ORIGINAL ARTICLE

Probiotics modulate testicular redox status, DNA oxidation, the pentose phosphate pathway, and Fas/CD95 signaling in dexamethasone-treated rats

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ABSTRACT

A continued global decline in semen quantity and quality has been reported in the last five decades, warranting scientific investigations to decipher the etiologies and mechanisms of identified causes in the race to develop effective management to halt the decline. The current investigation aims to further the existing understanding of the impact of dexamethasone (Dex) on testicular function and test the potential of probiotics (Pro) to mitigate such impacts. Twenty adult male Wister rats were divided into control (given 2 ml of normal saline), Dex (administered 4mg/Kg body weight), Pro (Lactobacillus acidophilus and Bifidobacterium bifidum every other day), and Dex/Pro (treated with Dex and probiotics. The treatments lasted 21 consecutive days. Blood samples and the right testes were used for biochemical assays; the left was used for histological analysis; and the right epididymides were used for sperm parameter evaluation. Circulating follicle-stimulating hormone (FSH) and testosterone dropped significantly ($p<0.05$) in the Dex group. Furthermore, lipid peroxidation, DNA oxidation and fragmentation, and glucose-6-phosphate dehydrogenase (G6PD) activities were significantly ($p<0.05$) increased while pro-inflammation cytokines were significantly ($p<0.05$) reduced in the Dex group. All these parameters were significantly improved in the Dex/Pro group. Probiotics promote testicular androgen secretion by improving Dex-induced suppression of the testicular antioxidant system, zinc concentration, and cytokine expression. Dex-induced DNA fragmentation and oxidation were improved by probiotics via reduced expression of Fas/CD95-mediated apoptotic signalling and increased utilization of the pentose phosphate pathway.

Keywords: Testis, Glucose-6-phosphate dehydrogenase Fas/CD95, DNA fragmentation, Probiotics, Dexamethasone

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Citing this article

Medubi L.J., Medubi O.O., Akinpelu F.A., Owolabi B.O., Ojo T.N. and Ugboh V.O. Probiotics Modulate Testicular Redox Status, DNA Oxidation, the Pentose Phosphate Pathway, and Fas/CD95 Signaling in Dexamethasone-Treated Rats. KIU J. Health Sci, 2025: 5(1);

Conflict of Interest: None is declared

INTRODUCTION

As male reproductive capacity continues to worsen (1, 2), the pharmacological use of glucocorticoids remains popular without a complete understanding of its impact on male reproductive function (3, 4). More importantly, alleviating glucocorticoid-induced male reproductive dysfunction depends on further the comprehension of the mechanisms that drive glucocorticoid-induced testicular dysfunction during stress and glucocorticoid treatment. An elevated circulating glucocorticoid is the conclusive proof of stress and the hormone by which stress initiates cascading events that ultimately result in dyshomeostasis (5). Understanding stress-induced glucocorticoid rise provides a scientific basis for pharmacologically mimicking stress by loading animals with glucocorticoids. In normal amounts, glucocorticoids play roles in metabolism, particularly gluconeogenesis, inflammation suppression, and modulation of secretions of other hormones (6).

The physiological, pharmacological, and cellular effects of glucocorticoids vary widely as soon as they become elevated. At pharmacological doses, for instance, dexamethasone (Dex) shows inflammation suppression efficacy (7, 8), but this does not guarantee that it will not initiate stress-like effects on other systems like reproduction. The stress-like effect of Dex makes it an ideal agent for examining how stress impairs spermatogenesis pharmacologically and tests the usefulness of mitigating stress-induced testicular function with probiotic supplementation. Early investigations have reported Dex-induced poor sperm parameters and testicular oxidative stress (9); however, elucidating the complete mechanism of stress-induced reproductive dysfunction requires more studies.

Testicular function proceeds on narrowly balanced cellular and biochemical activities susceptible to elevated glucocorticoids. While testosterone (TT) secretion is under the pulsatile luteinizing hormone (LH), Sertoli cells are dependent on follicle-stimulating hormone FSH) in concert with TT for cellular stimulation for the production of nutrients and regulatory molecules needed for spermatogenesis (10). Because of the massiveness of the number of germ cells undergoing cell division during spermatogenesis, the maintenance of spermatogenic DNA integrity is under tremendous regulatory pressure, requiring efficient molecular regulation of apoptosis to remove and recycle germ cells with pronounced DNA damage (11). To meet this need, an array of apoptotic pathways operational within the seminiferous tubules can be engaged by various pro-apoptotic molecules, such as Fas/CD95. The Sertoli cell membrane Fas ligand binds to the Fas receptor expressed by the spermatogenic cells and initiates apoptosis (12). Furthermore, damage to DNA can be assessed through the DNA fragmentation index and DNA oxidation by quantifying 8-hydroxy-2' -deoxyguanosine (8-OHdG) (13, 14).

