Lycopene alleviates inflammatory explosion and oxidative stress in lipopolysaccharide-induced reprodu ctive system injury of male rats

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Research Article

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Abstract

Male reproductive system infection and inflammation negatively impacts male infertility through various mechanisms, while the factors precipitating these mechanisms remain obscure. Lipopolysaccharide (LPS) causes damage to male reproductive system by causing fatty acid metabolism disorder and induces inflammation. It is reported that Lycopene (LYC) has anti-inflammatory and antioxidant activities, perhaps being a treatment option for male infertility. The present study aimed to investigate the effects of LYC on LPS-induced testicular injury in male rats. The rats were treated with LPS (5 mg/kg i.p.).LYC was given at 5 mg/kg, i.g. for 4weeks, starting the 24 hours after treatment of LPS. The levels of interleukin1alpha(IL-1α), interleukin1beta(IL-1β), tumor necrosis factor alpha(TNF-α), monocyte chemotactic pretein1(MCP-1) and interleukin6(IL-6) in serum, testis and epididymis. Testicular and epididymal glutathione peroxidase(GSH-Px), superoxide dismutase(SOD), catalase (CAT) were measured. Testicular histopathological examination and epididymal sperm quality were further evaluated. LPS impaired testicular function by increasing the level of inflammatory cytokines and decreasing antioxidant capacity. LYC supplement reduces cytokines levels in serum, testis and epididymis and increases antioxidant activity. In conclusion, LYC can protect male infertility by extenuating male reproductive system inflammatory burst, and enhancing antioxidant defense, which is a new treatment strategy for male reproductive system infection and male infertility.

Full Text

Male reproductive system infection generated by chronic nonspecific bacterial caused, including *Escherichia coli, Staphylococcus epidermidis, Staphylococcus albus, Streptococcus faecalis* and other, or mixed infection[1]. In addition, Sexually transmitted infections (STIs) has been seen a global resurgence. Concurrent with the increase in established STIs are emerging epidemics, such as *shigella, hepatitis A virus* and *Zika virus*[2]. Moreover, “non-classic” urogenital pathogens that cause hidden clinical syndromes, including *Ureaplasma urealyticum, Chlamydia trachomatis and Mycoplasma genitalium* have been found be the important pathogens of non-bacterial infections of reproductive system [3, 4].

Male reproductive system infections can be caused by a variety of bacteria and fungi, but the most common pathogens are *Escherichia coli* [5], in particular epididymitis[6]. *Escherichia coli* can damage spermatozoa through various mechanisms, the tight adherence between *Escherichia coli* and sperm can lead to immobilization and agglutination of sperm[7]. Another mechanism is that *Escherichia coli* produces and secretes soluble factors, such as lipopolysaccharide (LPS)[8]. LPS is the main component of the cytoderm of gram-negative bacteria and the major pathogenic factor.[9], LPS injecting can bring in an inflammatory response similar to infection and inhibit the production of testosterone steroid hormones in animals, leading to sperm dysfunction[10] , which has been widely used in animal inflammatory models[11, 12]. LPS-induced inflammation disrupted the blood-testosterone barrier, produced the anti-sperm antibodies, and induced apoptosis of sperm and spermatogenic cells[13]. Toll-like receptor4 (TLR-4) is a transmembrane protein capable of recognizing specific pathogen molecules such as LPS of Gram-negative bacteria. Aggregation of the TLR4–MD-2 complex after LPS binding leads

to the activation of multiple signaling components, and the subsequent production of pro-inflammatory cytokines [14, 15], which is an important approach to cause inflammatory injury of male reproductive function.

LYC is a non-pro-vitamin A carotenoid mainly found in tomato and tomato-based products, whereas it cannot be synthesized in the human body[16]. It has reported that Lycopene regulated LPS-induced oxidative stress by increasing total antioxidant and HDL-related PON-1 function as well as down-regulating the plasma level and inflammatory mediators expression[17]. Moreover, LYC not only quenches singlet reactive oxygen species (ROS), but also prevents lipid peroxidation. Furthermore, LYC can decrease sperm DNA fragmentation, as well as lipid peroxidation by its antioxidant activity in normospermic infertile men[16, 18]. In this regard, this study was conducted to investigate the possible protective role of LYC against the adverse effects induced by LPS on male reproductive system in rat.

