ORIGINAL ARTICLE

Improvement Prospects of Lycopene Supplementation in the L-NAME Toxicity on the Male Wistar Rat Reproductive System

[*]Nkanu, E.E.[1], Obeten, K. E.[2], Aja, P. M.[3], George, E.I.[4]

[1]Department of Physiology, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Uganda
[2]Department of Anatomy, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Uganda
[3]Department of Biochemistry, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Uganda
[4]Department of Physiology, University of Calabar, Calabar-Nigeria.

ABSTRACT

Non-selective nitric oxide synthase inhibitors, such as N (G)-nitro-L-arginine Methyl Ester (L-NAME), are typically prescribed to treat spinal injuries and hypotension. Many physiological processes, including the regulation of reproductive hormones, are typically linked to it. Bioactivity in physiological systems is influenced by the antioxidant lycopene. This study sought to determine the impact of lycopene supplementation and L-NAME administration on gonadal hormones and sperm parameters. Twenty male Wistar rats were used for this study and were divided into four groups containing 5 rats each. L-NAME (5mg/kg) was administered through drinking water and Lycopene (5mg/kg) was supplemented through oral gavage for four weeks. Using established protocols with Elisa Kits, plasma hormone levels were ascertained. Estradiol did not differ substantially across all groups, however, the group that received L-NAME had lower plasma concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and nitric oxide (NO). Lycopene supplements raised testosterone, FSH, LH, and NO levels while not affecting estradiol. The sperm profile was altered with L-NAME but ameliorated in Lycopene supplementation. We conclude that lycopene has the potential to protect against hormonal imbalance and male infertility resulting from Nitric oxide synthase (NOS) inhibition.

Keywords: L-NAME, Steroid androgens, Sperm, Lycopene, Nitric Oxide.

*Corresponding Author
Nkanu, Etah Etah, Department of Physiology, Kampala International University, Western Campus, Uganda. nkanuee@yahoo.com; nkanuee11@gmail.com; +2347030299991

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INTRODUCTION

Over time, experimental research on the various roles that nitric oxide (NO) plays in both healthy physiological states and illness states has garnered scholarly attention. Nitric oxide synthase (NOS) catalyzes the body's cells and tissues to produce nitric oxide, a signaling chemical. Certain body organs may develop severe diseases as a result of downregulating the NO/sGC/cGMP signaling (1, 2).

L-NAME depletes NO availability or acts as a precursor for its production when administered acutely or over an extended period (3). Yet studies have demonstrated that nitric oxide, in varying amounts, is important for reproductive processes such as spermatogenesis, follicular maturation, sperm maturation, and steroid synthesis (5). Nitric oxide is also important for the male and female reproductive systems. Sperm motility (6) and Leydig cell testosterone (7) are inhibited at extremely high NO levels. One theory links the appearance of all of these control activities to the reproductive process's connection with the regulation and maintenance of the hypothalamic-pituitary-gonadal axis (HPA). For example, hypothalamic or pituitary dysfunction might lead to low levels of LH. Male infertility due to testicular damage might be brought on by such hormonal imbalances (8). Reduced gonadotrophin concentrations, such as those of FSH and LH, are another symptom that might be indicative of the HPA change (9).

With the growing quest for identifying an alternative way to combat toxicity in our body tissues, interest has shifted from the use of chemical drugs with their relatively high cost and adverse effects to the use of plant products and nutraceuticals. Recent experimental reports have shown that natural food products possess antioxidant and anti-apoptotic effects on both cardiovascular and reproductive systems and help to ameliorate inflammatory disease and most other metabolic problems related to oxidative stress (10,11). β-carotene, α-carotene, lycopene, and β-cryptoxanthin are the main antioxidants present in this natural diet (12). These compounds are identified majorly in vegetables, watermelon, pink grapefruit, papaya, and tomatoes (13). Nutraceutical and lipophilic carotenoid lycopene are present in various tissues, with the testes, adrenal glands, liver, and prostate containing the largest quantities (14).