Oxidative stress secondary to elevated glucocorticoids has been reported (15, 16) and may be responsible for Sertoli cell dysfunction under stress (17). A previous study reported that stress-induced Sertoli cell dysfunction is associated with zinc loss and loss of Sertoli cell ability for phagocytosis of residual bodies (17). While germ cells are shown to have limited intracellular antioxidant capacity, the testis is packed with an antioxidant defense system to protect spermatogenic cells during spermatogenesis. Although used to a limited extent in the testis, catalase (CAT) is involved in free radical scavenging (18). Furthermore, superoxide dismutase (SOD) is strongly expressed in the testis in addition to glutathione

peroxidase (GPx) and glutathione-s-transferase (GST), with the non-enzymatic antioxidant in the testis being reduced glutathione (GSH) (19). It is not completely clear how testicular elevated glucocorticoids influence antioxidants. In addition, constitutive cytokine expressions, including tumor necrotic factor (TNF-α), interleukin 1 beta (IL-1β), and caspases 3, 6, and 10 have been demonstrated in the testis, suggesting they play some roles in spermatogenesis (20).

The pentose phosphate pathway (PPP), otherwise referred to as the hexose monophosphate shunt, offeres an alternative pathway to the glycolytic pathway that generates pentose, ATP, and fat as well as metabolites and cofactors used for GSH synthesis (21, 22). Increased utilization of the PPP signals an increased demand for nucleotides for DNA repair (21, 22). The production of reactive oxygen species (ROS) through the activities of xanthine oxidase under Dex and probiotic treatments remains to be investigated. Therefore, changes in testicular redox status, cytokine expression, G6PD, and XO activity, uric acid, and DNA oxidation marker, 8-OHdG during Dex load and treatment with probiotics stand to further current understanding of the mechanism of high levels of glucocorticoids into reproductive failure and how probiotics can mitigate Dex effect on the testis.

MATERIALS AND METHODS

Animals care, and grouping, experimentation.

The institutional Ethics Committee approved procedures, which conform to the National Institutes of Health's standards (NRC 1985), were followed. Twenty adult Wistar rats (220-230) g were randomized into the control, Dex, Pro, and Dex+Pro groups. The control group was given 2 ml of normal saline, while Dex dissolved in saline solution was administered intraperitoneally to the Dex group at a dose of 4mg/Kg body weight. Probiotic supplementation was done by feeding Pro and Dex/Pro groups with 10×10^6 colony-forming units of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* every other day. Dex and probiotic treatments lasted for 21 consecutive days. Following ketamine anesthesia, blood samples were collected through cardiac puncture into plain bottles to obtain serum after spinning for 15 min at 3000×*g*. Ten percent of the right testicular homogenates (centrifuged for 15 minutes at 3000×*g*) were prepared in ice-cold phosphate buffer solution and were used for hormonal and biochemical assays.

Enzyme-Linked Immune Assay for Testosterone, FSH, and LH

Just before assays, reagents and samples were were allowed to acquired the room temperature (18~25℃). Hormonal assays were done with commercially available ELISA kits (Elabscience). To measure testosterone concentration in the samples, the standard working solution or sample (50 µL) was added to each well to which 50 µL of Biotinylated Detection Ab was immediately added, covered with the provided sealer, and incubated at 37℃. for 45 min. The well content was then aspirated, soaked for approximately 2 minutes in the wash buffer, the liquid drained, and cleaned with absorbent paper. Following three repeated washes, Horseradish Peroxidase (HRP) conjugate working solution (100 µL) was added to each well, covered, and incubated for 30 min at 37°C. This was followed by aspiration, five repated washes before adding Substrate Reagent (90 µL) to each well, and 15 minute incubation at 37°C, after which the Stop Solution (50 µL) was added. The optical density was read with a microplate reader at 450 nm, and testosterone concentrations were calculated. Following the manufacturer's instructions, FSH and LH concentrations were similarly measured.

Estimation of malondialdehyde (MDA)

Lipid peroxidation was quantified as previously reported (23). Briefly, the thiobarbituric acid-trichloroacetic acid-HCl reagent was first made, to which equal parts of the reagent and testicular homogenate were added and warmed for 15 minutes at 80 °C in water. The resulting reaction mixture was centrifuged at 10,000 rpm for 10 min at 4ºC. The amount of MDA generated was measured at an absorbance of 532 nm.