Materials And Methods

Animals

A total of 40, aged 8 weeks, sex mature male Sprague Dawley (SD) rats weighting 280 ± 10g were obtained from the East General Hospital. Animals were maintained at temperature (24 ± 2°C), relative humidity (60% ± 5%) with a 12 hour light: dark cycle and with access to food and water ad libitum during the course of the study. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of East Region Military Command General Hospital (Date20220307/No.2022JLHSXJDWLS-0021).

Chemicals and Experimental design

Lipopolysaccharide (LPS, L2630-10MG) was purchased from Sigma-Aldrich, Co., (USA). Lycopene was obtained from the Nanjing Yuanjian Biotechnology Co., LTD (Nanjing, China).

After 2 weeks of acclimation, the experimental rats were divided into four groups (per n = 10): First group was blank control. Second group was control-received vehicle (CRV, olive oil, 5 ml/kg/d, 4weeks). Third group was treated with LPS (5mg/Kg, dissolved in 0.9% Sodium chloride injection, i.p.). And fourth group received LPS (5mg/Kg, dissolved in 0.9%Sodium chloride injection, i.p) and Lycopene (5mg/kg/d, dissolved in olive oil, i.g, 4weeks).

Epididymal sperm analysis

Detection of sperm parameters was performed according to the WHO laboratory manual for the Examination and processing of human semen (WHO, 2010). The right cauda epididymis were cut centrally and put in plates containing warm (37°C) 0.9%Sodium chloride injection (Chimin Health Management Co., LTD.). Sperm counting was performed using a Neubauer haemocytometer. The semen specimens were evaluated on dishes under a light microscope for sperm motility and sperm concentration. Sperm motility and count were completed by one researcher.

Histopathological Examination

The testicular tissue was preserved in 4% paraformaldehyde over 24 hours. The samples were dehydrated and embedded in paraffin and serially sliced, and they were prepared for hematoxylinand eosin staining. Then, 5-μm sections were cut from the paraffin- embedded tissues, stained with haematoxylin and eosin (HE), After staining, the sections were dehydrated in alcohol, cleared in xylene, and preserved finally. The pictures were examined by light microscope.

Measurement of cytokines

Serum, testis and epididymis tissue were used for the estimation the level of the following cytokines: interleukin1alpha (IL-1α, Cat num: ELK1148), interleukin1beta (IL-1β, Cat num: ELK1272), tumor necrosis factor alpha (TNF-α, Cat num: ELK1396), monocyte chemotactic pretein1(MCP-1, Cat num: ELK5504) and interleukin6(IL-6, Cat num: ELK1158) [19]. Those cytokines were detected in serum, testis and epididymis tissue with commercial kits (ELK Biotechnology CO., LTD, Wuhan, Hubei, China) according to the manufacturer's instructions.

Testis Antioxidants Biomarkers

Catalase (CAT, A007-1), glutathione peroxidase (GSH-Px, A005-1), and superoxide dismutase (SOD, A001-1) [20]were detected in testis homogenate with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed using statistical software SPSS 26.0. Data are presented as the mean ± SD. One-way analysis of variance (ANOVA) was used to determine differences between the normally distributed data. Kruskal–Wallis test was employed to compare non-normally distributed variables. Dunnett´s T3 was employed to compare non-equal variances variables. $P < 0.05$ was considered to be statistically significant.

Results

Sperm Analysis

Fig. 1 showed that LPS treatment caused the decrease in sperm motility (Fig.1A) compared with control and CRV group (all $P<0.05$), whereas LPS did not affect sperm count ($P>0.05, P>0.05$) (Fig.1B). Administration of LYC to LPS-treated rats also resulted in a significant decrease in sperm motility ($P<0.05, P<0.05$), but has no significant change in sperm count($P>0.05, P>0.05$). In addition, there was no significance between LPS and LPS+LYC in terms of sperm count and sperm motility($P>0.05, P>0.05$).

