ORIGINAL ARTICLE

EFFECTS OF PHOSPHODIESTERASE 5 INHIBITOR (VIAGRA) ON BIOCHEMICAL PARAMETERS OF L-NAME-INDUCED TESTICULAR TOXICITY IN ADULT MALE WISTAR RATS

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ABSTRACT

Testicular toxicity is a growing concern in today's world, with various factors contributing to its prevalence. Nitric oxide (NO) imbalance, often induced by N (gamma)-nitro-L-arginine methyl ester (L-NAME), is a significant factor associated with testicular dysfunction. Sildenafil (Viagra), a phosphodiesterase type 5 inhibitor, has shown promise in improving testicular function by modulating NO levels. This study aimed to investigate the role of Sildenafil (Viagra) on biochemical parameters of L-NAME-induced testicular toxicity in Wistar rats. Twenty-four adult male Wistar rats were divided into four groups: Control (physiological saline-treated), L-Name (L-Name-induced testicular toxicity), PDE (Sildenafil-treated), and L-Name + Sildenafil (co-treatment) and subjected to a 56-day treatment regimen. At the end of the administration, the animals were sacrificed, tissues collected and biochemical and histological assessments were performed. Findings revealed that L-Name administration led to a significant decreased in nitric oxide levels, follicle stimulating hormone, luteinizing hormone, testosterone and increase in oxidative stress when compared to the control group. Furthermore, histological analysis demonstrated structural alterations in the testes of L-NAME-treated rats, indicative of testicular toxicity. Rats treated with Sildenafil showed a slight reversal of these adverse effects. Also, slight reversals of impaired spermatogenesis were evident in the co-treatment group. This study provides compelling evidence for the potential therapeutic role of sildenafil in ameliorating L-NAME-induced testicular toxicity in adult male Wistar rats.

Keywords: Testicular toxicity, spermatogenesis, oxidative stress, testosterone.

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Conflict of Interest: None is declared

INTRODUCTION

Testicular toxicity is a major concern for male reproductive health since it can affect spermatogenesis and cause fertility problems. Several factors, including environmental contaminants and pharmacological agents, have been identified as potential causes of testicular toxicity (1). One such agent is N(gamma)-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor that has been employed in experiments to produce testicular toxicity (2). L-NAME treatment has been demonstrated to impair testicular function by blocking Nitric Oxide synthase (NOS), resulting in oxidative stress, inflammation, and hormonal abnormalities (3). L-NAME does not exist naturally in biological systems and was specially designed as a Nitric oxide synthase inhibitor for scientific purposes. Reactive nitrogen species, such as nitric oxide (NO), are important molecules in both physiological and pathological processes. In men, NO is important in sperm capacitation, acrosome response, steroidogenesis, and erectile function. Furthermore, NO modulates the blood-testis barrier and the interaction of germ cells with Sertoli cells (4).

Phosphodiesterase 5 (PDE 5) inhibitors such as sildenafil, tadalafil, and vardenafil are commonly used to treat erectile dysfunction (ED) (5). These drugs work by inhibiting the breakdown of cyclic guanosine monophosphate (cGMP), an important intracellular signaling molecule involved in smooth muscle relaxation and vasodilation (6). In addition to their effects on erectile function, PDE 5 inhibitors have been found to have protective properties in various organ systems, including the cardiovascular, nervous, and renal systems (7, 8). However, their potential role in mitigating testicular toxicity induced by L-NAME has not been extensively studied.

Limited research suggests that the PDE 5 inhibitor, sildenafil (Viagra) may offer protective effects against testicular toxicity. For example, in animal models of testicular toxicity, sildenafil has been demonstrated to enhance spermatogenesis, reduce oxidative stress, and restore hormonal balance (9).

Elevated levels of cGMP are thought to mediate these effects by strengthening antioxidant defense mechanisms and promoting the survival of testicular cells (10, 11). It is unknown, therefore, what precise pathways this PDE 5 inhibitor uses to protect against L-NAME-induced testicular damage. Furthermore, not enough research has been done on the effects of various PDE 5 inhibitors and how they compare in terms of biochemical indicators. Thus, the purpose of this investigation was to ascertain how the medication sildenafil (Viagra) affected the biochemical markers of testicular damage caused by L-NAME in Wistar rats.