Because of the high diene number and apoptosis of lycopene, it is believed to have a singlet oxygen scavenging property that makes it beneficial for treating diseases of the male reproductive tract, restoring spermatogenesis, and modulating oxidative stress (15). Despite the known biological benefits of lycopene, there have been some contradictory studies about its effectiveness in experimental testicular ischemia/reperfusion, suggesting that long-term lycopene treatment may not be successful in healing testicular injury (16). For this reason, we examined in this work how lycopene supplementation and L-NAME delivery affected sperm profiles and steroid testosterone levels in male Wistar rats.

MATERIALS AND METHODS

Experimental design

This study used twenty male Wistar rats, weighing between 150 and 180 grams. They were split up into four groups, each with five rats. Group 1 (control) was given water and food as usual. Group 3 received 5 mg/kg body weight of lycopene dissolved in tween 80 and given orally, while Group 2 received 5 mg/kg body weight of N (G)-nitro-L-arginine Methyl Ester (L-NAME) in drinking water. Group 4 was given only 5 mg/kg of lycopene. After the animals were given food and care for four weeks, blood samples were taken by heart puncture, and serum was extracted by centrifuging the blood for ten minutes at 1000 rpm. Using Elisa Kits and following the manufacturer's instructions, normal procedures were followed to determine the levels of steroids and testosterone. The study received ethical permission from the Animal Research Ethics Committee of the Faculty of Basic Medical Science at Cross River University of Technology, located in Calabar, Nigeria. The approval number for the study is CRUTECH/FBMS/REC/NIG/2023/029. For biochemical studies, the serum samples were kept at -10°C.

Collection of semen

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After macerating and well mixing the caudal epididymis in 0.8 ml of 1% trisodium citrate solution for seven to eight minutes, 1% trisodium citrate solution was added once more and thoroughly mixed for a minute. In 10% buffered formalin, the mixture was diluted 1:1. Using a standard hemocytometer and a light microscope, estimated functions of other sperm were carried out at room temperature (about 28 °C).

Sperm morphology.

Applying the method of (17), the proportion of morphologically aberrant spermatozoa was calculated on eosin–nigrosin (1.67% eosin, 10% nigrosin, and 0.1M sodium citrate) stained slides. A 400× magnification light microscope was used to study the slides. The percentages of spermatozoa with abnormalities in the head, tail, and overall were reported for each group.

Sperm motility.

Using a light microscope with a heated stage that was raised to 37°C, the percentage of motility of sperm from newly isolated epididymal tissue was calculated. Drops of Tris buffer solution, which contained 0.3M Tris (hydroxymethyl), 0.027M aminomethane, glucose, and 0.1M citric acid, were added, and these were mixed with a small drop of fluid from the left cauda epididymis. Using an x400 magnification, the percentage of motility of the sperm was visually assessed. The final motility score was determined by averaging the three subsequent assessments from three distinct fields (18).

Determination of sperm viability

The procedure outlined in (16) was applied. The nigrosin-eosin staining method was used, in short, to evaluate the vitality of the sperm. A microcentrifuge was used to thoroughly mix one drop of 50µl of semen with an equivalent volume of eosin–nigrosin suspension (1% aqueous solution of eosin-y and 10% aqueous solution of nigrosin). After that, the combination was examined under a 400X magnification on a glass slide. At least 200 cells are needed to assess the proportion of living (not stained) and dead (red) cells.

Hormonal assay evaluation

When the trial concluded, the animals were put to sleep with mild anesthesia. After obtaining whole blood via heart puncture, the serum for the hormonal assay was extracted and the blood was stored in a non-heparinized vacutainer spinning at 1000 rpm for 10 minutes. The following methods were used to determine the levels of hormones: (19) for testosterone and (20) for follicle-stimulating hormone. while luteinizing hormone level and estradiol were determined by the method of (21). The resulting color was measured using a spectrophotometer at 450 nm within 10 minutes.

Determination of NO

Serum nitric oxide concentrations were assessed by applying the procedure of (22). In short, each sample placed in a microtiter plate received 50 µL of modified Griess reagent (Sigma-Aldrich, St. Louis, MO, USA) combined with it. An Emax ELISA microplate reader was used to measure the nitrite content at 540 nm following a 15-minute incubation period. The NO determination processes were conducted in a dimly lit setting. Using known sodium nitrite values, a standard curve was created and used to quantify the nitrite concentrations (Sigma-Aldrich, St. Louis, MO, USA).