Measurement of Reduced Glutathione (GSH Level, Glutathione-S-Transferase (GST) Activities, Glutathione Peroxidase (GPx),

GSH concentrations were determined as earlier presecibed by Moron et al. (24). In comparison, The activity levels of GPx were quantified according to a previously reported method (25). Relying on the activity of glutathione-S-transferase on 1-chloro-2,4-dinitrobenzene as the second substrate, glutathione-S-transferase activity was assessed as previously published (26).

Estimation of SOD and CAT Activities

The activity levels of SOD activity were quantified as previously described (27). Summarily, samples were diluted 10 times with distilled water. To start the reaction, 0.3 mL of freshly made 0.3 mM adrenaline was added to the mixture, promptly inverted for thorogh mixing. The absorbance was read at 480 nm. Catalase activity was estimated as previously reported (28). The supernatant (16.6 µl) was added to the 1 mL of phosphate buffer (pH:7)-H2O2 solution in a cuvette. The reaction was recorded for 1 min at an absorbance of 240 nm to measure the decomposition rate of H2O2 spectrophotometrically.

Measurement of G6PD and Xanthine Oxidase Activities and Uric Acid Assay

The G6PD activity was quantified using a commercially availible assay kit (Elabscience, Wuhan, China), as described by De Angelis et al. (29). The principle involved oxidizing glucose-6-phosphate to generate a product that converts a nearly colorless probe to an intensely colored product whose optical density can be read at 450 nm. Adhering to the manufacturer's (Elabscience, Wuhan, China) instructions, Xanthine oxidase (XO) activity levels and uric acid concentrations were equally determined.

Measurement of Testicular Cytokines, Apoptosis, and Nuclear Oxidative Stress Markers

Using rat-specific enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Wuhan, China), quantitative measurements of testicular levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) and interleukin-1β (IL-1β), and tumor necrotic factor-alpha (TNF-α were made according to the kit manufacturer's instructions. Briefly, for TNF-α, 100µL of the standard or sample was added to the wells, incubated for 90 min at 37°C and the liquid discarded and the liquid was discarded, and 100µL Biotinylated Detection Ab working solution was immediately added to each well and further incubated at 37°C for 60 min. The plate was aspirated and washed three times. Then, 100µL HRP conjugate working solution was added and incubated for 30 min at 37°C, aspirated, and washed 5 times. Then, 90µL Substrate Reagent was added and incubated for 15 min at 37°C, after which the 50µL of Stop Solution was added. The absorbance was read at 450nm to determine the TNF-α concentration. Following the manufacturer's instructions, 8OHdG, IL-1β, and Fas/CD95 concentrations were similarly measured.

Assessment of Spermatid DNA Fragmentation

Spermatid DNA fragmentation was measured based on a previous report (30). Briefly, testicular tissue was homogenized in ice-cold PBS and centrifuged to obtain homogenization-resistant steps 17-19 spermatids to form pellets containing native DNA was re-suspended in 200

µL hypotonic lysis buffer to which 200 µL perchloric acid (0.5 M), 2 volumes of a solution containing 0.088 M diphenylamine, 98% V/V glacial acetic acid, 1.5 % V/V sulfuric acid and 0.5% acetaldehyde solution were added. The absorbance of the samples kept at 4°C for 24 hours was determined spectrophotometrically at 600nm. DNA FI was determined as OD 600 supernatant X 100 OD 600 supernatant + OD 600 pellet.

Zinc Assay Protocol:

Zinc concentration was measured colorimetrically with a zinc assay kit (Elabscience, Wuhan, China) according to the manufacturer's instructions. Briefly, sample depolarization was achieved by adding 50 µl of the 7% TCA solution to 50 µl each sample. Depolarized samples were centrifuged at 3000 rpm for 5 minutes before adding 20 µl of the samples to the plate wells. Then, 200 µl of zinc reaction mix was added to each standard and sample, incubated for 10 minutes at room temperature, and the absorbance read at 560 nm to calculate zinc concentration.

