RESEARCH ARTICLE

Lycopene alleviates microplastic-induced reproductive injury in male mice

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Received: June 9, 2026; **accepted:** October 14, 2026.

Microplastics (MPs) have emerged as a global environmental concern due to potential toxicity, yet effective protective strategies against MP-induced reproductive damage remain underexplored. Lycopene (LYC) is a natural carotenoid with high concentrations in ripe tomatoes and other red fruits and vegetables. Physiologically, LYC has been reported to prevent cancers, lower lipid levels, eliminate free radicals, enhance immunity, protect the prostate, and improve sperm vitality. This study evaluated the protective effects of LYC against male reproductive injury induced by polystyrene microplastics (PS-MPs) in Kunming mice, focusing on body weight (BW), testicular weight (TW), sperm count, and testicular histology. Male mice were exposed to PS-MPs (60 nm) at 100 μg/day with or without co-administration of LYC at low, medium, or high dosages for 4 weeks. The results showed that exposure to PS-MPs significantly reduced BW gain and sperm count, while inducing severe testicular histopathological damage including seminiferous tubule atrophy, spermatogenic cell disarray, vacuolization, inflammation, and apoptosis, but had no effect on absolute TW. Importantly, co-treatment with LYC effectively attenuated these adverse effects, restoring BW gain and sperm count, while ameliorating testicular histopathological alterations. The results indicated that LYC exerted significant protective effects against PS-MP-induced male reproductive injury in mice, thereby providing a theoretical foundation for potential application as a dietary intervention.

Keywords: polystyrene microplastics; lycopene; male reproduction; spermatogenesis.

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Introduction

Male infertility is considered a major reproductive issue, affecting about 15% of couples worldwide, and is often caused by impaired spermatogenesis and reduced sperm quality due to genetic factors, hormonal disorders, lifestyle habits, and exposure to environmental pollutants [1]. Microplastics (MPs) have recently become an increasingly serious environmental pollutant with potential risks to ecosystems and human health [2, 3]. MPs are plastic particles with a diameter of less than 5 mm that originate from human activities and are generally classified as either primary MPs released directly from industrial sources or secondary MPs formed through the physical, chemical, or biological degradation of larger plastic debris [2, 4, 5]. MPs are predominantly found in oceans, urban beaches, sediments, and

rivers, and their distribution continues to expand with the increasing use of plastic products [6, 7]. Owing to degradation resistance, MPs are gradually evolving into global environmental pollutants that are readily ingested by organisms and accumulate in tissues. With the increasing use of plastic products, contamination with MPs has become more extensive with intensifying adverse effects. The ingestion of MPs through the food chain has raised concerns about human health as MPs have been shown to accumulate in the gastrointestinal tract, liver, kidneys, brain, and testes of both aquatic organisms and mammals [8].

Studies have demonstrated that exposure to polystyrene microplastics (PS-MPs) reduces sperm velocity and induces concentration-dependent testicular abnormalities characterized by interstitial hyperplasia and seminiferous tubule disorganization in marine organisms [9, 10]. In rodents, exposure to PS-MPs decreases sperm count and quality and even disrupts the blood–testis barrier [11-13]. Beyond reproductive toxicity, PS-MPs also cause adverse effects in both aquatic and terrestrial organisms including physical deterioration, oxidative damage, intestinal barrier dysfunction, inflammation, and neurotoxicity [14-16]. Lycopene (LYC) is a naturally occurring carotenoid abundantly present in tomatoes and other red-pigmented fruits that possess diverse pharmacological properties including antioxidant, anti-inflammatory, antibacterial, antitumor, and immunomodulatory activities [17, 18]. Clinical studies have demonstrated that oral supplementation of LYC can improve seminal parameters in infertile men [19-21]. In rodents, LYC was reported to significantly enhance semen quality and testicular antioxidant capacity following exposure to cisplatin or radiation [18, 22]. Moreover, LYC supplementation was found to protect against reduced sperm motility, morphological abnormalities, acrosomal damage, and necrospermia. These beneficial effects are largely attributable to the capacity to scavenge reactive oxygen species (ROS) and inhibit lipid peroxidation-induced cellular injury. Despite accumulating evidence that exposure to MPs adversely affects reproduction, effective mitigation strategies remain limited because the specific mechanisms underlying impaired spermatogenesis and whether natural antioxidants such as LYC can counteract these effects have not been systematically investigated. Given the widespread distribution of MPs in the environment and the increasing incidence of male infertility, the development of preventive or therapeutic strategies against MP-induced reproductive injury is urgently needed. However, to date, no study has explored the protective effects of LYC against MP-induced male reproductive toxicity in mammals.

