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BIOCHEMICAL EVALUATION OF TESTOSTERONE PROPIONATE-INDUCED BENIGN PROSTATIC HYPERPLASIA IN WISTAR RAT

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ABSTRACT

The biochemical assessment of testosterone propionate (TP)-induced benign prostatic hyperplasia (BPH) in male Wistar rats was studied. Thirty-five mature male Wistar rats, with an average weight of 210 g, were randomly distributed into seven groups comprising five rats each. Male Wistar rats were induced by subcutaneous injection of various concentrations of TP. Group 1 received only food and water and was designated the control group, while Groups 2, 3 and 4 were respectively given 4, 6 and 9 mg kg^{-1}b.w of TP subcutaneously for 11 days. Groups 5, 6 and 7 received 4, 6 and 9 $mgkg^{-1}$ body weight respective doses for 21 days. The animals were fed with standard rat chow and clean water ad libitum. The animals in Groups 2, 3 and 4 were sacrificed at the end of 11 while Groups 2, 3 and 4 were sacrificed at the end of 21 days. Sperm morphology and characteristics were observed and measured. A variety of biochemical indices including oxidative stress markers were assessed. The volume (0.10 ± 0.00 ml) viability (86.5±11.5%), activity (47.5±12.5%), and sperm count ($56.0±12.3×10^6$) were significantly decreased ($p<0.05$) in group 2 when compared with the respective values obtained in group 1. Inducement with different concentrations of TP significantly ($p<0.05$) increased abnormal sperm features observed in group 2. Superoxide dismutase (0.60 ± 0.01 U/L) was significantly increased ($p<0.05$) in group 2, but the values were increased beyond normal after inducement with varying concentrations of TP for 21days especially in the tissue homogenate compared with the values of the antioxidants parameters in the blood. The biochemical and morphological changes observed in the serum and testis of rats in the study may have been caused by the administration of testosterone propionate.

Keywords: Testosterone propionate, Benign Prostatic Hyperplasia, Testis, Hypogonadism, infertility.

INTRODUCTION

Testosterone is a key male sex hormone that plays a significant role in the body by controlling libido, semen production, muscle mass, bone mass, and strength (Netam *et al*., 2023). As males age, there is a slow and continual reduction in testosterone levels, prompting a condition called hypogonadism (gonadal and hypothalamic-pituitary axis failure) (Rey, 2022). The decrease in the levels of testosterone is responsible for the age-related impairment in spermatogenesis, which results in infertility (Sharma *et al*., 2021; Pedestrian, 2021; Netam *et al*., 2023). The treatment of hypogonadism or low androgen concentration

involves testosterone supplementation treatment (TST) to normalize serum testosterone levels (Chioma and Cappa, 2021; Suare *et al*., 2021; Netam *et al*., 2023). Testosterone propionate (TP) is an effective testosterone ester injectable compound basically utilized as a testosterone supplementation therapy agent for the treatment of low testosterone levels in men. Current research has recommended that testosterone therapy further improves sexual activity, libido, erectile function, and fertility (Rizk *et al*., 2017; Netam *et al*., 2023). A few scientists have suggested that administering exogenous testosterone in various forms of TST may be unsafe for the liver, lower impact of insulin on lipid metabolism, and increase the threat of cardiovascular disease, stroke, and prostate cancer (Yabluchanskiy and Tsitouras, 2019; Linhares *et al*., 2022; Netam *et al*., 2023). Consequently, an alternative to TST is needed, ideally one that boosts endogenous testosterone production to standardize steroidogenic function in aging human males (Khodamoradi *et al*., 2021; Netam *et al*., 2023). The utilization of natural products in potentiating the male reproductive system has been connected with human civilization since antiquity (Rahimi *et al*., 2022). Everyman stands a danger of prostate issues because all men have a prostate (Onyegeme-Okerenta *et al*., 2022). The most common prostate problems are prostatitis, benign prostatic hyperplasia (BPH) and prostate cancer. In males under the age of 50, prostatitis is the most common prostate problem (Cui R *et al*., 2017). A number of studies have detailed that there is a potential risk of developing BPH as men grow older. Despite the fact that the pervasiveness of BPH differs because of variations in geographic regions and diagnostic methods (Egan 2016), it affects approximately 50% to 70% of males over the age of 50. By age 80 or older, this percentage rises to 90% (Napalkov *et al*., 1995). Pelvic discomfort is the most widespread symptom associated with prostatitis (Zhang *et al*., 2021).