Sperm Analysis

The method for sper, parameter evaluation was modified from WHO manual (31). The epididymal fluid ratio of 1:20 was prepared by adding 0.1 ml of the fluid to 1.9 ml of water. After mixing the dilution thoroughly, the improved Neubauer hemocytometer with a Leica D750 microscope was used to determine the sperm concentration as previously reported with spermatozoa concentration expressed in millions/ml. Sperm motility was assessed by dropping epididymal fluid onto a glass slide such that the spermatozoa were evenly distributed, coverslipped, and examined under the light microscope at a magnification of ×20 using several fields. Sperm motility was classified as progressively motile (PM), non-progressively motile (nPM), or immotile (IM). The viability and sperm morphology were determined following staining with nigrosine-eosin and sodium citrate by mixing an aliquot (5 µl) of epididymal fluid of the stain microscope slide. The stained spermatozoa were considered dead, and the unstained live. Morphology was classified as normal or with head defects, neck defects, and tail defects. Under a 100× microscope objective, 100 randomly chosen spermatozoa were used for viability and morphology evaluations, and the results were expressed as percentages.

Evaluation of Daily Sperm Production

DSP was quantified based on an earlier report (32), which relies on homogenization-resistant spermatids. Briefly, 10% of each testis was homogenized in 0.154 M NaCl (50 ml), 0.5% Triton X-100 (v:v), 2% sodium azide, and eosin Y. The number of sperm nuclei was quantified using a hemocytometer and expressed as sperm $\times 10^6$/g testis per day.

RESULTS

Dexamethasone-disruption of Reproductive Hormones Improved by Probiotic Supplementation

Figure 1 shows changes in TT, FSH, and LH levels following stress exposure and probiotic treatment. All three hormones decreased significantly in the Dex group compared with the other groups. The Pro group maintained statistically similar levels of these reproductive hormones as the control, while the Dex/Pro group showed increased hormonal levels compared with the Dex group, so much so that the serum TT level in the Dex/Pro group was not statistically different from the control.

Dex-induced Prooxidant-antioxidant Imbalance Attenuated by PROBIOTIC supplementation

The Dex group had a very significantly higher MDA level compared with any other group, but treatment with probiotics significantly reduced the Dex/Pro group's MDA level compared with

the Dex group (Figure 2). Prooxidants parameters, SOD, CAT, and GSH, decreased significantly in the Dex group compared with the other group but treatment with probiotics brought GSH level and SOD activity to almost the control level. However, the significantly depressed serum and testicular Zn concentrations did not improve with probiotics treatment.

Probiotics Reduce the Increased Activation of the Pentose Phosphate Pathway During Stress

The activity of G6PD (Figure 3) increased significantly in the Dex group but decreased significantly in the Dex/Pro group compared with the Dex group.

Probiotic Attenuated the Dexamethasone-induced Cytokine Downregulation

The TNF-α and IL-1β expression decreased significantly in the Dex group but increased significantly in the Dex/Pro group compared to the Dex group, such that IL-1β was statistically at the same level as the control.

Probiotic Modulates Stress-induced Altered XO/Uric Acid Signaling

While the Dex group exhibited significantly higher XO activity in the Dex group (Figure 5), the uric acid levels in the group were significantly reduced. In addition, XO activity was significantly reduced, while uric acid was significantly increased in the Dex/Pro group compared with the Dex group.

Probiotics Decreased DNA Fragmentation and Oxidation During Dex Treatment

As shown in Figure 6, the Dex group had a significantly higher amount of DNA fragmentation compared to all other groups, but it was significantly reduced in the Dex/Pro group compared with the Dex group. The pattern of DNA oxidation estimated with the level of 8-OHdG is the same as that of DNA fragmentation.

Probiotics Attenuated the Apoptotic Pathway During Stress Exposure

Pro-apoptosis protein, Fas, is expressed significantly more in the Dex group compared with any other group (Figure 7), but the suppression is significantly reduced in the Dex/Pro group compared with the Dex group.

Probiotics Improved Sperm Parameters and Daily Sperm Production During Psychological Stress

Sperm concentration declined significantly by about 53% in the Dex group compared with the control. Treatment with probiotics alone increased sperm concentration, although not with a significant margine compared with control. In the Dex/Pro group, sperm concentration is significantly higher by about 35% compared with the Dex group. Vitality is significantly reduced in the Dex group by approximately 20% compared to the control. Furthermore, daily sperm production (DSP) decreased significantly in the Dex group by 60% compared with the control whereas, DSP increased significantly by 85% in the Dex/Pro group compared with the Dex group.