The aim of this study was to investigate the protective role of LYC against reproductive injury to male Kunming mice induced by PS-MPs. The findings of this research not only provided a theoretical basis for the application of LYC as a dietary intervention against environmental pollutant-induced reproductive injury but also offered new insights into the development of low-cost and easily accessible nutritional strategies to address the growing threat of MPs to male fertility and reproductive health.

Materials and methods

Experimental animals and treatments

A total of 60 male Kunming mice, aged 5 weeks, were obtained from the Experimental Animal Center of Zhengzhou University (Zhengzhou, Henan, China) and housed under a controlled temperature of 25°C and relative humidity of 50 - 60% with a natural light-dark cycle and fed a standard rodent diet with free access to water. Following an acclimation period of 2 days, the mice were randomly allocated to one of six groups of control, MPS, LYC, MPS + LYC L (2 μg/g body weight (BW)/day), MPS + LYC M (4 μg/g BW/day), and MPS + LYC H (8 μg/g BW/day). PS-MPs with diameter of 60 nm were obtained from Tianjin BaseLine ChromTech Research Centre (Tianjin, China), and a stock suspension was prepared using ultrapure water. LYC was

purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The mice in the treatment groups were first exposed to PS-MPs at 100 μg/L followed by LYC treatments at different dosages. The LYC-alone group received LYC at 4 μg/g BW/day, while the control group received an equivalent volume of oil. All treatments were administered daily for four consecutive weeks. All procedures of this research were approved by the Institutional Animal Care Committee of Huanghuai University (Zhumadian, Henan, China).

Indicator measurements and histological observation

The BW of each mouse was measured weekly throughout the experimental period. At the end of the experiment, all mice were euthanized by cervical dislocation, and the bilateral testes were dissected and weighed to determine the testicular weight (TW). Briefly, following disinfection with alcohol swabs, the abdominal cavity was opened using sterile dissection instruments. The bilateral epididymides and testes were located with tweezers, and the epididymides were carefully removed. After removal of adherent adipose tissue, each epididymis was placed into 1 mL of physiological saline pre-warmed to 37°C to prepare a sperm suspension. The sperm count was performed using a ML-608JZ-II sperm quality analyzer (Nanning Songjing Tianlun Bioengineering Co., Ltd., Nanning, Guangxi, China) [23, 24]. The bilateral testes collected from each mouse were fixed in Bouin's solution (Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China). After 12 h, the testes were removed from the fixative, rinsed with distilled water, and dehydrated with a graded series of ethanol at 70%, 80%, 95%, and 100% concentrations for 10 min at each concentration. The tissues were then cleared in xylene for 30 min and embedded in liquefied paraffin. Following paraffin solidification, the tissues were cut using a Leica RM2016 microtome (Leica Instruments Ltd. Shanghai, Shanghai, China) into 5 μm-thick sections, which were then heated in an oven at 55°C, dewaxed with xylene, and stained with hematoxylin and eosin (H&E). The stained sections were visualized and imaged using an Eclipse E100 optical microscope equipped with a DS U3 camera (Nikon Precision (Shanghai) Co., Ltd., Shanghai, China).

Statistical analysis

Prism 8 software (GraphPad Software, LLC, San Diego, CA, USA) was used in this study for statistical analysis. Group comparisons were performed using one way analysis of variance (ANOVA). A *P* value less than 0.05 was considered statistically significant.

Results

Effects of LYC on PS-MP-induced changes to BW and TW

The results showed that the 60 nm PS-MPs appeared spherical, uniform in size, and distributed evenly, indicating good uniformity and dispersion. After 4 weeks of continuous exposure to the PS-MPs and LYC, mice in the MP-alone group exhibited significantly slower BW gain compared to the other groups ($P < 0.05$) (Figure 1A). In contrast, there were no significant differences in BW among the control, LYC, and the three LYC co-treatment groups. The results indicated that LYC effectively mitigated PS-MP-induced suppression of BW gain in male mice. There were no significant differences in absolute TW among the control, MPS, LYC, and three LYC co-treatment groups (Figure 1B). These findings indicated that, under the current experimental conditions, neither exposure to PS-MPs alone nor supplementation with LYC, whether administered alone or in combination, altered the TW of male mice.