Benign prostatic hyperplasia (BPH) is characterized by non-malignant enlargement of the prostate. It entails an increase in the quantity of both stromal and epithelial cells in the transitional area of the prostate and can lead to lower urinary system tract symptoms, which includes frequency, urgency, dysuria, suprapubic pain and urinary incontinence (Wang *et al*., 2020). It is estimated that 50% of males will have histologic evidence of BPH by age 50 and 75% by age 80. It has likewise been reported that in about 40 to 50% of these men, BPH becomes clinically significant (Liao *et al*., 2016; Kaufman *et al*., 2019). BPH also increases the risk of developing lower urinary tract symptoms (LUTS) (Li *et al*., 2018).

There is no recognized anti-BPH medicine that can totally heal BPH without severe negative effects. Consequently, efforts to foster brand-new drugs have been on the rise. To test new anti-BPH drugs, animal models are needed for in vivo efficacy research studies. The idea that androgens are important for the maintenance of prostate disease directs the standard of care for BPH (Makary *et al*., 2018; Netam *et al*., 2023). Nevertheless, to our knowledge, there are no experimental reports available on the enhancement of the reproductive system by the activity of testosterone propionate (TP) in animals or humans, whether young or aged (Netam *et al*., 2023). Thus, this current study was designed to study the biochemical implication of TP-induced BPH on various reproductive parameters in male Wistar rats.

MATERIALS AND METHODS

Experimental Design and Animal Study

Thirty-five male Wistar rats weighing between 150 to 250 g were obtained from the Animal farm of the Department of Biochemistry, University of Port Harcourt, Choba, Rivers State, Nigeria and were acclimatized for 15 days. The animals were given food and water ad libitum and were kept in an environment with ambient temperatures of 25 ± 3º C, relative humidity of 60 ± 5%, and a 12-hr light/dark cycle.

Testosterone Propionate (A high-grade SB (Batch number 47) was procured from Sigma-Aldrich, US. The rats were randomly divided into 5 groups of 5 animals each based on their body weight. The dose of TP was selected based on earlier studies (Jeremy *et al.,* 2019; Sulistyoningrum 2017; Cui *et al.,* 2017). Group 1 received only food and water and was designated the control group, while Groups 2, 3 and 4 were respectively given 4, 6 and 9 mg kg^{-1}b.w of TP subcutaneously for 11 days. Groups 5, 6 and 7 received 4, 6 and 9 mg kg^{-1}b.w of TP subcutaneously for 21 days. The animals in Groups 2, 3 and 4 were sacrificed at the end of day 11 while Groups 5, 6 and 7 were

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sacrificed at the end of day 21. Normal saline and water, and sesame oil were used as vehicles for administering TP. The detailed experimental design is given in (Table 1).

The behavioral and body weight changes were monitored throughout the experiment. At the end of the experiment, the animals were sacrificed by ethyl ether inhalation. The testes and other accessory reproductive organs (epididymis, prostate, and seminal vesicle) were quickly dissected and washed with ice-cold 0.9% normal saline. The organs were weighed and transferred to sterile containers and stored at -70^{o} C for tissue homogenate study. Blood samples were obtained by cardiac puncture, and serum was collected and stored at – 20^{o} C until required for further analysis. C-reactive proteins (CRP) and Prostate Specific antigen (PSA) were assayed using serum and rat-specific ELISA kits (Elabscience, China), following the kit manufacturer's instructions.

Sperm Parameters

The epididymal sperm count assessment was done using the hemocytometer method with certain modifications (Widyastuti *et al.,* 2020). Briefly, the outer covering of the cauda epididymis was removed, minced, and homogenized in 1 ml of 0.9% NaCl and 0.05% triton-X solution. The resultant sperm suspension was then diluted 10 times and centrifuged at 8000 RPM for 2 min. The homogenate (10 µl) was placed in a hemocytometer and sperms were counted at 400 × magnification.

