Peer Review File

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Review comments-Reviewer A

Male fertility can be hampered by systemic and testicular infections and inflammation, which can lead to impaired spermatogenesis that often cannot be reversed by antibiotic treatment. There has been some suggestion that lycopene (LYC) may be useful in the preservation of fertility, although its mechanisms are complex. In the manuscript "Lycopene alleviates lipopolysaccharide-induced testicular injury in rats by activating the PPAR signaling pathway to integrate lipid metabolism and the inflammatory response", authors examined the therapeutic efficacy of LYC on testicular damage and its underlying mechanisms. Couple questions are required to be answered before it will be accepted.

Comment 1: What were the roles of PPAR signaling pathway in inflammation? Please state in the introduction.

Reply 1: We thank the reviewer for the nice summary of our study firstly, and thank the comment and suggestion. We have stated the role of PPAR signaling in inflammation in the introduction according to the suggestion (see Page 4, line 112-122, in the marked copy).

Changes in the text: PPARs are ligand-activated transcription factors belonging to the nuclear receptor family. PPARs not only bind to DNA and regulate gene expression, but also act as intracellular receptors to bind active lipid molecules. The role of PPARs in inflammation is particularly relevant to disorders of lipid metabolism, such as metabolic syndrome and atherosclerosis, which have potential inflammatory factors in their pathogenesis. Furthermore, the mechanisms of PPARs in inflammation are diverse. PPAR can regulate key genes, and these genes have inflammation regulatory effects. For example, PPAR-α upregulates IKB expression, there by blocking nuclear translocation and activation of the pro-inflammatory transcription factor NF-κB. Alternatively, PPARs also regulate inflammatory processes by altering lipid metabolism,

such as the lipid regulatory mechanism in human dendritic cells.

Comment 2: It was advised to add related reference (Ann Transl Med. 2021 Apr;9(8):631) about the lycopene gin inflammation in the introduction.

Reply 2: We thank the reviewer for the reference suggestion. The reference has been integrated in the new version of the manuscript (see Page 4, line 136-139, in the marked copy)

Changes in text: LYC promotes the activation of Nrf2/HO-1 pathway and further inhibits NLRP3 inflammasome by enhancing autophagy in kupffer cells, thereby alleviating hepatic ischemia-reperfusion injury (19).

Comment 3: The LYC was the crucial topic in the study. Please make a brief introduction, including its functions.

Reply 3: We thank the reviewer for this constructive suggestion. In accordance with this suggestion, we introduce lycopene in the introduction, including basic information and main functions, as well as research progress (see Page 4, line 125-130, in the marked copy).

Changes in text: Lycopene (LYC, C40H56) is a natural carotenoid with 11 conjugated double bonds. LYC, which is mainly found in red fruits and vegetables, cannot be synthesized by the human body and needs to be ingested from the daily diet. LYC is a powerful antioxidant with various biological functions, such as anti-inflammatory, anti-cancer, lowering blood lipids and protecting the liver.

Comment 4: Please state clearly the name of used rat strain, and the body-weight in the methods.

Reply 4: We thank the reviewer for the detailed points to clarify our methods and improve the manuscript. Information about rat strain and body weight has been integrated in the corresponding sentences (see Page 5, line 152, in the marked copy).

Changes in text: A total of 40 sprague-dawley (SD) male rats (6–8 weeks old) weight 260±20g were obtained from Jinling Hospital and housed under a conventional 12-hour

light/dark cycle, at a constant temperature of 24±2℃, and relative humidity of 60%±5%.

Comment 5: How to determine the dose of LYC? Please provide supported references.

Reply 5: We thank the reviewer for raising important concerns about our methods. The dose of LYC used in this study was 5mg/kg (5mg/ml LYC at 1ml/100g body-weight). This dose was determined for two reasons. First, from the reference (see Page 5, line 165, the 23rd reference in the marked copy), the corresponding reference has been integrated into the revised version. The second is from the investigation of other researchers in our group, which mainly focuses on the treatment of prostatic hyperplasia.

Changes in text: Gupta, P., Bansal, M. P., & Koul, A. (2013). Lycopene modulates initiation of N-nitrosodiethylamine induced hepatocarcinogenesis: studies on chromosomal abnormalities, membrane fluidity and antioxidant defense system. Chemico-biological interactions, 206(2), 364–374. https://doi.org/10.1016/j.cbi.2013.10.010

Comment 6: In the figure legends, please state clearly the scale bar showed in the figures.

Reply 6: The scale bar (*100μm*) is included in figure legends and is shown in the lower right corner of each image. In addition, we also integrated *100μm* in the figures annotations, combined with text to make the picture easy to read (see Page 19, line 620, in the marked copy).

Changes in text: all the scale bar as shown is 100μm.

Comment 7: The rats were injected intraperitoneally with LPS to induce inflammation of the testes. How to identify the testes inflammation. Please state in the results.

Reply 7: We thank this reviewer for raising this concern. The identification of LPS-induced testicular inflammation in this study was mainly through two points. First, LPS was able to induce increased levels of cytokines in testes tissues, including pro-inflammatory cytokines IL-1α, IL-1β, TNF-α, and IL-6, indicating that testicular inflammation is induced by LPS, as described in the article (see Page 9, line 282, in

the marked copy). Secondly, LPS caused histological changes in the testis, such as widening of the interstitial space and increased inflammatory exudation, which was identified as an inflammatory response in the testicular tissue (see Page 9, line 298, in the marked copy).