MATERIALS AND METHODS

Animals: Twenty-four (24) male Wistar rats of average weights of 175 g were used. The rats were housed in Four (4) standard metabolic cages in the Animal House of the college of Health Sciences, University of Ilorin, Nigeria. The cages were well-ventilated, clean, secured from predators, in a suitable environment, and with a removable waste disposal. The animal beddings were changed always to prevent the animals from feeding on it. All groups were fed with 100 g/cage of grower mash feed (pellets) and about 200 ml of water. Water intake remained constant. The rats were maintained at room temperature (25 ± 20°C and humidity of 65 ± 5% with free access to normal pelleted standard rat diet and water which were changed every day. They were maintained as well in a 12-hour cycle of light and dark. The University of Ilorin's Guide for the Care and Use of Laboratory Animals was followed when treating the animals. Additionally, the Declaration of Helsinki's guiding principles for animal research as well as the Guiding Principles for the care and use of animals were applied. The twenty-four (24) male Wistar rats were divided into four (4) groups (A-D), with six (6) rats in each group.

Dosage and administration: Group A served as a control group and was administered 0.5 ml of physiological saline daily. The group B served as Sildenafil (Viagra) group and received 50 mg/kg bw of Viagra solution daily. Group C served as the L-NAME group and received 20 mg/kg bw of L-NAME solution daily while group D served as Sildenafil (Viagra) co-treated with the L-NAME group and received 50 mg/kg bw and 20 mg/kg bw of Viagra and L-NAME solutions respectively. All administrations were done orally using a cannula for 56 days.

Tissue collection and sample preparation: After the last day of administration, the rats were sacrificed by anesthetized with 80 mg/kg of ketamine hydrochloride. Blood was withdrawn from the apex of the heart (left ventricle) and dispensed in red-topped sample bottles. After the collection of the blood sample, whole-body perfusion was done with 10ml of normal saline and 10ml of neutral buffered formalin

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successively for fixation. The testes were identified, excised, and fixed in neutral buffered formalin.

Biochemical analysis: Testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and Glutathione peroxidase (GPx) levels were determined using an ELISA kit. Nitric oxide (NO) was measured by non-enzymatic colorimetric assay.

Histological analysis: The testes tissues were fixed in neutral buffered formalin for 48 hours and then embedded in paraffin. Sections of 5µm thickness were prepared, attached to slides, and deparaffinized with hematoxylin and eosin stain (H & E) for histological examination. Histological sections were viewed through a light microscope and photographed using a USB Amscope camera and then comparisons were made between the control and other groups. Pictures were taken at a magnification of x100.

Statistical Analysis

Version 8.0.2 of GraphPad Prism was utilized for all statistical analyses. The student's t-test was used to examine group differences, and all results were reported as Mean ± SEM. A P value of less than 0.05 was deemed statistically significant

RESULTS

Effects of Sildenafil and L-NAME on Glutathione Peroxidase (GPx) Levels

The mean value for glutathione peroxidase (GPx) concentration was significantly decreased in the L-NAME-only treated group ($p<0.05$) when compared with the PDE-only treated group and the group co-treated with both L-NAME and PDE. Moreover, a significant decrease in glutathione peroxidase (GPx) concentration was observed in the L-NAME-only treated group ($p<0.01$) when compared with the control group. No significant difference in glutathione peroxidase (GPx) concentration was seen among the control, PDE-only treated group and the group co-treated with both L-NAME and PDE (Figure 1).

Effects of Sildenafil and L-NAME on Luteinizing Hormone (LH) Levels

As shown in figure 2, there was a significant decreased ($p<0.05$) in luteinizing hormone (LH) concentration in all the treated groups (PDE only, L-NAME only and L-NAME and PDE co-treated groups) when compared with the control group. Moreover, no significant difference was seen among the treated groups.

Effects of Sildenafil and L-NAME on Follicle Stimulating Hormone (FSH) Levels

Mean values of FSH concentration among experimental groups has shown in figure 3 revealed a significant decreased ($p<0.05$) in follicle stimulating hormone (FSH) concentration in all the treated groups (PDE only, L-NAME only and L-NAME and PDE co-treated groups) when compared with the control group. Moreover, no significant difference was seen among the treated groups (Figure 3)

Effects of Sildenafil and L-NAME on Testosterone Levels

Mean values of testosterone concentration among experimental groups revealed significant increase ($p<0.01$) in testosterone concentration in PDE only treated group when compared with the control, L-NAME only and group co-treated with L-NAME and PDE. Furthermore, no significant difference was seen among the control, L-NAME only treated group and group co-treated with L-NAME and PDE (Figure 4).