Statistical Analysis

The statistical analysis of the data collected for this study was conducted using the graph pad version 5 software. Mean ± standard error of the mean (Mean ± SEM) is the expression used to represent the results. The collected data was subjected to analysis of variance (ANOVA) and Bonferroni multiple comparison post hoc tests to determine the degree of significance that separated the experimental and control groups. A p-value of less than 0.05 was deemed significant.

RESULTS

Effect of L-NAME administration and Lycopene supplementation on Estradiol in Wistar rats.

No discernible change in estradiol levels was observed across all groups when L-NAME and lycopene were consumed. as displayed in Figure 1.

Effect of L-NAME administration and Lycopene supplementation on Testosterone level in Wistar rats

Comparing L-NAME to the control, the testosterone level was considerably ($p<0.05$) lower. Testosterone levels were considerably ($p<0.05$) raised by lycopene supplementation. Figure 4 illustrates this.

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Effect of L-NAME Administration and Lycopene Treatment on Follicle-Stimulating Hormone (FSH).

As illustrated in Figure 2, FSH levels were considerably ($p<0.01$) lower in the L-NAME group but higher in the L-NAME+LYCO and LYCO-only groups when compared to the Control and L-NAME groups, respectively.

Effect of L-NAME Administration and Lycopene Treatment on Luteinizing Hormone (LH).

In the L-NAME group, there was a significant ($p<0.01$) decrease in LH levels. As seen in Figure 3, it was higher in the L-NAME+LYCO and LYCO-only groups compared to the Control and L-NAME groups alone, respectively.

Effect of L-NAME administration and Lycopene supplementation on Nitric Oxide:

Comparing the administration of L-NAME to the control and experimental groups, there was a substantial ($p<0.05$) decrease in nitric oxide levels. When lycopene supplementation was given to the experimental groups, their levels of nitric oxide were considerably ($p<0.05$) higher than those in the control and L-NAME groups. This outcome is shown in Figure 5.

Effect of L-NAME administration and lycopene treatment on Sperm Profile in Wistar rats

Morphology: When comparing the L-NAME groups to the control, the percentage value for sperm morphology was considerably ($p<0.01$) lower. However, when comparing the L-NAME groups to the Co-treatment of lycopene and lycopene alone, the percentage value increased (Table 1)

Motility, Vitality & Agglutination: When L-NAME and Lycopene were administered together, there was a significant ($p<0.01$) increase in the percentage of motility and vitality, but a significant ($p<0.01$) decrease when L-NAME was administered alone. In the groups treated with lycopene, there was no discernible variation in the proportion of agglutination.

DISCUSSION

NG-nitro-L-arginine Methyl Ester (L-NAME) has been shown to block Nitric Oxide Synthase (NOS), which is one of its documented harmful effects in people or experimental animals. This action prevents the enzyme (NOS)from catalyzing the conversion of L-arginine nitric oxide in various physiological systems and processes including reproductive hormone regulation (23). In this work, we looked at the antioxidant influence of lycopene supplementation and the toxicological effect of L-NAME on the sperm profile and male steroid androgens in Wistar rats. Based on our data, we observed a noteworthy reduction in testosterone serum concentration in the L-NAME treatment group in contrast to the control group. This result does not agree with the report of Ibukun and his colleagues (24,10) who reported an increase in serum testosterone levels. This variation could be a result of the treatment approach.

Furthermore, this study also demonstrated that L-NAME administration impacts the activity of NOS and thus reduces the availability of nitric oxide in circulation as well as gonadotrophins (FSH, LH) and Testosterone levels respectively. Nitric oxide at normal levels is important in the control of reproductive processes and facilitates Luteinizing hormone–releasing hormone (LHRH) (25). At very low levels it may cause testicular degeneration, inhibition of testosterone, and infertility due to its reaction with free radicals (7).

Following the supplementation of lycopene, we found an optimal increase in NO, FSH, LH, and testosterone. Essentially, LH is released from the pituitary as a result of activation of the hypothalamus by kisspeptin leading to the release of gonadotropin-releasing hormone (GnRH) via the HPA pathway which eventually stimulates the pituitary to release LH. Impaired activity of the pituitary or hypothalamus may lead to LH deficiency and dysfunction (7). The main function of LH is to promote testosterone production from the Leydig cells in the testis. The testosterone further maintains spermatogenesis (26), Sertoli-spermatid adhesion, and sperm release (13,27).