Effects of LYC on PS-MP-induced changes to sperm quality

Exposure to the PS-MPs significantly decreased the sperm count compared to the control group ($P < 0.05$). In addition to PS-MPs exposure, administration of LYC at the low, medium, and high dosages significantly attenuated this effect as evidenced by a marked increase in sperm

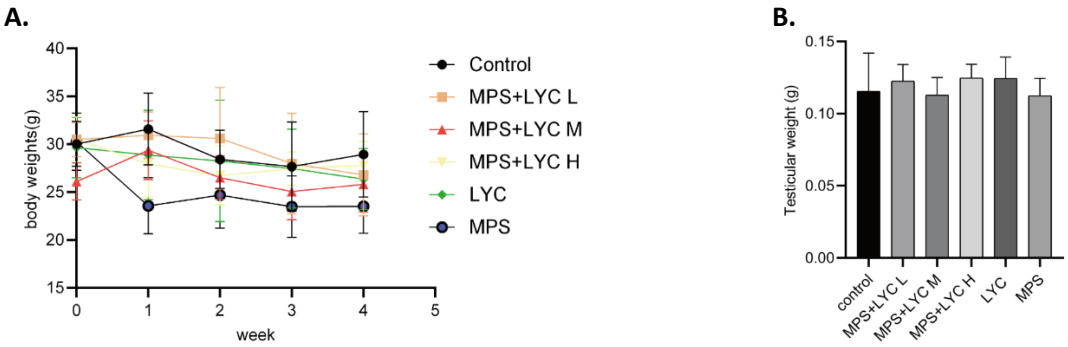


Figure 1. The body weight (BW) and testicular weight (TW) of mice in different groups. **A.** BW in each group of mice was weighed weekly. **B.** TW of mice in different groups weighed on 30th day.

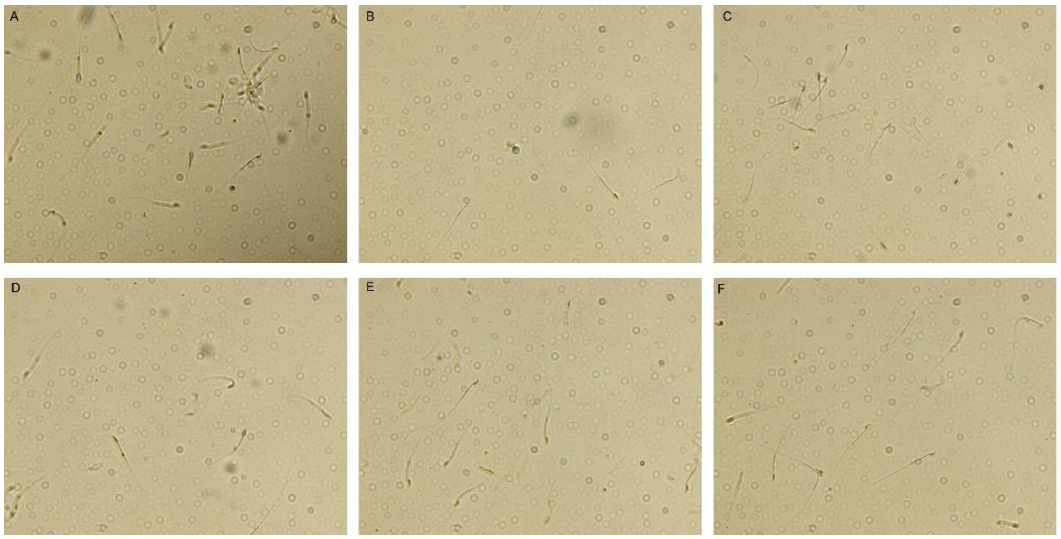


Figure 2. Lycopene prevented the reduction in sperm count caused by microplastics. **A.** Control. **B.** MPS. **C.** LYC. **D.** MPS + LYC L. **E.** MPS + LYC M. **F.** MPS + LYC H.

count relative to the MP group ($P < 0.05$ for all three dosages) (Figure 2). Notably, no significant difference in sperm count was observed between the LYC and control groups (Figure 3). Collectively, these findings demonstrated that LYC effectively ameliorated PS-MP-induced spermatogenic impairment by partially restoring sperm production.