Histopathological Evaluation

Testicular sections from control group showed healthy architecture consisting of uniform, well organized seminiferous tubules with normal arrangement of spermatogonia and Sertoli cells resting on intact basement membrane(Fig.2A&B). LPS provoked marked pathological alternations including edema in interstitial space, spermatogenic severe reduction and chaotic arrangement in seminiferous tubules (Fig.2C). On the other hand, rats treated with LPS and LYC group showed visibly restore of the spermatogenic layers of the seminiferous tubules with the reappearance of the mature sperms in the lumen and disordering of the spermatogenic lineage remains in some of the seminiferous tubules. (Fig.2D). The detailed figure and description is indicated in the Appendix to the present study.

Serum cytokines' level

LPS-treated rats exhibited significant increases in serum IL-1α, IL-1β, TNF-α,MCP-1 and IL-6(Fig.3A-E) (all *P<0.001*), respectively. Following administration of LYC to LPS-treated rats, there was significant decrease in those serum cytokines when compared with the LPS group (all P<0.001,except for MCP-1 which is P< 0.01).

Testis and epididymis Cytokines

LPS-treated resulted in a significant increase in testicular IL-1α, IL-1β, TNF-α, MCP-1 and IL-6 levels (Fig.4A-E) when compared with the control and oil group (all *P<0.001*). Administration of LYC to LPS-treated rats decreased the testicular IL-1α, IL-1β, TNF-α, MCP-1 and IL-6 levels when compared with the LPS group (*P<0.001, P<0.05, P<0.001, P<0.001* and *P<0.05*, respectively). The cytokine levels in epididymis (Fig.4F-J)) were consistent with the results in testis.

Antioxidants Biomarkers

Fig. 5A-C showed that LPS-treated resulted in a significant decrease in testicular GSH-Px, SOD and CAT activities when compared with both of control and CRV group (all *P<0.001*). Administration of LYC to LPS-treated rats increased the level of testicular GSH-Px, SOD and CAT activity when compared with the LPS group (all *P<0.05*). Compared with both of control and CRV group administration LYC to LPS-treated rats did not significantly change SOD(P*0.05*, P*0.05*), and decreased in testicular GSH-Px (*P<0.001, P<0.05*) and CAT activities (*P<0.05, P<0.05*).

Fig. 5D-F showed that the epididymal GSH-Px, SOD and CAT activities decreased in LPS-treated compared with control and CRV group (all *P<0.001*, except for CAT which is *P<0.01*). Administration of LYC to LPS-treated rats increased the level of epididymal GSH-Px and SOD activity when compared with the LPS group (*P<0.05*, *P<0.05*),whereas the level of CAT activity was not significantly changed(P*0.05*).

Discussion

Various microorganisms, including bacteria, viruses and protozoa, can infect the male reproductive tract such as the testis, epididymis and impair male fertility. And most of these pathogens can be sexually transmitted. In addition, these pathogenic infections play a disruptive and hidden role in male

reproductive failure[21]. Infection or inflammation may affect fertility by inhibiting reproductive hormone synthesis[22], reducing the sperm number and motility, and promoting apoptosis of sperm and spermatogenic cells[5]. Different kinds of pathogen are often able to interfere with reproductive function in both sexes and lead to infertility[23–25].

LPS is the main pathogenic factor of Gram-negative bacteria and could cause various inflammatory reactions in the body, which has been widely used to study the impact of inflammation on testis physiology[26].Animals and cells treated with LPS exhibit testicular dysfunction due to infection and inflammation[10]. This inflammatory response of the genitourinary tract to the invasion of microorganisms activates leukocytes and inflammatory mediators such as cytokines and ROS, which play significant roles in sperm DNA fragmentation and male infertility. As a natural occurring carotenoid, LYC has a strong antioxidant capacity and can decrease oxidative damage to cells[27]. Hence, the present study is designed to clarify the possible ameliorative role of Lycopene against the LPS toxicity after given to rats.