Effects of Sildenafil and L-NAME on Nitric Oxide (NO) Levels

Has revealed in figure 5, the mean value for nitric oxide (NO) concentration was significantly decreased ($p<0.05$) in all the treated groups (PDE only, L-NAME only and L-NAME and PDE co-treated groups) when compared with the control group. Moreover, no significant difference was seen among the treated groups.

Histological analysis

Figure 6 depict the histoarchitecture of the testes of Wistar rat in groups A, B, C and D stained with H&E. The testicular slides of groups A and B, which were treated with PDE, showed no abnormalities. These groups' characteristics included normal spermatogenic cell development, Leydig cell presence in the interstitial spaces, and spermatozoa in the lumen.

Group C, which received L-NAME treatment, showed multiple visible degenerative alterations, including fragmented basement, pyknotic Leydig, enlarged lumen which is devoid of spermatozoa, and maturation arrest of spermatogenic cell line in multiple seminiferous tubules.Furthermore, group treated with co-administration of L-Name and PDE showed well-structured testicular histoarchitecture characterized testicular histoarchitecture, defined by the presence of Leydig cells in the interstitial spaces, Sertoli cells at the lumina border, spermatozoa-filled

seminiferous tubule lumens, and a large number of spermatogonia cells that have developed into many spermatocytes in seminiferous tubules.

DISCUSSIONS

N(Gamma)-nitro-L-arginine methyl ester, commonly known as L-NAME is a class of compounds known as nitric oxide synthase (NOS) inhibitors. Nitric oxide synthase is an enzyme responsible for the production of nitric oxide (NO) in the body. Nitric oxide is a signaling molecule that has a variety of physiological uses, such as immune response modulation, neurotransmission, and vasodilation (blood vessel relaxation). L-NAME and other nitric oxide synthase inhibitors are used to investigate the function of nitric oxide in various biological processes. By competing with L-arginine, the substrate for nitric oxide synthase, L-NAME selectively suppresses the synthesis of nitric oxide. Because L-NAME inhibits NOS, it prevents nitric oxide from being synthesized. Testicular toxicity has been linked to this inhibition.

The primary mode of action of phosphodiesterase 5 (PDE5) inhibitors is the inhibition of the PDE5 enzyme. PDE5 plays a role in the degradation of cyclic guanosine monophosphate (cGMP), a signaling molecule. These drugs raise cGMP levels by inhibiting PDE5, which causes vasodilation and an increase in blood flow. Given that cGMP is a nitric oxide's downstream mediator, PDE5 inhibitors have been shown to have an indirect impact on nitric oxide signaling. Testicular function is influenced by nitric oxide, and elevated amounts may be beneficial. Therefore, using adult male Wistar rats, this study examined the potential functions of this PDE 5 inhibitor in modifying the biochemical parameters linked to L-NAME-induced testicular damage.

This present study showed that L-NAME cause a significant decreased in GPx, LH, FSH, testosterone and NO concentration following 20 mg/kg bw administration of L-NAME only for 56 days. L-NAME administration impairs the activities of antioxidant enzymes, such as glutathione peroxidase (GPx), further exacerbating oxidative stress (12). These findings are in accordance with santos et al, 2020 work where they reported increase in oxidative stress parameters after the use of L-NAME The study's findings suggesting a decrease in GPx concentration could be the consequence of L-NAME's inhibitory actions on NO concentration. Reduced nitric oxide, which is generated by NOS and possesses antioxidant and vasodilatory qualities, lowers antioxidant defenses. Free radicals can be scavenged by NO, which also shields cells from oxidative damage. GPx is associated with multiple facets of male reproductive health and is an essential marker of oxidative stress (13). Testicular tissues are particularly susceptible to oxidative stress because their cell membranes contain a large amount of polyunsaturated fatty acids. Oxidative stress arises when the delicate balance between the production of reactive oxygen species (ROS) and antioxidant defense systems is disrupted (14). The testes, which secrete hormones and produce sperm, are vulnerable to oxidative stress, which can have a deleterious effect on reproductive health and fertility in general. (15).

In addition to oxidative stress, L-NAME disrupts hormonal balance in the testes. This study found a significant decrease in the concentrations of testosterone, LH, and FSH. These results were in line with a study by Zhang et al 2014, that claimed L-NAME-induced testicular toxicity has a substantial impact on testosterone, the main male sex hormone required for spermatogenesis. When L-NAME is administered, testosterone levels are lowered and other hormone levels, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are altered (16).