DISCUSSION

Psychological stress is an empirically documented risk factor for impaired male fertility that continues to attract scientific investigations to decipher the mechanisms involved and for the development of efficacious therapeutic management for stress-induced male infertility. Stress suppression of male fertility is known to be activated through the hypothalamic-pituitary-testicular (HPT) axis. A significant suppression of the pituitary

gonadotrophins (LH and FSH) in the Dex group was observed in this report, which was believed to be secondary to elevated glucocorticoids. Studies have shown that elevated glucocorticoids can suppress pituitary FSH and LH output.

The suppression of LH and FSH directly impacts testicular steroidogenesis and function. In the current study, TT levels dropped significantly following the Dex administration. As testosterone is secreted in response to LH binding to the androgen receptor on the Leydig cells, a corticosterone-induced suppression of LH will consequently limit the amount of TT that can be elaborated by the Leydig cells. Reduced FSH and TT levels directly impair spermatogenesis because both hormones are critical to Sertoli cell function and germ cell development.

Treating rats with probiotics significantly mitigated the impact of Dex on pituitary gonadotrophin release and testicular TT production, as seen in the Dex/Pro group. Previous reports have shown that gut microbiota modulates stress response and testicular function (33). Furthermore, stress has been shown to disrupt the gut microbiota (33). Augmenting the gut microbiota, as done in this study, boosts the gut ecology during stress and might explain the improved reproductive hormonal status of the animals in the Dex/Pro group.

Stress is energetically demanding in and of itself, as it represents demands for additional resource expenditure to maintain the organism's integrity in the face of environmental stimuli. The activation of the HPA axis is thought to arise to meet this demand by raising glucocorticoid levels to recruit metabolic resources. A consequence of increased energy production and utilization is the generation of free radicals as metabolic byproducts. As measured in this study, the MDA level, a proxy for lipid peroxidation by free radicals, became significantly higher in the Dex group relative to the other group, suggesting the existence of oxidative stress. Typically, there exists a balance in the levels of free radicals known as prooxidants and antioxidants; however, when more free radicals are produced than could be quickly removed by antioxidants, the cell recognizes a state of oxidative stress. Data from the current study show that Dex induces oxidative stress by producing excessive free radicals such as reactive oxygen species (ROS) and depleting the testicular antioxidant system. The reduced SOD and CAT activity levels plus the low level of GSH represent significant attenuation of testicular antioxidant response during stress. These antioxidants scavenge ROS such as O^{-} and H_2O_2 by donating electrons and reducing them to water. When this scavenging activity is diminished in the testis, Sertoli cell germ cell-nurturing capacity becomes impaired as their cell membrane, which maintains contact with the germ cells, is oxidized. Furthermore, during a state of oxidative stress, germ cells become very vulnerable to oxidative attacks, which may trigger cascades of events such as apoptosis and consequently lead to reduced sperm production, as seen in the DSP data in Table 1.

The rate-limiting enzyme in the PPP is G6PD. Branching from glycolysis, the PPP is a metabolic pathway that begins with glucose-6-phosphate and yeild glyceraldehyde-3-phosphate and fructose-6-phosphate (F6P), serving primarily to produce nicotinamide adenine dinucleotide phosphate (NADPH) and nucleotides critical to the antioxidant defense system against (ROS) and DNA synthesis To maintain cellular redox homeostasis; the PPP is activated in rapidly proliferating cells to supply ribonucleotides for DNA synthesis and repair caused by oxidative damage (34). As shown in

the current study, there was a significant elevation in the G6PD activity suggestion and increased utilization of the PPP, which further suggests increased demand for either or both nucleotides for DNA synthesis or repair. The trend in G6PD activity paralleled MDA levels and those of DNA fragmentation index and 8-OHdG. 8-OHdG provides a measure of DNA damage through oxidation

In the current investigation, treatment with Dex reduced sperm concentration by more than 50%, but when stressed animals were supplemented with probiotics, sperm concentration was only reduced by about 30%. Similarly, sperm progressive motility and normal morphology were significantly reduced in the Dex group but improved in the Dex/Pro group. Although the epididymis is often considered a sperm reservoir, it is during the transit through the epididymis that spermatozoa acquire their motility and fertility capacity, suggesting that changes continue to occur in spermatozoa after exiting the testis. Therefore, epididymal sperm concentration and quality can be influenced by epididymal cellular integrity. As no biological organ is immune to the negative effects of stress, it is possible that the hormonal disruption seen in this report had a causal effect on epididymal sperm quantity and quality.