Cytokines exist in testicular tissue and can be produced by interstitial cells, sertoli cells and macrophages. Under physiological conditions, they can ensure the normal function of reproductive system and are essential regulatory factors in spermatogenesis[28]. When inflammation occurs, the cytokines burst beyond the normal range, which negatively affect spermatogenesis and maturation, further damage male fertility. Previous data point to a proapoptotic effect of LPS on premeiotic germ cells (GCs) mediated by inflammatory factors such as NO, IL-1, IL-6, TNFα and IL-18 produced by macrophages, Sertoli cells and Leydig cells[29]. Testicular macrophages and Sertoli cells respond to LPS by secreting proinflammatory cytokines IL-6 and IL-1α, while Leydig cells respond by secreting TNF-α, IL-1β and transforming growth factor beta1(TGF-β1)[30, 31]. We also found significantly elevated levels of the cytokines, including IL-1α, IL-1β, TNF-α, MCP-1 and IL-6 in testis of LPS-treated rats. The levels of these cytokines in epididymis were consistent with the results in testis. The increase of these cytokines in testis can directly lead to spermatogenic cell damage and indirectly affect testosterone production and spermatogenic function of testis[10, 13].

Cytokines dysregulated influence spermatogenesis and spermatozoa functions, and can modulate pro-oxidant and antioxidant activities in the male genital tract. Positive correlations have been observed between ROS production and pro-inflammatory cytokines in the seminal plasma, including IL-6 and IL-8[32]. Indeed, this study revealed that LPS caused histopathological damage of the testis and reduced the number of epididymal sperm in rats, which agreed with a previous report[33]. The decrease of sperm motility was not statistically significant and perhaps affected by different degrees of self-healing in rats. Previous report clearly demonstrated that LYC effectively inhibited the production of inflammatory mediators TNF-α and IL-1β in testis[20]. This is in agreement with present work, in addition to TNF-α and IL-1β, in which LYC inhibited the increase of IL-1α, MCP1 and IL-6 in rat testis treated by LPS. To the best of our knowledge, the current study is the first comprehensive report on the identification of cytokines in the field of male reproductive infection. Meanwhile, the present study proves the anti-inflammatory effects of lycopene in inflammation of male reproductive system.

The activities of GSH-Px, SOD and CAT were measured as an index for evaluating the antioxidant activity in testicular tissue. SOD and CAT are parts of the antioxidant defense system, which are used as markers for antioxidant capacity. GSH-Px can perform the peroxide removal such as ROS, H_2O_2, and malondialdehyde (MDA). Previous studies indicated that LPS increased the content of MDA and H_2O_2 while decreased the activity of CAT, SOD, and GSH-Px enzymes in testis[34]. Consistent with previous results, our date showed that LPS reduced the anti-oxidative enzymes, including GSH-Px, SOD and CAT activities of testis and epididymis. Similarly, in a study of Zelen et al. [35], the activities of SOD and CAT were significantly lower in the seminal plasma of the oligozoospermic, astenozoospermic, and teratozoos-permic patients compared to the fertile controls, while the level of MDA was higher in the infertile subjects. The antioxidant activities of LYC have demonstrated in previous studies[34, 36]. Previous data have provided insight into the protective effect on lycopene of testicular toxicity induced by various factors through suppression of oxidative stress and enhancement of antioxidant capacity. Emre Kaya et al[37] evaluated that the protective effects of lycopene on diethylnitrosamine-induced testicular lipid peroxidation and on the associated changes in spermatological parameters and histopathological architecture of rat testis. HAA Aly[38] reported gentamicin treatment induced testicular toxicity as evidenced by increased oxidative stress and apoptosis. These alterations were effectively prevented by lycopene pretreatment. Moreover, in a study of experimental model of varicocele, supplement with LYC significantly increased testosterone levels and testes weight, improved tubular structure and decreased MDA levels. The data suggested that LYC use might be considered a novel strategy for the treatment of varicocele[39]. Here, we also found that administration of LYC at the dose of 5 mg/kg for 4weeks increases the anti-oxidative defense such as SOD, GSH-Px and CAT, suggesting that LYC had a protective role in LPS-induced testicular redox state disorders by increasing the anti-oxidative enzyme activities.