For the assessment of sperm abnormalities, a piece of the cauda epididymis was minced, 1 ml of 0.9% saline and 1 ml of 10% neutral buffer saline were added. The suspension was further diluted with water followed by the addition of 1 ml of eosin stain (1%) and incubated at room temperature for 1 hr. The sperm smear was prepared by pouring a drop of the suspension on the slide and examined at 400 × magnification using a trinocular light microscope (Olympus Microscopes, Tokyo, Japan). On each slide, 200 sperm cells were analyzed for various head and tail abnormalities, and results were expressed as percentage abnormalities. To measure sperm motility, the distal end of the epididymis was removed and minced in 2 ml of Dulbecco's phosphate buffer saline and was kept at 36-38 ^{o}C for further analysis. The minced cauda was placed in a water bath and kept for 1-5 min to disperse the sperms. The sperm suspension (5-10 µl) was placed into the counting chamber, and the number of motile sperms were counted. The haemocytometer was then placed at 40-50 ^{o}C for 1 min to kill the sperm, and the total number of dead sperms were counted. The results were presented as percentage motility (Aksu *et al.,* 2021).

Antioxidant Assay on Serum and Testis Homogenate

A 10% (w/v) phosphate buffer (pH 7.4 + 150 mM KCl) ice-cold solution was used to homogenize the testis. The lipid peroxidation assay using malondialdehyde (MDA) and reduced glutathione (GSH) were determined from one part of the homogenate while another part of the homogenate was centrifuged at 9000 RPM to obtain the supernatant (S9) fraction. The S9 fraction was used to calculate total protein, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) concentrations. The LPO and GSH levels in the testis were estimated by the previously used methods (Bhattacharjee *et al.,* 2014; Selmi *et al.,* 2015; Chen *et al*., 2022). The activities of SOD and CAT were measured by the protocols described by Mohammadi *et al.,* (2020) and Grami *et al.,* (2020) while GPx, GST, and GR were assessed by the methods described by Grami *et al.,* (2020) and Shah *et al.,* (2017).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 20.0. The data were analysed using one-way analysis of variance (ANOVA) and expressed as mean ± standard error of the mean (SEM). Significant differences were

determined using the Post Hoc least significant difference tests for multiple comparison at $p < 0.05$.

RESULTS

Sperm Parameters

The results showed a significant ($p < 0.05$) decrease in the epididymal sperm counts in the TP administered groups as compared to the control group. The injection of TP significantly reduced sperm cells in groups 3 (22.0±5.10), 5 (22.10±4.90), 6 (37.50±11.50) and 7 (24.0±3.90%). A significant ($p < 0.05$) decrease in the sperm motility was observed in groups 3 (10.00±0.20%) and 6 (15.00±2.10%) when compared to the control group (56.7±8.04%). Testosterone propionate administration also showed a non-significant ($p > 0.05$) decrease in sperm activeness in group 2 (47.50±12.50%) compared to group 1 (48.70±6.10%). The results of sperm abnormalities are summarized in (Tables 1) which shows the various types of sperm head and tail abnormalities. The results show a significant decrease in sperm abnormality with various head and tail abnormalities in the other groups except group 2 (5.5±0.20%), indicating the deleterious effect of TP. The TP-treated groups showed lower sperm abnormalities when compared to the control group. Similar trends were observed for groups where lower sperm head and tail abnormalities were observed as compared to the normal group.

Serum inflammatory markers and Antioxidant enzymes

The results from Figures 1 and 2 showed the C - reactive protein (CRP) and Prostate Specific Antigen (PSA) levels respectively. There was a significant increase ($p <0.05$) in PSA levels for groups 2 to 7 with respect to group 1. The findings revealed a significant ($p< 0.05$) increase in the concentrations of CRP and PSA in the serum but impacted mostly on the PSA in the exposure groups in comparison to the control group. TP inducement significantly elevated PSA concentrations in the serum.

Several inhibitory responses to the testicular antioxidant status were observed on TP exposure (Figures 3 and 4). The rats exposed to TP had significantly ($p < 0.05$) increased levels of the antioxidant enzymes SOD, CAT, and non-enzymatic antioxidant GPX and MDA in both blood and testis homogenate (Figures 5 and 6) when compared to the control group (group 1).