The observed increase in nitric oxide and steroid androgens following lycopene supplementation may have been due to its rich natural phytonutrients, strong antioxidant properties, and notable scavenging properties in the handling of oxidative stress and reversing testicular damage both in vitro and in vivo (28). Thus, lycopene is said to play a significant protective or ameliorative action on the sperm profile and oxidative balance. In a similar study, it has also been

reported that lycopene administration increases testosterone levels in varicocele-treated rats and plays a significant role in restoring hormonal dysfunction and infertility (29). This goes on to confirm the reversal potential of lycopene in testicular function as shown in this study.

Investigation into sperm characteristics in this study showed that L-NAME treatment caused agglutination, reduced sperm morphology, sperm motility, and sperm vitality. Available reports have demonstrated also that lycopene administration protects the testes, increases sperm motility, sperm morphology, and vitality (30), and thus reduces apoptosis and improves steroid production (31)

CONCLUSION

This study shows that lycopene has the potential to protect against hormonal imbalance and male infertility resulting from nitric oxide synthase inhibition.

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REFERENCE

1. Kelly, D., Bart P., Charlotte B., Robrecht T., Emmanuel S.B., Peter B, Johan V.V. (2017). Erectile Dysfunction in Heme-Deficient Nitric Oxide-Unresponsive Soluble Guanylate Cyclase Knock-in Mice. J Sexual Med. 14(2), 196-204
2. Shalini, M.K, Jan, R.K, frank, E., Agnes, B, Peter, S (2018). The impact of the Nitric oxide (NO)/Soluble Guanylyl cyclase(sGC) signaling cascade on kidney health and disease: A preclinical perspective. Int. J Mol Sci.19(6)1712 Doi: 10.3390/ijms19061712
3. Kopincová, J, Púzserová A, Bernátová I (2012) L-NAME in the cardiovascular system – nitric oxide synthase activator? Pharmacol. Rep (64), 511-520
4. Herrero M, Lamirande E, Gagnon C. (2003) Nitric Oxide Is a Signaling Molecule in Spermatozoa. Curr Pharm Design. 9:419–25. doi: 10.2174/1381612033391720
5. O'Bryan MK, Schlatt S, Gerdprasert O, Phillips DJ, de Kretser DM, Hedger MP. (2000). Inducible Nitric Oxide Synthase in the Rat Testis: Evidence for Potential Roles in Both Normal Function and Inflammation-Mediated Infertility. BiolReprod. 63:1285–93. doi: 10.1095/biolreprod63.5.1285
6. Wang J, He Q, Yan X, Cai Y, Chen J. (2014). doi: 10.1186/0717-6287-47-44
7. Soni Y, Softness K, Arora H, Ramasamy, Yin Yang. (2020). Role of Nitric Oxide in Prostate Cancer. Am J Men's Health. 14:1557988320903191. doi: 10.1177/1557988320903191
8. Poongothai, J, Gopenath, T. S, Manonayaki, S and Poongothai, S. (2009). Genetics of human male infertility. Singapore Medical Journal, 50(4), 336-347
9. Lenzi A, Balercia G, Bellastella A, Colao A, Fabbri A, Foresta C, et al. (2009). Epidemiology; Diagnosis, and Treatment of Male Hypogonadotropic Hypogonadism. J Endocrinol Invest; 32(11)934–8. doi: 10.1007/BF03345775
10. Kim, J. K. & Park, S. U. Recent insights into the biological and pharmacological activity of lycopene. EXCLI J. 2022; 21, 415–425
11. Khan, U. M. (2021) Lycopene: Food sources, biological activities, and human health benefits. Oxid. Med. Cell. Longev.; 2713511
12. Gerster H. (1997). The potential role of lycopene for human health. J. Am. Coll. Nutr. 16:109–126. doi: 10.1080/07315724. 10718661.
13. Bin-Jumah, M. N. et al. (2022) Lycopene: A natural arsenal in the war against oxidative stress and cardiovascular diseases. Antioxidants, 11, 232.
14. Aly H.A.A. (2019) Testicular toxicity of gentamicin in adult rats: Ameliorative effect of lycopene. Hum. Exp. Toxicol.; 38:1302–1313. doi: 10.1177/0960327119864160.
15. Güzel M., Sönmez M.F., Baştuğ O., Aras N.F., Öztürk A.B., Küçükaydin M., Turan C. (2016). Effectiveness of lycopene on experimental testicular torsion. J. Pediatr. Surg. 51:1187–1191. doi