Elucidating the possible protective mechanism of LYC is beneficial for its effective application. Former study showed that LYC-alleviated di(2-ethylhexyl) phthalate induced the increase of Nuclear factor erythroid 2-related factor 2 (Nrf2) downstream antioxidant genes Kelch-like ECH-associated protein 1 (Keap1) and Hypoxia-induced factor-1α (HIF-1α) mRNA and protein expression to antagonizes oxidative stress of Leydig cells[36]. Nrf2, as the main transcriptional activator, can activates many antioxidant genes in response to the intracellular ROS increase to protect cells from stress[40]. Nrf2 is severally combined with Keap1 and HIF-1α, which can mediate the degradation of Nrf2 and to elicits its biological functions[41, 42]. Similarly, Yuan Tian et al[43] showed that LYC can reduce testicular fluorosis-induced oxidative stress and spermatogenic cell apoptosis through the suppression of Jun N-terminal kinase (JNK) and extracellular signal-regulated protein kinase (ERK) phosphorylation. JNK and ERK signaling pathways play a critical role in the mechanism of apoptosis which might be essential in fluorosis-induced impairment of reproductive system[43]. Although the mechanism by which lycopene plays a protective role in LPS-induced testicular injury remain obscure, it is possible that previous studies may inspire further research.

Conclusion

In summarization, the current study demonstrated that LPS impaired testicular function by increasing the level of inflammatory cytokines and decreasing antioxidant capacity. Our results also manifested that the beneficial effects of LYC supplement, enhancing anti-inflammatory and antioxidant defense on LPS-induced male reproductive toxicity, which provided a new strategy for LYC protection against injury induced by infection and inflammation in male reproductive system.

Declarations

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Declaration of interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xuejun Shang, Yu Li and Jinde Zhu. The first draft of the manuscript was written by Yu Li. The writing - review & editing was done by Hongcai Cai and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

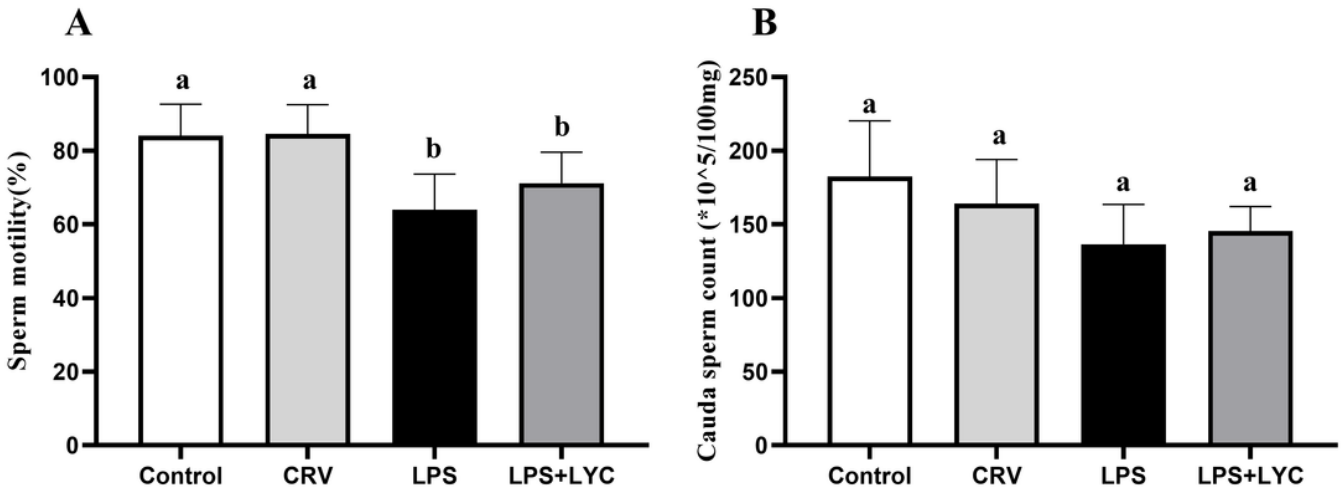

Figure 1

Effects of LPS and/or LYC on epididymal sperm motility (A) and sperm count (B) in male rats. Control: blank control. CRV: control-received vehicle. LPS: lipopolysaccharide. LPS+LYC: lipopolysaccharide and Lycopene. Means within the same column in each category carrying different letters are significant at (*P<0.05*) where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