Table 1: **Semen Analysis of male Wistar rats induced with Testosterone Propionate**

Groups	motility	Active	Sluggish	Dead	TSC	Head Def	Mid piece	tail	Via	Test
1	56.7±8.04	48.7±6.1	8.0±0.6	43.3±4.1	66.0±7.9	3.0±0.2	1.7±0.03	2.7±0.1	92.7±11.1	5.6±0.2
2	54.5±10.2	47.5±12.5	3.0±0.2*	45±6.3	56.0±12.3	5.5±0.2	2.5±0.06	5.5±0.3#	86.5±11.5	7.6±1.0
3	10.0±0.2*	7.5±1.1*	2.5±0.01*	40.0±11.0	22.0±5.1*	0.0±0.0*	1.0±0.001	2.0±0.003	48.5±10.1*	4.3±0.05
4	50.0±9.2	30.0±7.7*	20.0±4.3#	50.1±11.2	73.2±12.1	3.2±0.03	1.1±0.01	1.0±0.01*	94.2±15.2	7.4±0.5
5	41.5±11.1	33.5±6.2*	8.0±0.8	58.5±12.11	22.1±4.9*	3.0±0.06	4.5±0.12#	0.01±0.0*	92.5±14.5	7.6±1.2
6	15.0±2.1*	12.5±1.2*	2.5±0.04*	85.0±15.0#	37.5±11.5*	1.5±0.02*	4.5±0.03#	0.02±0.00*	94.0±15.0	10.1±1.5*
7	0.0±0.0*	0.0±0.0*	0.0±0.0*	100.1±12.1#	24.0±3.9*	1.0±0.01*	3.0±0.03#	1.0±0.01*	95.1±12.3	9.5±1.2*

Values expressed as mean±SEM; n=5 * $p< 0.05$ significantly low when compared to the control (Group 1); # $p< 0.05$ significantly high when compared to the control.

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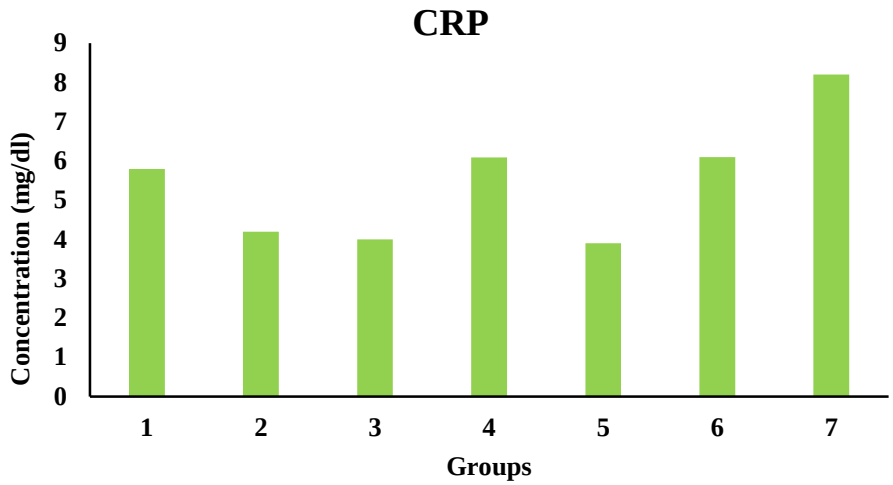