10.1016/j.jpedsurg.2015.11.008.
16. Fernandes, G. S. A., Fernandez, C. D. B., Campos, K. E., Damasceno, D. C., Anselmo-Franci, J.A., &Kempinas, W. D. G. (2011). Vitamin C partially attenuates male reproductive deficits in hyperglycemic rats. Reproductive Biology and Endocrinology, 9, 1–9. https://doi.org/10.1186/1477-7827-9-100
17. Makler A. (1978). A new chamber for rapid sperm counts and motility determination. Fertil Steril. 1978; 30:313–8. https://doi.org/10.9734/ijbcrr/2015/14318
18. Rochette L, Lorin J, Zeller M, Guilland JC, Lorgis L, Cottin Y, et al. (2013) Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: Possible therapeutic targets? PharmacolTher.140:239–57
19. Bjorndah, L. I., Soderlund, U. Kvist. (2003) Evaluation of the one-step eosin nigrosin staining technique for human sperm vitality assessment.Human Reprod. 18: 813-816
20. Akorede, G. J. (2020). Protective effect of vitamin C on chronic carbamazepine-induced reproductive toxicity in male Wistar rats. Toxicology Reports, (7): 269–276. https://doi.org/10.1016/j.toxrep.2020.01.017
21. Midgley A. R. (1967). Radioimmunoassay for human follicle stimulating hormone. J ClinEndocrinolMetab. 27:295–299
22. Don S. Schalch, Albert F. Parlow, Robert C. Boon, and Seymour Reichlin. (1968) Measurement of human luteinizing hormone in plasma by radioimmunoassay. J Clin Invest. Mar; 47(3): 665–678. doi: 10.1172/JCI105762
23.
24. Ibukun P. Oyeyipo,, Y. Raji and Adeyombo F. Bolarinwa. (2015). N -nitro-L-arginine Methyl Ester Protects Against Hormonal Imbalances Associated with Nicotine Administration in Male Rats.N Am J Med Sci. 2015 Feb; 7(2): 59-64. doi: 10.4103/1947-2714.152080
25. Milsom AB, B.O. Fernandez, M.F. Garcia-Saura, J. Rodriguez, M. Feelisch. (2012). Contributions of nitric oxide synthases, dietary nitrite/nitrate, and other sources to the formation of NO signaling products. Antioxidants Redox Signal., 17 (2012), pp. 422-432
26. Sortino MA, Aleppo G, Scapagnini U, Canonico PL. (1994). Involvement of nitric oxide in the regulation of gonadotropin-releasing hormone release from the GT1-1 neuronal cell line. Endocrinology. 134:1782-7
27. Kumar P, Chaturvedi CM. (2008). Correlation of Nitric Oxide (NO) Activity and Gonadal Function in Japanese Quail, Coturnix Coturnix Japonica Following Temporal Phase Relation of Serotonergic and Dopaminergic Oscillations. Anim. Reprod. Sci . 2008; 106:48–64. doi: 10.1016/j.anireprosci.2007.03.025
28. Yan HHN, Mruk DD, Lee WM, Cheng CY.(2008) Blood-Testis Barrier Dynamics are Regulated by Testosterone and Cytokines via Their Differential Effects on the Kinetics of Protein Endocytosis and Recycling in Sertoli Cells. FASEB J. 22(6):1945–59. doi: 10.1096/fj.06-070342
29. Cohen LA. (2002). A review of animal model studies of tomato carotenoids, lycopene, and cancer chemoprevention. ExpBiol Med (Maywood).227(10):864–868.
30. Pietro Antonuccio, Antonio Micali, Domenico Puzzolo, Carmelo Romeo,1 Giovanna Vermiglio,2 Violetta Squadrito,3 Jose Freni,2 Giovanni Pallio,3 Vincenzo Trichilo,3 Maria Righi,2 Natasha Irrera,3 Domenica Altavilla,2 Francesco Squadrito, Herbert R. Marini,3 and Letteria Minutoli3(2020). Nutraceutical Effects of Lycopene in Experimental Varicocele: An “In Vivo” Model to Study Male Infertility. Nutrients. 12(5): 1536. doi: 10.3390/nu12051536
31. Hekimoglu A, Kurcer Z, Aral F, Baba F, Sahna E, et al. (2009). Lycopene, an antioxidant carotenoid, attenuates testicular injury caused by ischemia/reperfusion in rats. Tohoku J Exp Med. 218:141–7.