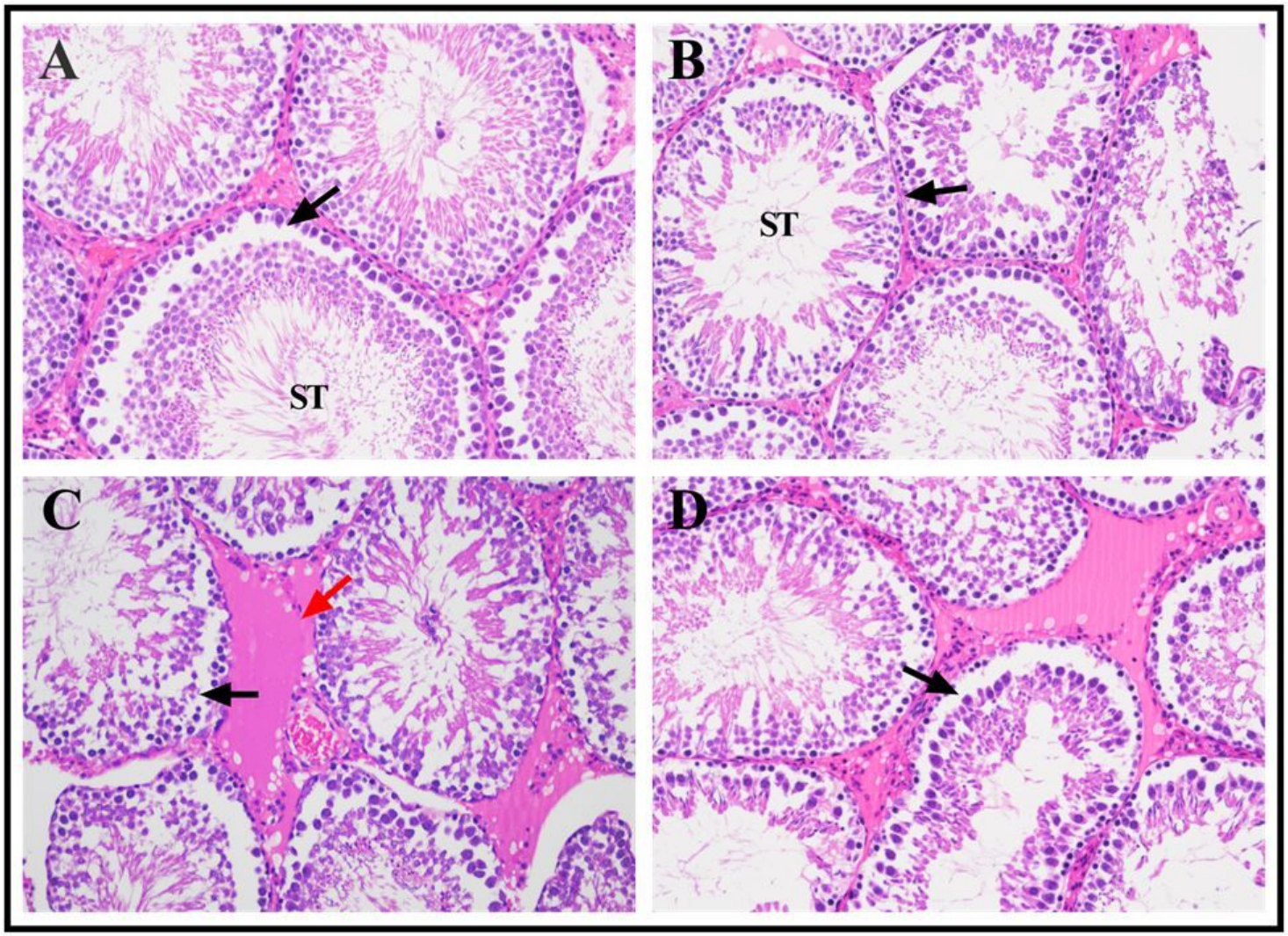

Figure 2

Histopathological observations (testis sections stained with Hematoxylin & Eosin, magnification×200) showing effects of LYC on LPS-induced testicular toxicity. (A&B)Control and Control-received vehicle (CRV) group showing normal intact seminiferous tubules (ST) with normal spermatogenic lineage. (C) LPS group illustrating edema in interstitial space(the red arrow), spermatogenic lineage significantly reduced , disordered arrangement and disappeared level in ST(the black arrow). (D) LPS and LYC group showing significant improvement of histological derangements as the arrangement of spermatogenic cells is relatively orderly and spermatogenic arrest in a few seminiferous tubules.