Effects of LYC on PS-MP-induced changes to mouse testicular tissue

H&E staining results showed that testicular tissues from mice exposed to the PS-MPs displayed varying degrees of pathological damage, characterized by inflammation, apoptosis, and a significant reduction in both spermatogenic cells and sperm count compared to the control group. Furthermore, treatment with the PS-MPs induced marked structural abnormalities including seminiferous tubule atrophy and damage, loose arrangement of spermatogenic cells, and the presence of intratubular vacuoles. In contrast to the pronounced testicular damage observed in the MP-exposed group, H&E staining of testicular sections from the LYC-alone group showed no significant histopathological changes compared to the control group, suggesting that LYC administration alone did not adversely affect the testicular structures. More importantly,

treatment with LYC at low, medium, and high dosages significantly alleviated the pathological alterations induced by administration of the PS-MPs. Relative to the MP group, the LYC treatment groups exhibited marked histological improvement including restored seminiferous tubule morphology, increased numbers of spermatogenic cells, heightened sperm production, reduced vacuole formation, and diminished signs of inflammation and apoptosis (Figure 4). These findings demonstrated that LYC effectively ameliorated PS-MP-induced testicular injury and partially restored spermatogenesis, highlighting its potential as a protective agent against environmental reproductive toxicants.

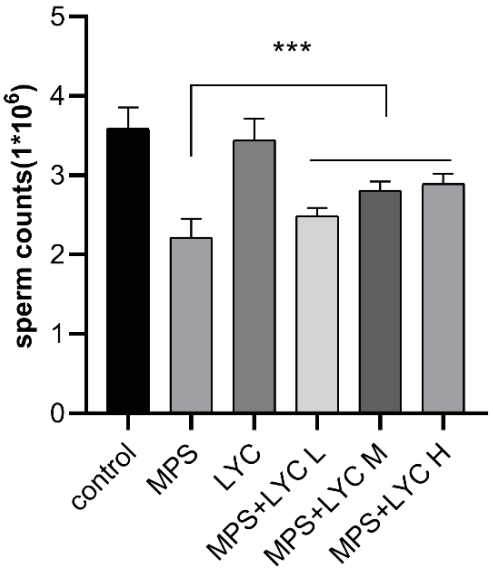


Figure 3. The statistics of sperm count in different groups. ***: $P < 0.001$.

Discussion

In the present study, the protective effect of LYC against male reproductive injury induced by PS-MPs in Kunming mice was investigated. The results revealed that co-administration of LYC not only effectively alleviated PS-MP-induced reductions in BW gain and sperm count but also markedly attenuated testicular histopathological damage including seminiferous tubule atrophy, spermatogenic cell disarray, vacuolization, inflammation, and apoptosis. Taken together, these findings demonstrated that LYC, a naturally occurring antioxidant carotenoid, exerted potent protective effects against PS-MP-mediated male reproductive toxicity. Consistent with a previous report [13], the present study confirmed that exposure to PS-MPs impaired male reproduction. Earlier studies have demonstrated that PS-MPs accumulate in testicular tissues and promote oxidative stress, leading to impaired spermatogenesis and reduced sperm quality [25, 26]. In agreement with these observations, this study found that exposure to PS-MPs significantly decreased the BW and sperm count of the mice and caused pronounced testicular histopathological alterations. More importantly, this study extended these findings by showing that LYC, a well-characterized dietary antioxidant, effectively alleviated these adverse effects. The protective role of LYC in male reproduction has been well documented under various pathological conditions. LYC supplementation has been reported to improve semen quality in infertile men and to alleviate cisplatin- or radiation-induced testicular damage in animals [22, 27, 28]. This study was the first to demonstrate the protective effects of LYC against PS-MP-induced male reproductive injury. The findings of this study filled a critical gap concerning potential dietary interventions against the toxicity of PS-MPs.