Table 1: Showing the effect of L-NAME administration (5mg/kg) and Lycopene (2mg/k) supplementation on sperm profile.

Sperm parameters	GROUPS			
	Control	L-NAME	L-NAME +LYCO	LYCO
Sperm morphology (%)	35.0±0.200	26.60±0.400*	40.40±0.400*,b,d	44.40±0.600*,b
Sperm motility (%)	53.00±1.225	41.00±0.66*	54.00±1.800b	59.00±1.000*
Sperm Vitality (%)	61.00±0.447	54.80±0.800*	63.40±1.03d	69.00±1.000*
Sperm agglutination	9.40±0.2	13.80±0.489*,c,d	8.20±0.200	8.60±0.244

Values are expressed in Mean ± SEM; n=5; *=P<0.05 vs Control; b=p<0.05 vs L-NAME; d=p<0.05 vs Lyco

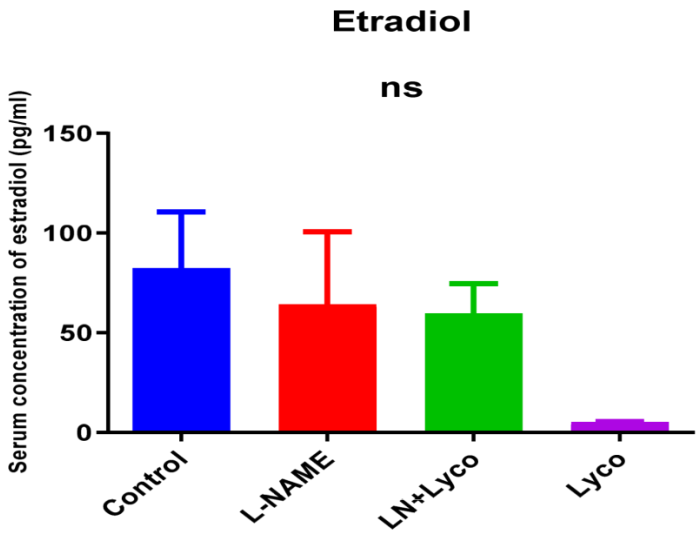

FIGURE. 1: Showing effect of lycopene supplementation on estradiol level in Nitric oxide synthase inhibitor (L-NAME) treated rats. Values are expressed in Mean ± SEM ns= not significant

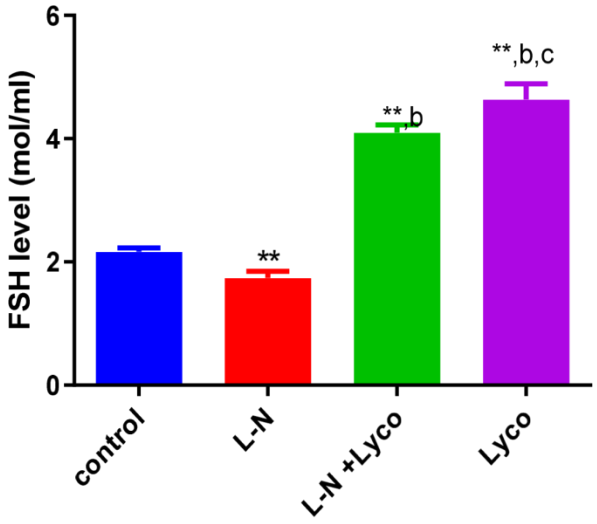

FIGURE. 2: Showing effect of lycopene supplementation on follicle stimulating hormone level in Nitric oxide synthase inhibitor (L-NAME) treated rats. Values are expressed in Mean ± SEM n=5, **= p<0.001 vs control; b=p<0.01 vs L-NAME; c=p<0.01 vs L_NAME +Lycopene

