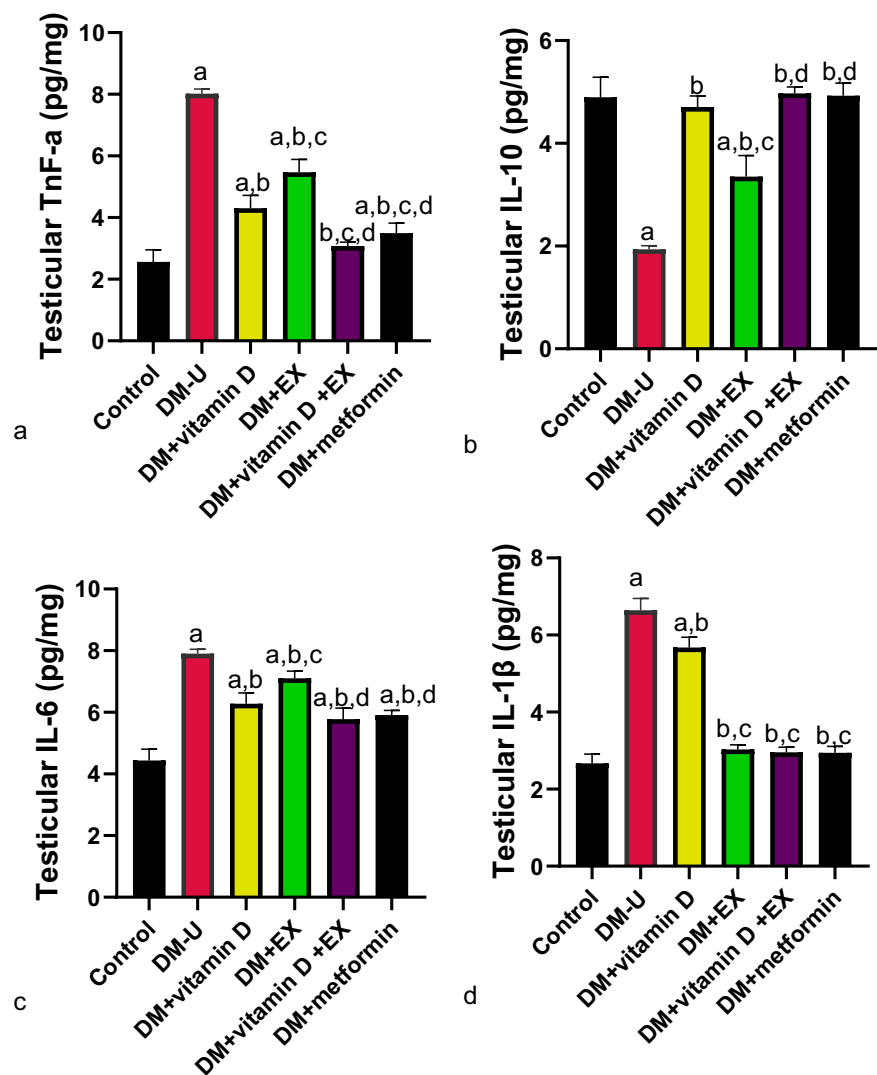


Fig. 7 Effect of vitamin D and exercise on testicular (a) xanthine oxidase (b) uric acid. ^a $P < 0.05$ vs control, ^b $P < 0.05$ vs DM-U; ^c $P < 0.05$ vs DM+vitamin D; ^d $P < 0.05$ vs DM+exercise, ^e $P < 0.05$ vs DM+vitamin D+ exercise. Data were analyzed by one way ANOVA and Tukey's posthoc test. DM-U Diabetes untreated, DM-diabetes