Notably, exposure to PS-MPs significantly suppressed BW gain compared to the control group. In contrast, co-administration of LYC effectively restored BW gain to control levels, suggesting that exposure to PS-MPs induced systemic toxicity affecting overall growth and that the protective effects of LYC were not limited to the reproductive system. Interestingly, no significant differences in absolute TW were observed among the experimental groups, which aligned with previous reports that TW might remain unchanged even in the presence of pronounced spermatogenic impairment [29]. As a plausible explanation, TW was a relatively insensitive endpoint following short-term exposure to PS-MPs, and that longer exposure

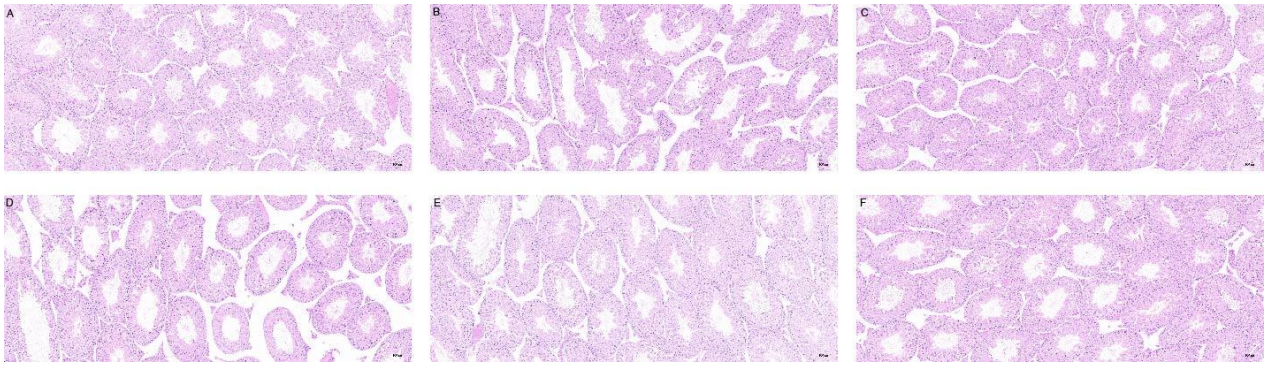


Figure 4. The protective effect of lycopene against microplastics-induced testicular tissue damage. HE staining of mice testes in different groups. **A.** Control. **B.** MPS. **C.** LYC. **D.** MPS + LYC L. **E.** MPS + LYC M. **F.** MPS + LYC H. The images were 200x.

periods or higher dosages might be necessary to detect significant alterations to TW. Alternatively, these results might indicate that the damage induced by exposure to PS-MPs primarily targeted spermatogenic function and testicular architecture rather than overall testicular size. The most striking findings of the present study were the substantial reduction in sperm count and severe testicular histopathological damage following exposure and the significant reversal by co-treatment with LYC. Specifically, H&E staining revealed that exposure to PS-MPs induced atrophy to the seminiferous tubule, disorganization of spermatogenic cells, vacuolization, infiltration of inflammatory cells, and the formation of apoptotic bodies. These morphological alterations were consistent with induced oxidative stress and testicular inflammation as previously reported [30, 31]. Co-treatment with low, medium, and high dosages of LYC ameliorated these pathological changes in a dose-dependent manner and effectively restored the sperm count. These protective effects were likely mediated by the potent antioxidant properties of LYC. As a highly efficient singlet oxygen quencher and free radical scavenger, LYC could neutralize ROS generated by exposure to PS-MPs, thereby inhibiting lipid peroxidation-induced cellular injury [32]. In addition, LYC has been shown to activate the Nrf2/ARE signaling pathway, leading to upregulation of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase [33, 34]. This enhanced antioxidative defense likely protects spermatogenic cells from ROS-mediated apoptosis and supports the preservation of normal spermatogenesis. Although the present study investigated the protective effects of LYC against PS-MP-induced male reproductive injury, the underlying mechanisms were not directly investigated. Hence, future studies should include biochemical assays to assess the extent of oxidative stress, inflammation, and apoptosis.

This study demonstrated that LYC effectively protected against PS-MP-induced male reproductive injury in mice as evidenced by restored BW gain, ameliorated testicular histopathological damage, and normalized sperm count. These findings provided a theoretical basis for the potential use of LYC as a dietary supplement to alleviate PS-MP-mediated reproductive toxicity. Given the widespread and increasing environmental contamination by PS-MPs, this study highlighted the importance of developing effective and safe nutritional strategies to protect human reproductive health.

Acknowledgements

This work was financially supported by the Zhumadian Youth Science and Technology Innovation Special Project (Grant No. QNZX202404), the Major Science and Technology Project of Zhumadian City (Grant No.

ZMDSZDZX2023007, ZMDSZDYF2024002), and the Zhumadian City Biomedical Industry Research and Development Joint Fund Project (Grant No. CY2522005).

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