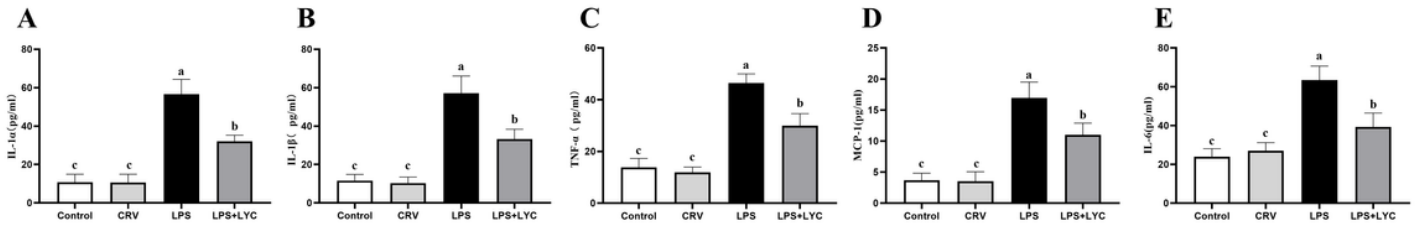

Figure 3

Effect of LPS and/or LYC on cytokines' levels in serum. A: Serum IL-1α level. (B) Serum IL-1β level. (C) Serum TNF-α level. (D) Serum MCP-1 level. (E) Serum IL-6 level. Means within the same column in each category carrying different letters are significant at (*P<0.05*) where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

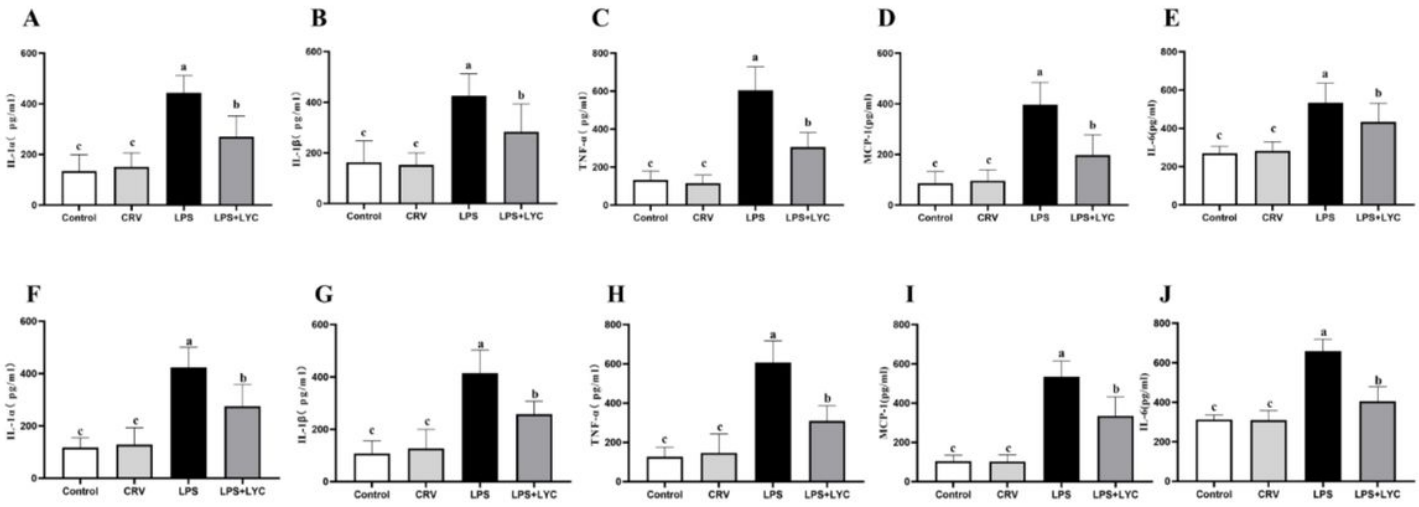

Figure 4

Effects of LPS and/or LYC on levels in testis(A-E) and epididymis(F-J). (A) Testis IL-1α level. (B) Testis IL-1β level. (C) Testis TNF-α level. (D) Testis MCP-1 level. (E) Testis IL-6 level. (F) Epididymis IL-1α level. (G) Epididymis IL-1β level. (H) Epididymis TNF-α level. (I) Epididymis MCP-1 level. (J) Epididymis IL-6 level. Means within the same column in each category carrying different letters are significant at (*P< 0.05*) where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