Changes in text:-

Comment 8: It was better to test the effect of LYC on PPAR signaling pathway.

Reply 8: We thank the reviewer for this constructive suggestion. In this study, as part of a series of studies, in addition to exploring the effects of lycopene on the testis, we also investigated the effects of lycopene on plasma and epididymis (see Page 5, line 141, in the marked copy). Therefore, this series of studies is rich in content, and accordingly, the workload is huge. Now we have selected several key genes in the PPAR signaling pathway to test the effect of LYC on PPAR signaling. Almost all of these genes come from the identification results of this study, so this study not only enriches the basic treatment of inflammatory infertility, but also provides a reliable theoretical basis for further research.

Changes in text: LYC was found to attenuate LPS-induced epididymis injury in our previous study. And its regulatory mechanism is related to the changes of plasma lipidome (22).

Review comments-Reviewer B

Abstract

Comment 1: Please defined PPAR in the abstract.

Reply 1: We thank the editor for the detailed point in the abstract. We have defined PPAR as peroxisome proliferator-activated receptors in the new revision (see Page 2, line 50).

Changes in the text: The expression of retinoid X receptor alpha (RXR) in the peroxisome proliferator-activated receptor (PPAR) signaling pathway was significantly upregulated after LYC treatment, which activated the RXR/PPAR easy dimer.

Reporting Checklist

Comment 2a): For item 5, we cannot find any blinding method in the experiment process. If it is not applicable, please fill with N/A.

Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Only the experimental designer knows

Reply 2a: We thank for the correction. We have filled with N/A in the Reporting Checklist according to the suggestion.

Changes in the text: -

Comment 2b): Please also describe this information in method section of Main Text.

To maximize the objectivity of the presented study, a protocol was prepared before the study. And this protocol was uploaded to the Open Science Frame work (https://osf.io/89cd7/).

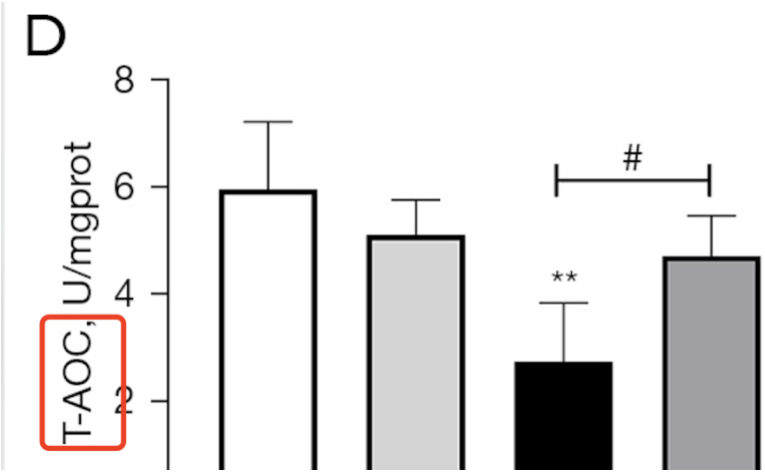
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	To maximize the objectivity of the presented study, a protocol was prepared before the study. And this protocol was uploaded to the Open Science Framework (https://osf.io/89cd7/).

Reply 2b: Thank for the comment and suggestion. We have inserted this sentence in method section of Main Text (see Page 6, line 172-174).

Changes in the text: -

Comment 3: Figure 2

Please unified the format, TAOC or T-AOC?



Reply 3: We thank for the correction. We revised T-AOC to TAOC in the *Figure 2-revised (See page 18, line 612)*

Changes in the text:-

Comment 4: Figure 6

Please explain PPAR in the legend.

Reply 4: Thank for the comment and suggestion. We have explained PPAR in the legend as other abbreviations (See page 22, line 686).

Changes in the text: PPAR, peroxisome proliferator-activated receptor

Comment 5: Ethical Approval

We found that the ethical approval ID (2022JLHSXJDWLS-0021) has been used in another study (Li Y, Zhu J, Zhao X, et al. Oral Lycopene Administration Attenuates Inflammation and Oxidative Stress by Regulating Plasma Lipids in Rats with Lipopolysaccharide-Induced Epididymitis. J Inflamm Res. 2022 Nov 30;15:6517-6531. doi: 10.2147/JIR.S380785. PMID: 36474518), please explain why the current study shares the same ethical approval ID with another study.

Usually, one ID matches with one study.

Reply 5: We thank this editor for raising this concern. We explain this concern in detail below. The experimental animals used in these two articles were the same rats treated with the same experimental protocol, including model establishment, treatment strategy, and therefore the same ethical approval ID was applied. However, the contents of these

two articles are completely different, and each has its own emphasis and highlights. The aim of this study is to investigate the lipidome and transcriptome profiles of the testis in order to elucidate the protective mechanism of lycopene against LPS-induced testicular injury. In another study, the correlation between epididymis damage and plasma lipid changes was investigated to analyze the effect of lycopene-induced systemic lipid changes on male reproductive damage. Therefore, these two articles each belong independently to two parts of this series of studies.

Changes in the text:-