Additionally, the group that only received oral Sildenafil (Viagra) at a dose of 50 mg/kg bw caused a significant decrease in GPx, LH, FSH, and NO levels as well as an increase in testosterone concentration when compared to the control group. However, there was a variable degree of increase in these parameters when comparing the L-NAME-only treated group with the Sildenafil (Viagra) only group. The oxidative effects of sildenafil in testicular tissues are shown in the reduction of GPx concentration, which is suggestive of increased oxidative stress. These results support the findings of Speranza et al. (2008), who reported that administering PDE5 significantly increased ROS levels. The decrease of antioxidant measure parameters in this investigation may be due to sildenafil's capacity to improve NO signaling through inhibiting the degradation of cyclic guanine monophosphate (cGMP) (18). The reduction in NO after 56 days observed in this experiment does not agree with previously reported cases that suggest a prolonged action of NO (6). The increased testosterone after 56 days agrees with previously reported cases (22).

Moreover, concomitant administration of 50 mg/kg bw of Sildenafil (Viagra) and 20 mg/kg bw of L-NAME for 8 weeks show varying degrees of increase in GPx, LH, testosterone and NO levels when compared to the L-NAME group, though

not statistically significant.

Photomicrograph of PDE group show seminiferous tubules, basement membrane, lumen, Leydig cells, Sertoli cells and spermatogonia suggests that sildenafil does not have detrimental effects on sperm production as previously reported (19, 20). Photomicrograph of L-NAME only group show several visible degenerative alterations marked by fragmented basement and pyknotic Leydig cells, enlarged lumen devoid of spermatozoa, and maturation arrest of spermatogenic cell line in several seminiferous tubules. This suggests impaired spermatogenesis as previously reported (2). A well-organized testicular cytoarchitecture is visible in the photomicrograph of the L-NAME co-administered with PDE group. This cytoarchitecture is characterized by the presence of Leydig cells in the interstitial spaces, Sertoli cells at the lumina border, and spermatozoa-filled seminiferous tubule lumens. Numerous Spermatogonia cells within the tubules have developed into numerous Spermatocytes.

Conclusively, the results of this work indicate that Sildenafil (Viagra) and L-NAME may have a detrimental effect on biochemical and hormonal functions (GPx, LH, FSH, testosterone and NO) in adult male Wistar rats, as evidenced by the alterations in biochemical and hormonal parameters. Moreover, the detrimental effects conferred on biochemical and hormonal functions by Sildenafil (Viagra) is not enough to cause testicular toxicity at the histological level as the group treated with Sildenafil (Viagra) only shows improvements in testicular cytoarchitecture. Thus, caution should be taken when using Sildenafil (Viagra) as it can have slightly detrimental effects on biochemical and hormonal functions.

REFERENCE

1. Aitken RJ, Krausz C. Oxidative stress, DNA damage, and the Y chromosome. Reproduction, 2001;122(4), 497–506
2. Salama AF, Abd-Elaziz HR, Elgebaly HA, Hassan SM, El-Mas MM. Role of endothelial nitric oxide synthase and peroxisome proliferator-activated receptor-gamma in vascular and renal protection exerted by Sildenafil in diabetic rats. Pharmacological Reports, 2016;68(6), 1253–1262.
3. Fathy SA, Shehata MA, Abdelghany AH, Mohamed MA, Abo-Youssef AM & Ibrahim SA. Role of naringenin in ameliorating hepatic and testicular oxidative stress induced by L-NAME administration in rats. Toxicology Mechanisms and Methods, 2018; 28(9), 653–663.
4. Dutta S, Sengupta P. The role of nitric oxide on male and female reproduction. The Malaysian Journal of Medical Sciences: MJMS, 2022;29(2), 18.
5. Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A. The international index of erectile function (IIEF): A multidimensional scale for assessment of erectile dysfunction. Urology, 2004;49(6), 822–830.
6. Corbin JD, Francis SH, Webb DJ. Phosphodiesterase type 5 as a pharmacologic target in erectile dysfunction. Urology, 2005;65(2), 269–272.
7. Vardi Y, Dayan L, Appleman M, Gruenwald I, Yehuda I, Yankova L. Penile revascularization in diabetic patients with erectile dysfunction treated by Sildenafil. Urology, 2007; 69(2), 166–169.
8. Nunes KP, Labazi H, Webb RC. New insights into hypertension-associated erectile dysfunction. Current Opinion in Nephrology and Hypertension, 2012; 21(2), 163–170.
9. Abdel-Raheem IT, El-Sahar AE, Ahmed HH, Allam AA, Ashour MB. Sildenafil citrate affects male fertility through the oxidative defense system of testicular tissues in rats. Andrologia, 2015; 47(1), 92–99.
10. Abdel-Moneim AM, Saad MM, El-Twab SM. Sildenafil ameliorates testicular damage in rats exposed to lead. Andrologia, 2014; 46(10), 1092–1100.
11. Saad MM, El-Twab SM, Abd-El-Fattah MM. Modulation of the oxidative stress by Sildenafil in the heart of rats exposed to gamma radiation. Environmental Science and Pollution Research International, 2017; 24 (35), 27566–27432.
12. Zhang L, Xu T, Wang S, Yu L, Liu Z, Zhu Y, Wang Y, & Liu J. L-NAME causes ovarian oxidative stress and impairs ovarian response to gonadotrophins in mice. Reproduction, Fertility, and Development, 2014; 26(1), 143–150.
13. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J