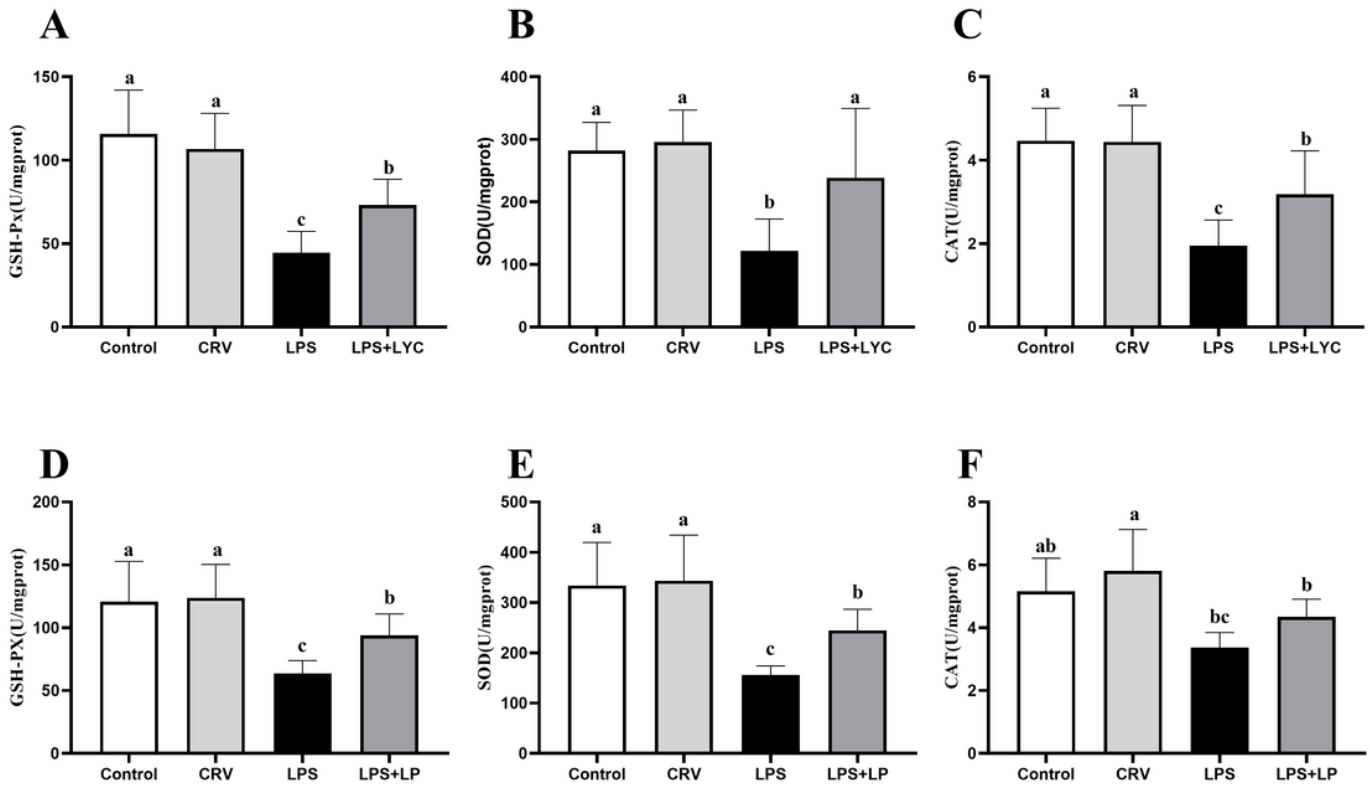

Figure 5

Effects of LPS and/or LYC on antioxidant enzymes activities of testis and epididymis. (A) Testis GSH-Px activities. (B) Testis SOD activities. (C) Testis CAT activities. (D) Epididymis GSH-Px activities. (E) Epididymis SOD activities. (F) Epididymis CAT activities. GSH-Px=glutathione peroxidase, SOD=superoxide dismutase, CAT=catalase. Means within the same column in each category carrying different letters are significant at (*P<0.05*) where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.