The DSP analysis indicates that stress significantly reduced sperm production. DSP is often a measure of Sertoli cell efficiency. As noted concerning testicular redox status, increased ROS production can impair Sertoli cell capacity due to the lipid peroxidation activity of ROS. It has been shown that the Sertoli cell number is established just before puberty; consequently, DSP is fixed because the number of germ cells that each Sertoli cell can nurture is fixed. Nonetheless, DSP can decrease when Sertoli cells come under significant ROS attack, as seen in the current study, leading to functional reduction in their ability to support the maximum number of spermatogenic cells. Besides ROS-induced Sertoli cell dysfunction, ROS can directly attack spermatogenic cells, causing DNA oxidation, as demonstrated by the result of 8-OHdG in this study. DNA damage can initiate apoptosis and weed out spermatogenic cells with severely damaged DNA. The current study's analysis of Fas/CD95 shows that germ cells expressing Fas increased in number and most likely underwent apoptosis through the Fas/Fas L systems.

In this study, reduced Sertoli cell capacity and direct apoptotic elimination of spermatogenic cells are believed to have caused a significant reduction in DSP. This could partly explain the reduced epididymal sperm concentration, decreased progressive motility, and normal morphology in the Dex group. Animals treated with probiotics during stress showed significantly reduced ROS, 8-OHdG, and Fas/CD95, partly explaining the improved sperm parameters in the Dex/Pro group.

XO is a ROS-generating enzyme implicated in conditions such as cardiovascular injuries. XO catalyzes the hypoxanthine oxidation to xanthine and further converts the xanthine to uric acid, consequently generating O_2^- Studies have shown that XO activity significantly increased in certain brain regions of psychologically depressed patients, and XO has been reported to mediate cognitive impairment secondary to stress (35)

Increased utilization of the PPP leads to more purine production, producing xanthine as the substrate upon which XO can act. As observed in the current study, testicular attempts to mend oxidative damage to DNA by G6PD upregulation in the Dex group may be suboptimal when some of the purines generated through the PPP are hijacked by XO and converted to uric acid and free radicals. While

the current study cannot confirm this, its plausibility favors linking the significant DNA fragmentation and oxidation, despite significant G6PD activity, to increased XO activity. This is even more so with a significant elevation in testicular uric acid concentration. Uric acid is sometimes portrayed as having antioxidant capacity, yet the preponderance of evidence points to elevated uric acid as a product of cell death rather than optimization of the antioxidant defense system (36). Holding this view, the significant spike in uric acid level in the Dex group can be understood to erode testicular functional integrity further and contribute to spermatogenesis's overall impairment. On psychological well-being, it has been shown that the rise in uric acid level passively correlated with anxiety level in athletes, suggesting that elevated uric acid may not be helpful (37). Since the current investigation concurs with the previous report on the putative testicular expression of XO, its detrimental effect on testicular function is most likely due to its overexpression secondary to Dex-treatment. Thus, this investigation shows that Dex impairs spermatogenesis through the XO/uric acid pathway, possibly by hijacking part of the purines generated through the G6PD activity in the PPP. The immediate consequence is reduced available nucleotides for DNA synthesis and repair. Even in the absence of oxidative stress, XO oxidation of xanthine will create oxidative stress and supplant cellular ability for DNA repair.

The current study reveals that treating animals with probiotics significantly lowered XO activity during stress. This observation aligns with a previous study that demonstrated that administration of *Lactobacillus* strains has lowered XO expression and reduced depression symptoms in mice (38). Since increased XO activity is connected to high-stress reactivity, lowering it with probiotics might have promoted anxiolytic feelings in the animals, leading to reduced stress impact on the HPT axis.

TNF-α exerts negative effects on claudin-11 expression, a protein involved in the maintenance of the blood-testis-barrier. It has been reported that TNF-α through yet-to-be-deciphered mechanisms, promotes matrix metalloprotease-9 (MMP-9) transcription, causing the cleavage of the basement membrane collagen network, consequently impairing the Sertoli cell tight junction (39) An earlier report indicated that during sepsis in th rat, TNF-α suppressed steroidogenesis by inhibiting steroidogenic acute regulatory (StAR) protein (40). Furthermore, TNF-α has been shown to downregulate the Fas ligand, thus preventing germ cell apoptosis (41).

The current work is limited by being directly applicable to the animal model used because rat gut microbiota is significantly different from that of humans. Nevertheless, this work provides the basis for future studies to test the relevance and efficacy in humans by a clinical trial using appropriate probiotics as an adjunct to Dex to protect male reproductive function.

In conclusion, this work shows that probiotics significantly mitigate Dex-induced testicular dysfunction through mechanisms that involve the increased utilization of the PPP, leading to reduced DNA oxidation, increased GHS availability, and reduced pro-inflammatory and apoptotic markers.