Men's Health. 2014; 32(1):1-17.
14. Zirkin BR. Aging and Declining Testosterone: Past, Present, and Hopeful Future. The Journal of Clinical Endocrinology & Metabolism, 2016; 101(12), 4789–4796.
15. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochem Cell Biol. 2007; 39(1), 4-84.
16. Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB, & Pereira CM. Protective effect of Sildenafil on Nω-nitro-L-arginine methyl ester-induced oxidative stress in the rat testis. Journal of Andrology, 2013; 34(4), 389–397.
17. Speranza L, Franceschelli S, Pesce M, Vinciguerra I, De Lutiis MA, Grilli A, Patruno A. Phosphodiesterase type-5 inhibitor and oxidative stress. International journal of immunopathology and pharmacology, 2008; 21(4), 879-889.
18. Calebiro D, Nikolaev VO, Gagliani MC, de Filippis T, Dees C, Tacchetti, Lohse MJ. Persistent cAMP-signals triggered by internalized G-protein–coupled receptors. PLoSBiology, 2010; 8(8), e1000562.
19. El-Sakka AI, Hassoba HM, Pillarisetty RJ, & Dahiya R. Sildenafil for treatment of erectile dysfunction in men with diabetes: A randomized controlled trial. Diabetic Medicine, 2014; 31(2), 205–212.SS
20. Sokol RZ, Langevin CB, Mielnik A, Palacios JM. Observations of enhanced spermatogenesis in men taking Sildenafil citrate. Urology, 2003; 61(1), 199–203.
21. Santos-Neto ATD, Silva IS, Ivo ML, Rodrigues CT, Parisotto EB, Ramalho RT & Monteiro GM. Effects of oxidative stress on liver, brain and spinal cord of rats using L-NAME and treated with hydroxyurea. A model of sickle cell complication. Acta Cirurgica Brasileira, 2020; 35, e202000301.
22. Zhang M, Xin J, Zou Q, Shen JW. A research on the relationship between ejaculation and serum testosterone level in men. J Zhejiang Univ Sci. 2003 Mar-Apr;4(2):236-40.