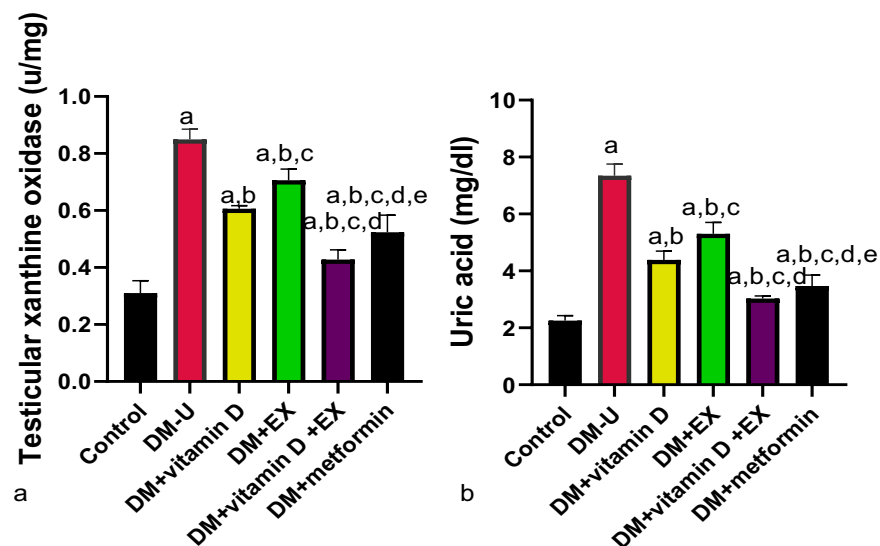


Fig. 8 Effect of vitamin D and exercise on testicular (a) nuclear factor erythroid 2-related factor 2 (Nrf2) (b) Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B). ^a $P < 0.05$ vs control, ^b $P < 0.05$ vs DM-U; ^c $P < 0.05$ vs DM+ vitamin D; ^d $P < 0.05$ vs DM+ exercise, ^e $P < 0.05$ vs DM+ vitamin D+ exercise. Data were analyzed by one way ANOVA and Tukey's posthoc test. DM-U Diabetes untreated, DM-diabetes

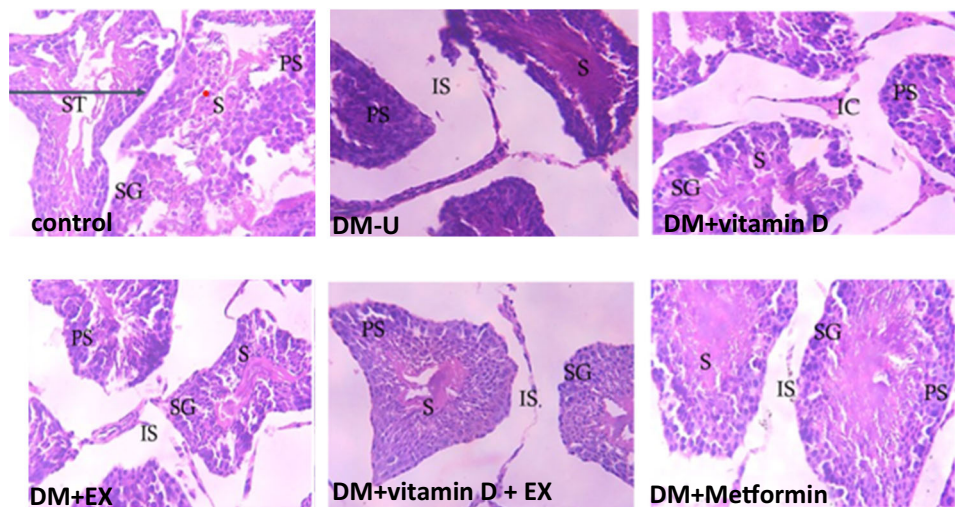
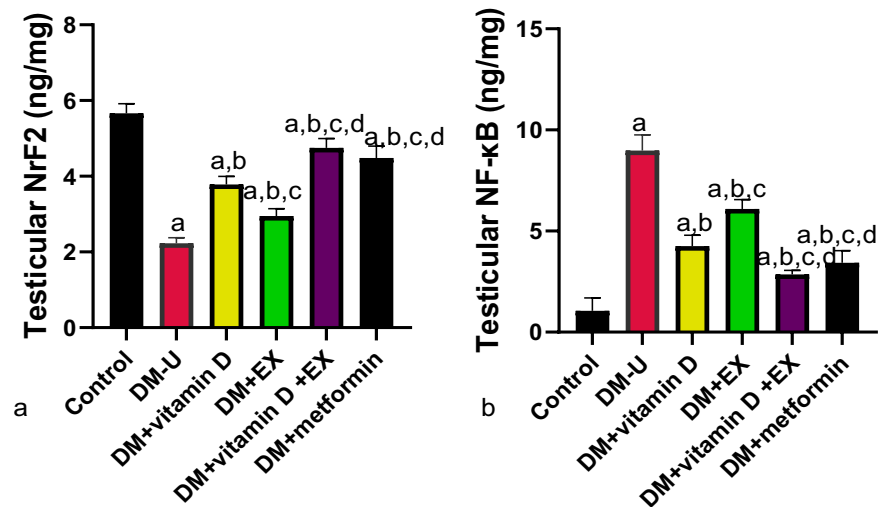