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TABLES AND FIGURES

Table 1: Epididymal sperm parameters and DSP following Dexamethasone and probiotic treatment

	Ctrl	Dex	Pro	Dex/Pro
Sperm Conc ($\times 10^6$/ml	127.59±11.61	60.49±7.39*	132.52±10.64	81.37±8.05*#
Vitality (%)	93.74±7.09	75.02±7.21*	93.91±8.29	87.41±7.22
NM (%)	81.06±7.31	65.45±6.72*	85.37±6.37	76.28±6.90
PM (%)	70.82±6.91	36.39±5.27*	72.48±7.03	66.94±5.75*#
DSP ($\times 10^6$/g testis)	31.85±5.79	12.70±1.92*	32.94±6.08	23.51±5.92*#

* is significantly (P<.0.05) different from the control and other groups while # indicates a significant (P<0.05) from Dex. Ctrl- control, Dex= dexamethasone, Pro= probiotics, PM= progressive motility, NM= normal morphology, DSP= daily sperm production

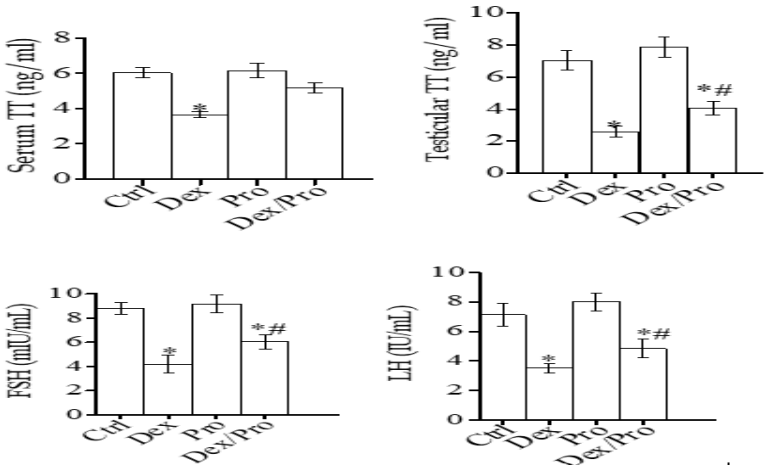

Figure 1: Testosterone and gonadotrophin levels following Dex and probiotic treatment. * is significantly (P<.0.05) different from the control and other groups. Ctrl- control, Dex= dexamethasone, Pro= probiotics

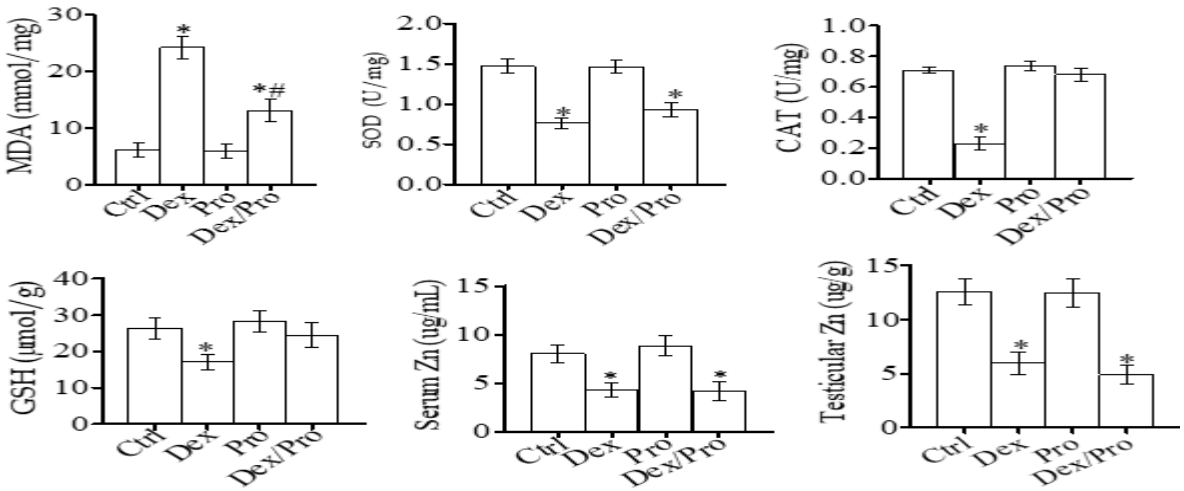

Figure 2: Testicular redox status and zinc levels following exposure to Dex and probiotic treatment. * is significantly (P<.0.05) different from the control and other groups while # indicates a significant (P<0.05) from Dex. Ctrl- control, Dex= dexamethasone, Pro= probiotics.