FIGURES

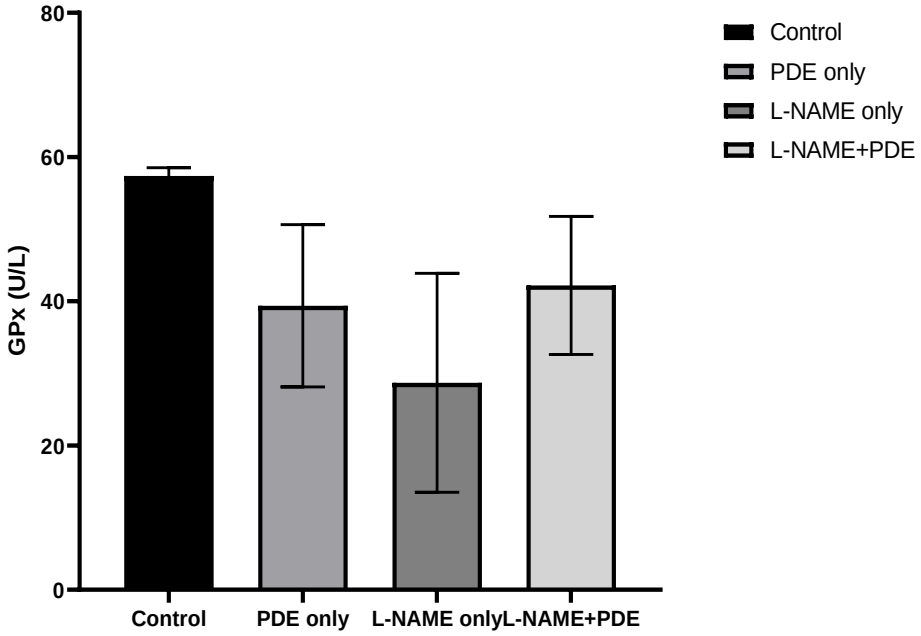

Figure 1: Effects of sildenafil and L-NAME on glutathione peroxidase (GPx) levels

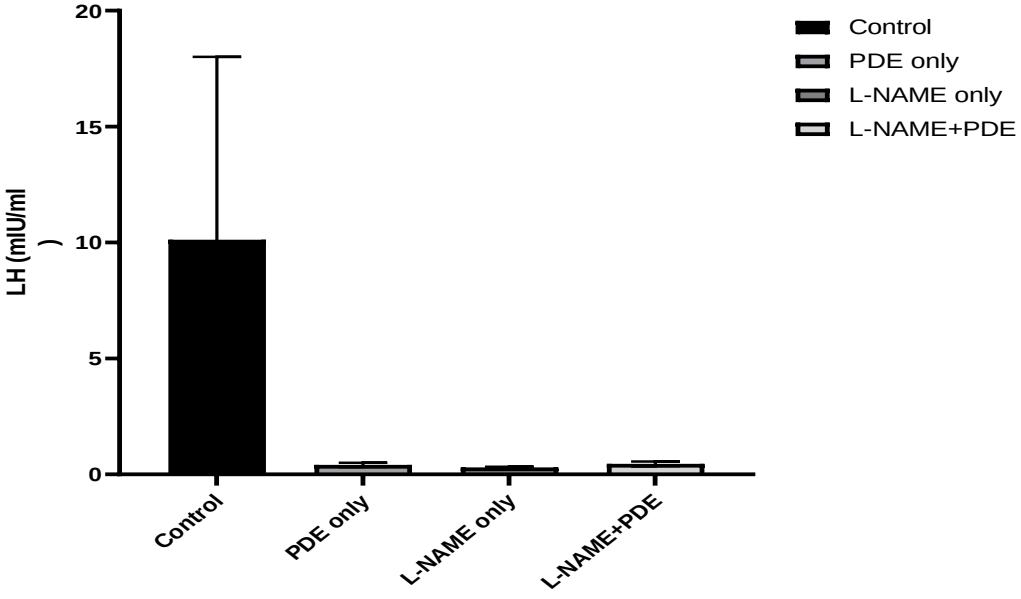

Figure 2: Effects of sildenafil and L-NAME on luteinizing hormone (LH) levels

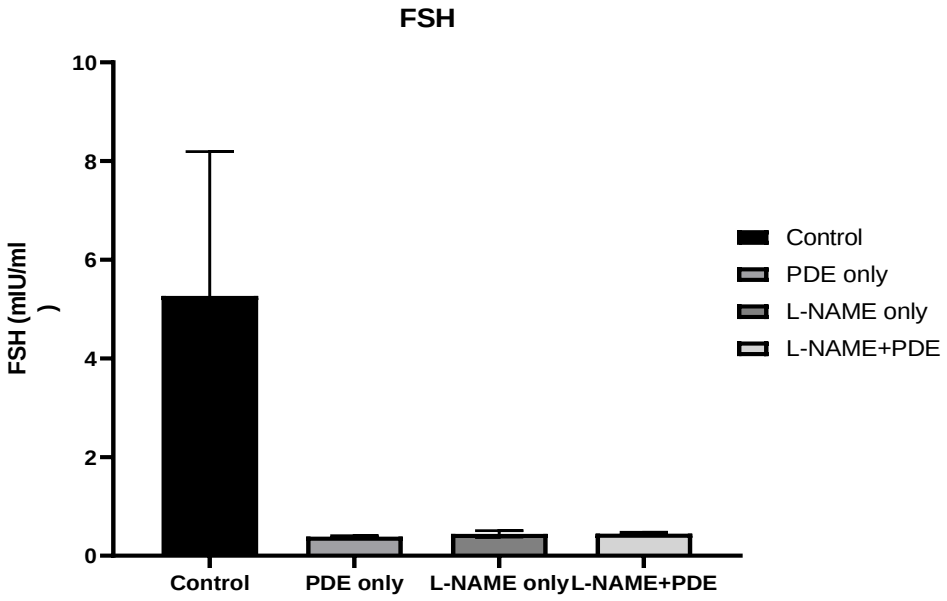

Figure 3: Effects of sildenafil and L-NAME on follicle stimulating hormone (FSH) levels