Fig. 9 Histology of the testis. Control: Photomicrograph of control testis showing seminiferous tubules (ST) lined with series of spermatogenic cells; spermatogonia (SG), primary spermatocytes (PS) and spermatids (S). Tubules are surrounded by basement membrane. DM-U: Severe histopathological changes of the seminiferous tubules (ST) and scanty Interstitial space (IS). DM+ vitamin D: Shows mild pathology; vacuolated and degeneration of germinal epithelium and thinning of the basement membrane. DM + EX: Shows mild

pathology; vacuolated germinal epithelium and degenerated seminiferous tubules DM+ vitamin D + EX: shows nearly preserved architecture of the seminiferous tubule with atrophy. DM+Metformin: Alteration of seminiferous tubules (ST) and degeneration of spermatogenic cells. Mg X 800. ST Germinal epithelium/Seminiferous tubule (ST), S Spermatids, IC Interstitial cells, IS Interstitial space, SG Spermatogonium, PS Primary spermatocytes, DM-U Diabetes Mellitus Untreated, DM Diabetes Mellitus, EX exercise

has been reported to stimulate different transcription factors leading to inflammatory pathways activation [47]. These findings agree with previous findings [23] that have reported an increase in ROS generation and inflammatory response in T2DM.

The observed oxido-inflammatory response could be linked with T2DM-induced XO/UA signaling upregulation. An increase in XO and, consequently, UA has been shown to be positively related to lipid peroxidation [48]. While it is important to identify the antioxidant effect of UA [49], its pro-oxidant activities, when produced in excess, cannot be overlooked. When in excess, UA can

react with other oxidants to generate radicals capable of distorting cellular activities. The resulting lipophilic environment from the accumulation of lipids, in turn, overwhelms the antioxidant activities of UA, leading to its conversion to oxidants by the oxidized lipids. Also, the accumulation of UA can lead to the activation of a pro-inflammatory response, thereby leading to an increase in inflammatory markers [49]. Since the XO/UA upregulation can lead to an oxido-inflammatory response, which could be responsible for the observed direct testicular damage, then it is plausible that T2DM-induced testicular injury is mediated via XO/UA signaling.

Another important pathway that might be responsible for T2DM-induced testicular damage is Nrf2/Nf- κ B signaling. The excessive ROS resulting from XO/UA upregulation could account for the decrease in testicular Nrf2 and increase in testicular Nf- κ B observed in this study following T2DM induction. Increased ROS generation has been shown to decrease antioxidant activities and increase inflammatory response [50]. Nrf2 is a major regulator of endogenous antioxidants responsible for maintaining redox balance [10]. Similar to other findings [51], the observed decrease in testicular Nrf2 activities could account for the observed increase in testicular MDA and decrease in testicular SOD and CAT following T2DM induction.