Figure 1: C-Reactive Protein levels of male Wistar rats induced with TP

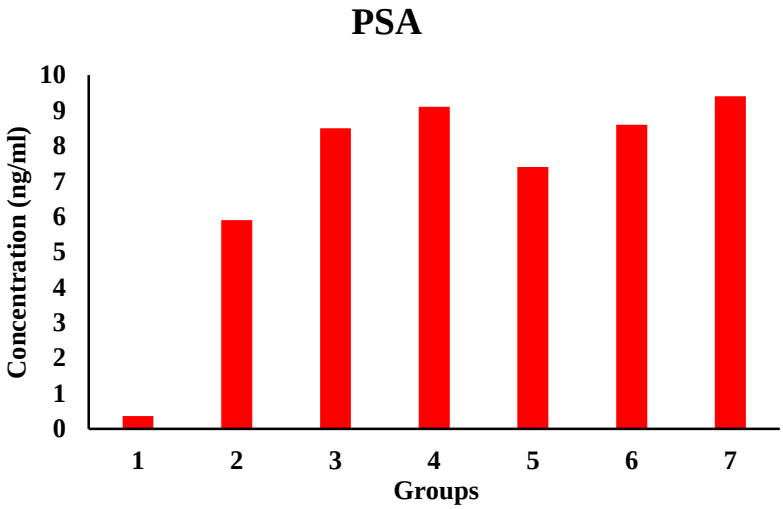

Figure 2: Prostate Specific Antigen levels of male Wistar rats induced with TP

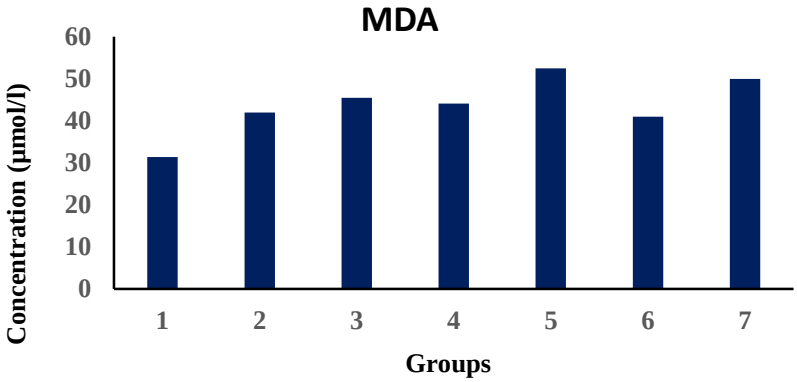

Figure 3: Lipid peroxidation marker in the serum of male Wistar rats induced with TP

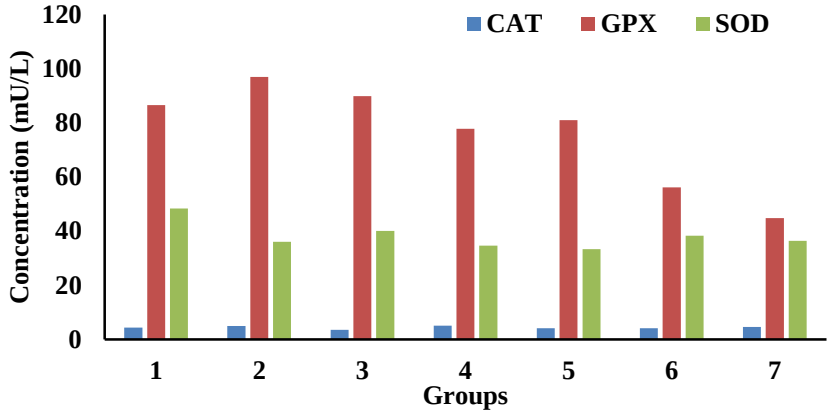

Figure 4: Oxidative stress enzyme marker levels in the serum of male Wistar rats induced with TP

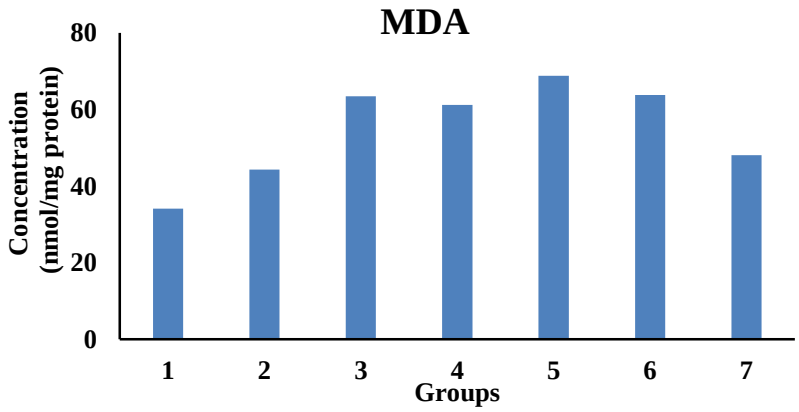

Figure 5: : Lipid peroxidation marker in the testis homogenate of male Wistar rats induced with TP

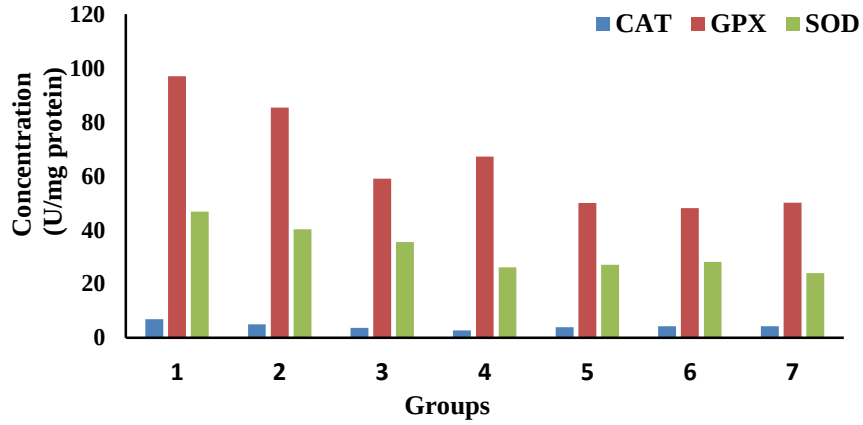

Figure 6: Oxidative stress enzyme marker levels in the testis homogenate of male Wistar rats induced with TP