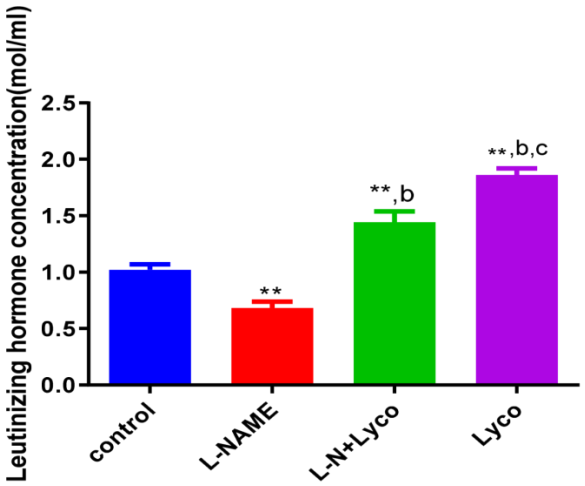

FIGURE. 3: Showing effect of lycopene supplementation on leutinizing hormone level in Nitric oxide synthase inhibitor (L-NAME) treated rats. Values are expressed in Mean ± SEM n=5, **= p<0.01 vs control; b=p<0.01 vs L-NAME; c=p<0.01 vs L_NAME +Lycopene

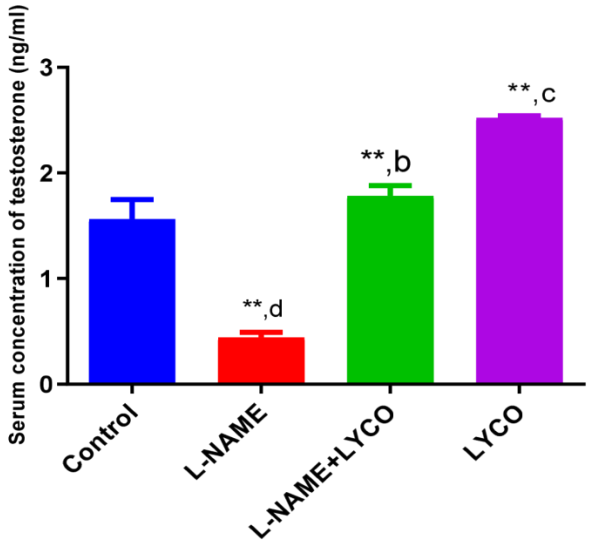

FIGURE 4: Showing effect of lycopene supplementation on testosterone level in Nitric oxide synthase inhibitor (L-NAME) treated rats. Values are expressed in Mean ± SEM;
**=p<0.01 vs control; b=p<0.01 vs L-NAME; c=p<0.01 Vs L-NAME+LYCO; d=p<0.01 vs Lycopene

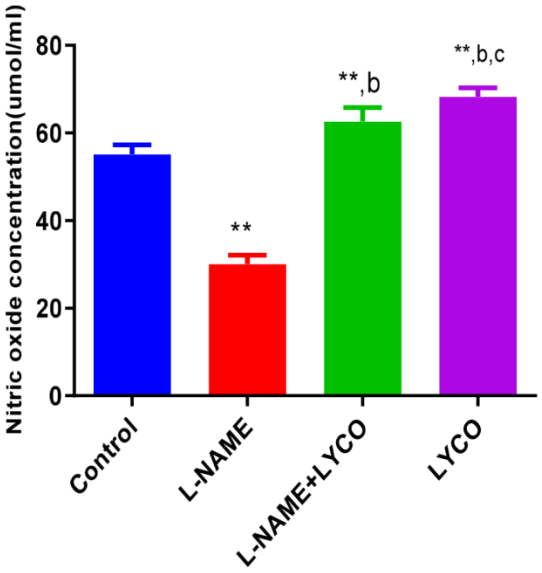

FIGURE. 5: Showing effect of lycopene on nitric oxide concentration in Nitric oxide synthase inhibitor (L-NAME)treated rats.
Values are expressed in Mean ± SEM n=5, **=p<0.05 vs control, b= L-NAME+LYCO Vs L-NAME; c=p<0.05 Vs L-NAME+LYCO