Additionally, Nrf2 can act as an anti-inflammatory agent via its inhibitory role on NF- κ B [52]. This inhibitory effect prevents inflammatory response because NF- κ B stimulates pro-inflammatory markers (such as IL-1 β) release. Hence, it is also plausible that the observed T2DM-induced oxido-inflammatory response could result from the observed T2DM-induced decrease in Nrf2 and the consequent increase in NF- κ B activities.

The novelty of this research extends beyond demonstrating the role of testicular XO/UA and Nrf2/Nf- κ B signaling in T2DM-induced testicular injury. Another key finding is establishing vitamin D and exercise's beneficial role in T2DM-induced testicular dysfunction. This present study revealed that combining vitamin D with exercise alleviated T2DM-induced impairment in testicular function by modulating testicular XO/UA and Nrf2/Nf- κ B-induced testicular redox imbalance, thus decreasing the levels of testicular injury and inflammatory markers. Although this seems to be the first study to suggest the synergistic effect of vitamin D and exercise in testicular function, the finding that vitamin D and exercise maintained redox balance agrees with previous findings that reported that vitamin D and exercise exert anti-inflammatory response and antioxidant effects [53, 54]. Although our anti-inflammatory effects of EX disagreed with that of [55], but the observed increase in SOD, CAT, GPx, and IL-10 and decrease in TNF- α , IL-1 β , and IL-6 concentration in the testicular tissues of vitamin D + exercise rats showed that vitamin D + exercise-driven repression of UA production via the downregulation of XO activities possibly inhibited the modulation of Nrf2/Nf- κ B signaling [56], leading to the inhibitory response on the transcription of genes encoding pro-inflammatory cytokines and oxidative response.

Conclusion

This study revealed that vitamin D and exercise synergistically ameliorated T2DM-induced hormonal imbalance, oxidative stress, and inflammatory response via the

modulation of XO/UA and Nrf2/NF- κ B signaling. These results will open another therapeutic window for the management of T2DM by providing a possible insight into the protective molecular mechanisms of vitamin D and exercise against T2DM-induced testicular dysfunction.

Limitations

XO, UA, Nrf2, and NF- κ B act by modulating the expression of downstream target genes to maintain oxido-inflammatory balance. Unfortunately, these target genes were not estimated in this study. This limitation in the present study opens a gray area for future exploration. Nonetheless, the significant decrease in Nrf2 and increase in XO, UA, and NF- κ B concentration accompanied by redox imbalance and inflammatory response is an indication of XO/UA and Nrf2/Nf- κ B-mediated oxido-inflammatory response.

Data availability

The materials for the manuscript, including any relevant raw data, will be freely made available by the corresponding author (O.A.F.) to any researcher who needs them for non-commercial study while protecting participant confidentiality.

Author Contributions A.O.F., A.H.A., and L.A.O.: Conceptualization, A.O.F., A.H.A., O.T.F., T.A.A., B.E.A., A.N.O., L.A.O.: Methodology, O.A.F.: Data curation, O.A.F.: Writing- Original draft preparation. A.O.F., A.H.A., O.T.F., T.A.A., B.E.A., A.N.O., L.A.O.: Visualization, Investigation. A.O.F., A.H.A., and L.A.O.: Supervision. A.O.F., A.H.A., and L.A.O.: Software, Validation.: A.O.F., A.H.A., O.T.F., T.A.A., B.E.A., A.N.O., L.A.O. Writing- Reviewing and Editing

Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

Ethics Approval and Consent to Participate The animals were carefully handled as stated by the National Institute of Health (NIH), and ARRIVE guidelines for reporting experimental findings were strictly followed. The experimental research protocol was designed according to the National Research Council's guidelines for the Care and Use of Laboratory Animals, and ethical approval was obtained from the University of Ilorin's ethical review committee before the commencement of the research work.

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