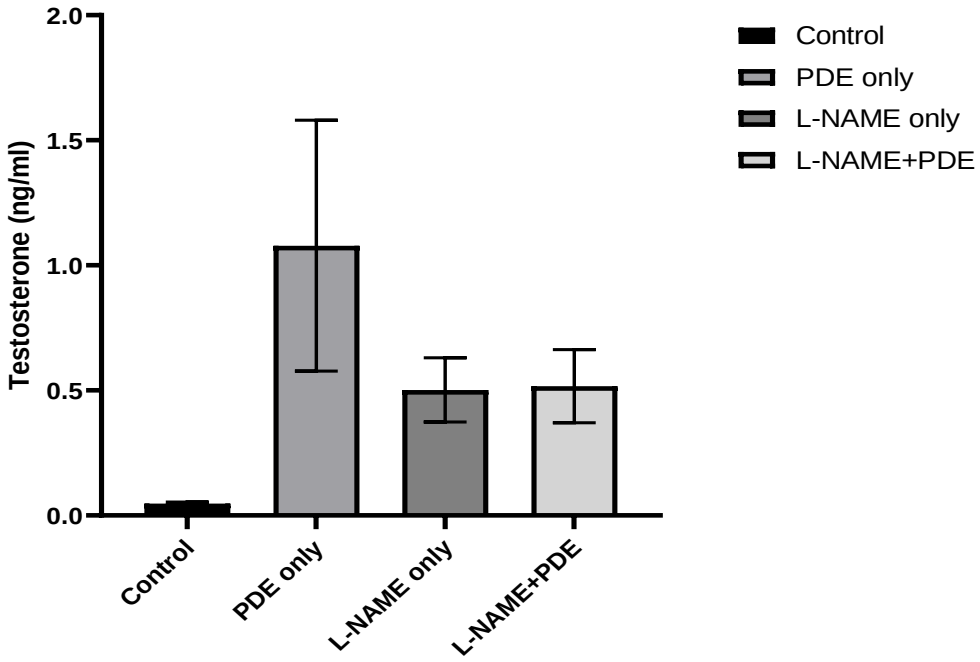

Figure 4: Effects of sildenafil and L-NAME on testosterone level

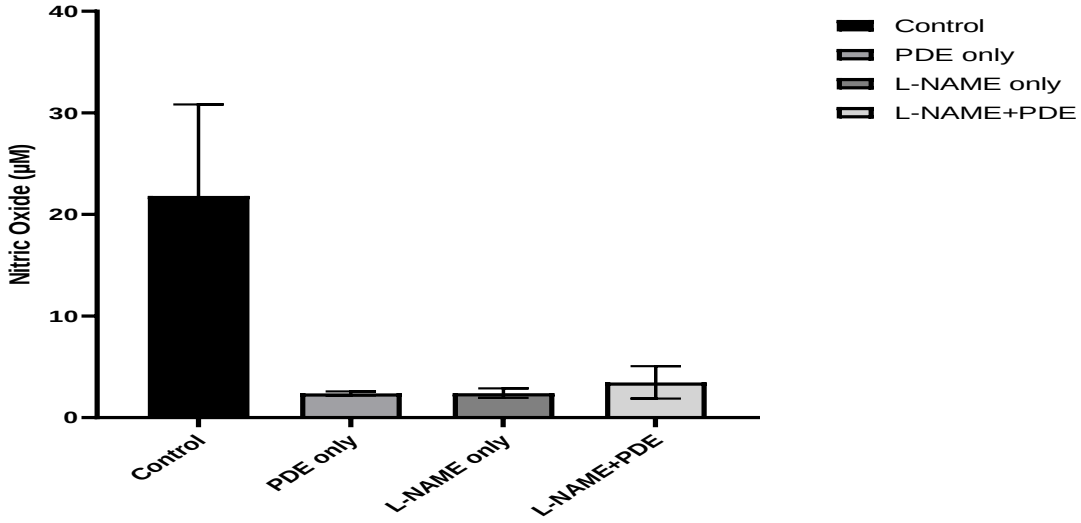

Figure. 5 Effects of sildenafil and L-NAME on nitric oxide (NO) levels