DISCUSSION

Sperm cell analysis is the most reliable technique for the detection of male reproductive disorders. Our results indicated that direct exposure to testosterone propionate resulted in a reduction in sperm count, and an increase in the number of sperm cells with abnormal morphology. The decrease in sperm count might be triggered by testosterone propionate-induced oxidative stress, which

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might prompt a low androgen hormone production triggering germ cell apoptosis (Walker, 2021; Netam *et al*., 2023). The rise in sperm abnormalities were potentially because of an increase in peroxidative damage to the sperm membrane as it is made up of lipids with high levels of sphingomyelin, polyunsaturated fatty acids and plasmalogen. Excessive reactive oxygen species (ROS) production might lead to a loss of DNA and membrane integrity in spermatozoa, hence hindering their fertilization capability. The results of this study on low sperm count, motility, increased sperm abnormalities, and the toxic effects of TP are simultaneous with several prior reports.

After direct exposure to testosterone propionate, the antioxidant enzyme activities (SOD, CAT, GPx, MDA) and PSA levels increased with a significant increase in SOD levels. It is well-documented that the male reproductive organs are particularly prone to the damaging impacts of ROS. The enhanced SOD level could be because of TP-mediated oxidative damage to the testicular membrane layer due to similarly higher unsaturated fatty acid levels in this tissue than others. The decreased levels of first-line antioxidant defence enzymes – SOD, CAT, and GPx – in the tissue homogenate reflects much less testicular mitochondria and microsome capacity to eliminate H_2O_2 produced by TP in oxidative stress. GSH and GPx are the other prime antioxidant enzymes that have important roles in cellular defence from oxidative stress. The TP-induced rat aging model showed a decrease in several enzymatic and non-enzymatic antioxidants which may be as a result of increased oxidative stress (Netam *et al*., 2023; Onyegeme-Okerenta *et al*., 2023). These results show an imbalance of antioxidants with a rise in the production of free radicals which may be responsible for numerous observed degenerative changes in the testis. The result findings correlate with a research study by Netam *et al*., (2023) which revealed that D-gal caused a decrease in antioxidant enzyme activities and caused numerous testicular histopathological impairments. The findings from this work suggest that TP exerted harmful effects on rat testis via increased oxidative stress, triggering several testicular structural damages. Earlier research has additionally shown various structural changes such as decreased epithelium layers, damaged seminiferous tubules, and decreased spermatogenic cells in histopathological examinations of mice subjected to D-gal (Taba *et al*., 2019; Netam *et al*., 2023). Changes in male reproductive systems caused by D-gal, such as testicular damage, reduced sperm count, and minimized androgen production with increased serum LH and FSH, are like normal ageing (Gunes *et al*., 2016). Based on the present findings, it can be presumed that exposure to TP triggered several alterations in the male reproductive system via increased production of ROS, enhanced inflammation cascade, and minimized androgen production. The TP inducement increased the serum CRP and PSA levels. This could likewise be interpreted as an increased testosterone level. This may be due to increased steroidogenesis, as evident from increased activities of PSA. Our research study supports previous research findings that testosterone therapy could trigger several adverse effects like symptoms of benign prostatic hypertrophy, liver toxicity, hyperviscosity, erythrocytosis, sleep apnoea, or cardiac arrest (Yabluchanskiy and Tsitouras, 2019).

CONCLUSION

The study shows that testosterone propionate caused several reproductive impairments through increased oxidative stress and decreased testosterone synthesis, affecting sperm count, motility and morphology. TP reduced oxidative stress in tissue homogenate, and decreased sperm count. From the findings, it could be suggested that maintaining androgen levels through exogenous testosterone treatment may help in sustaining gonadal activity. According to this study, the testosterone level endogenously increased antioxidant activity but did not restore normal spermatogenesis. Further study is needed to ascertain the various target protein of TP to

elucidate its exact mechanism of action. The study concludes that testosterone propionate affects the overall semen profile.

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