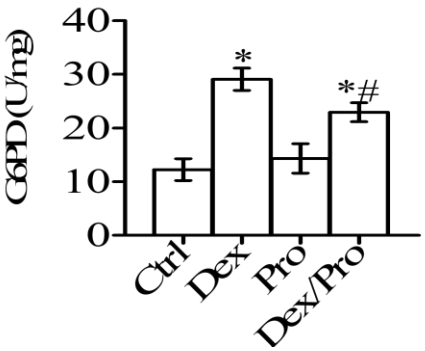

Figure 3: Testicular G6PD activity following Dex treatment and probiotic treatment. * is significantly (P<.0.05) different from the control and other groups while # indicates a

significant (P<0.05) from Dex. Ctrl- control, Dex= dexamethasone, Pro= probiotics

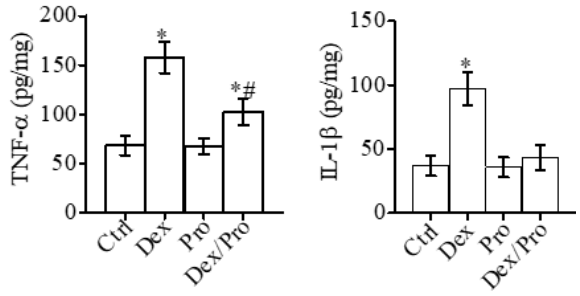

Figure 4: Testicular expression of cytokines following Dex and probiotic treatment. * is significantly (P<.0.05) different from the control and other groups while # indicates a significant (P<0.05) from Dex. Ctrl- control, Dex= dexamethasone, Pro= probiotics

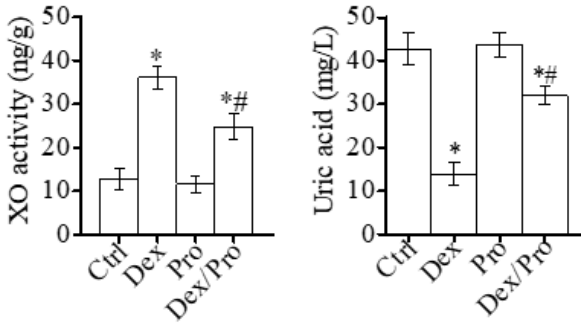

Figure 5: Testicular xanthine oxidase activity and Uric acid concentration following Dex and probiotic treatment. * is significantly (P<.0.05) different from the control and other groups while # indicates a significant (P<0.05) from Dex. Ctrl- control, Dex= dexamethasone, Pro= probiotics

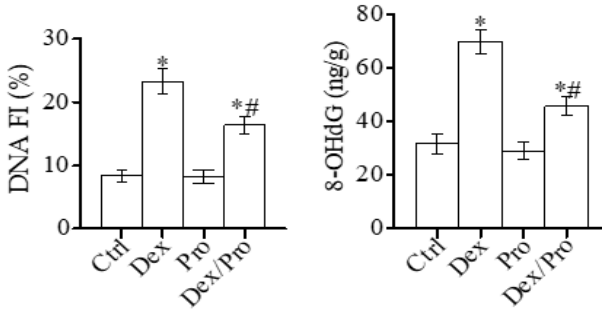

Figure 6: Spermatid DNA fragmentation and oxidation levels following Dex and probiotic treatment. * is significantly (P<.0.05) different from the control and other groups while # indicates a significant (P<0.05) from Dex. Ctrl- control, Dex= dexamethasone, Pro= probiotics.

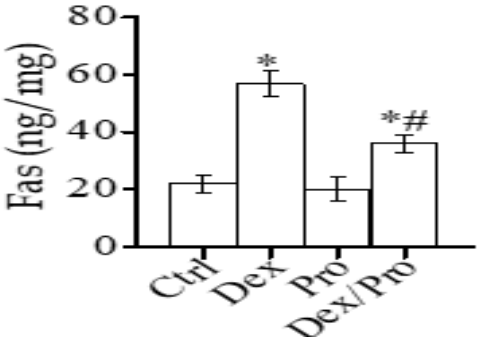

Figure 7: Testicular Fas expression following Dex and probiotic treatment. * is significantly (P<.0.05) different from the control and other groups while # indicates a significant (P<0.05) from Dex. Ctrl- control, Dex= dexamethasone, Pro= probiotics