Histological analysis

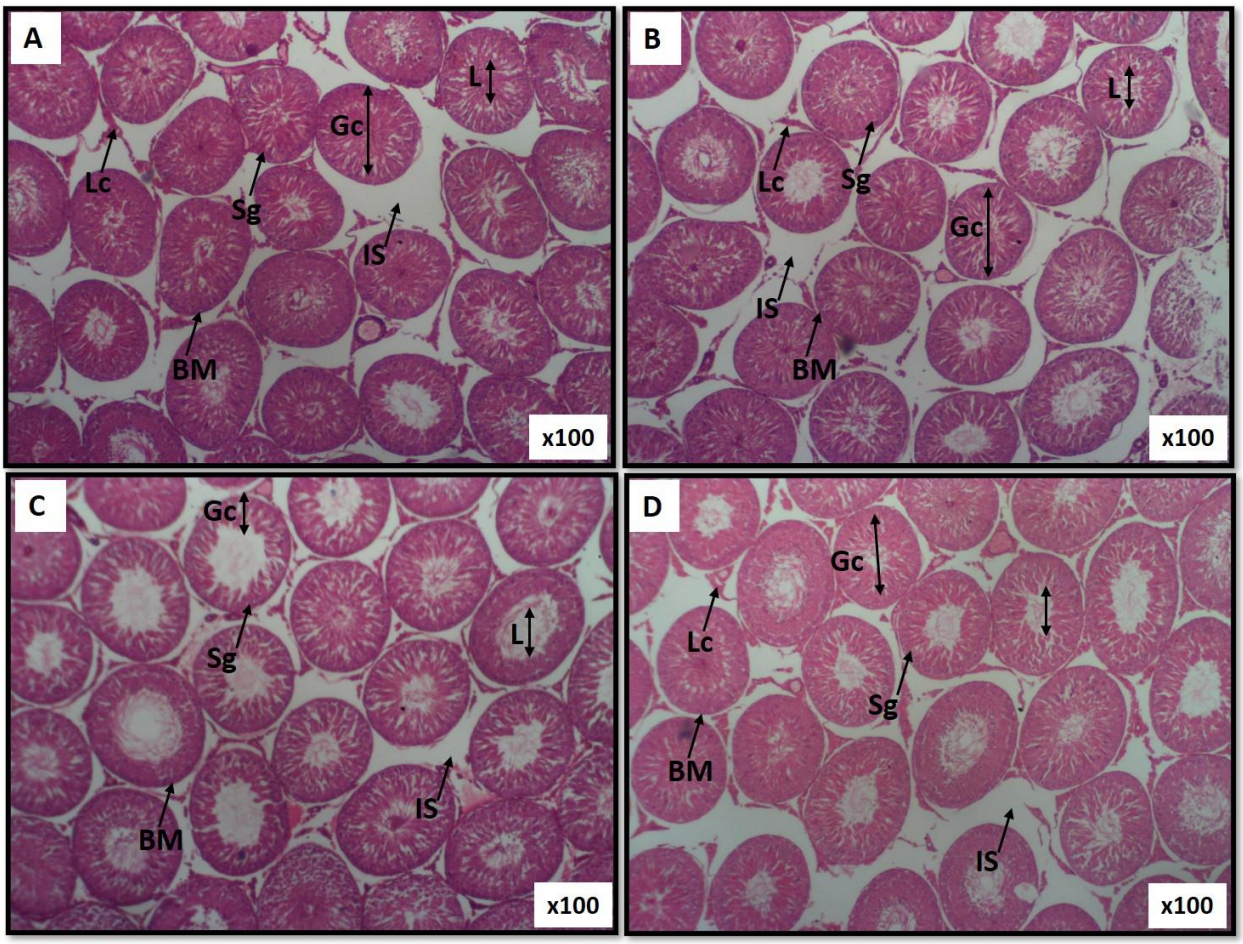

Figure 6: Photomicrograph of testicular architecture of control group, group treated with PDE, group treated with L-Name and group treated with co administration of L-Name and PDE.