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Original Article

Curcumin Ameliorates Microplastic-Induced Testicular Inflammatory Damage by Suppressing NLRP3 and WNT/B-Catenin Signaling Pathway Activation

Bingru Luo[1], Li Li[1], Lili Huang[1], Li Deng[2]*

[1]Reproductive Medicine Center, Hunan Provincial People's Hospital, (The First-affiliated Hospital of Hunan Normal University), Changsha, 410078, Hunan, China

[2]Reproductive Medicine Center, Xiangya Hospital, Central South University, Changsha, 410078, Hunan, China

[*]Corresponding Author: Li Deng

Abstract:

Background. While the reproductive toxicity of microplastics (MPs) and associated mechanisms have been extensively documented, the specific signaling pathways underlying MPs-induced testicular injury remain incompletely elucidated. Current studies focus on identifying molecular mechanisms by which MPs disrupt testicular homeostasis through inflammatory and developmental signaling cascades.

Methods. This study investigated the molecular mechanisms of MPs-induced testicular injury using a rodent model exposed to environmentally relevant concentrations of MPs. The synergistic roles of the NLRP3 inflammasome and WNT/β-catenin signaling pathways in mediating inflammatory damage were analyzed through biochemical assays, cytokine profiling, and histopathological evaluation. The therapeutic potential of curcumin was assessed by measuring its effects on NLRP3 inflammasome activation, WNT pathway hyperactivation, and functional recovery of Sertoli cells.

Results. MPs exposure triggered NLRP3 inflammasome assembly and IL-6 release, initiating a localized inflammatory cascade. Concurrently, MPs activated the WNT/β-catenin pathway, disrupting spermatogenic microenvironment homeostasis and exacerbating testicular tissue injury. Curcumin treatment suppressed NLRP3 inflammasome activation, blocked inflammatory cytokine release, and downregulated key WNT pathway effectors. These interventions restored Sertoli cell physiological function and mitigated MPs-induced structural damage.

Conclusion. This study reveals a novel mechanism by which MPs induce testicular injury through synergistic activation of NLRP3 and WNT/β-catenin pathways, and demonstrates curcumin's efficacy as a multi-target therapeutic agent. The findings provide critical insights into environmental pollutant-induced reproductive toxicity and highlight the potential of natural compounds in clinical intervention. Future research should explore crosstalk between these pathways and validate translational strategies for human applications.

Keywords: microplastics, NLRP3, β-catenin, curcumin

1. Introduction

Microplastics (MPs), defined as plastic fragments or particles with diameters <5 mm, are often termed "PM2.5 of the ocean." Common types of MPs include polystyrene (MPs), polyethylene (PE-MP), and polypropylene (PP-MPs), among others. The toxic effects of microplastics—particularly polystyrene MPs—on the testes and reproductive system have been demonstrated through multiple animal studies and mechanistic investigations. Their impacts encompass endocrine disruption, oxidative stress, and cellular

damage. MPs can enter the reproductive system via ingestion, inhalation, or placental transfer, accumulating in testicular tissues and leading to reduced testicular weight (decreased gonadosomatic index), thinning of the basement membrane, and disorganization of seminiferous tubules(Zangene et al., 2024) (T. Zhao et al., 2023). Chronic MP exposure (4–12 weeks) systematically compromises spermatogenic outcomes, characterized by marked reductions in sperm count and motility alongside elevated sperm morphological abnormalities. These deteriorations are mechanistically associated with MP-induced blood-testis barrier (BTB) structural destabilization and mitochondrial bioenergetic disturbances(Jin et al., 2025; Wei et al., 2021). MPs impair testicular reproductive function through multiple pathological mechanisms, primarily mediated by endocrine disruption, oxidative stress induction, BTB compromise, and mitochondrial dysfunction. Their toxicological effects demonstrate distinct dose- and time-dependent toxicity profiles, with potential synergistic interactions when co-exposed with environmental co-pollutants, thereby amplifying reproductive toxicity through multi-target biological disruptions.

MPs induce excessive reactive oxygen species (ROS) production in testicular tissues, leading to diminished mitochondrial membrane potential, reduced ATP synthesis, and subsequent apoptosis of spermatogenic cells(Fang et al., 2024) (Liu et al., 2022). Concurrently, MPs suppress pituitary-testicular axis function by decreasing serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, thereby disrupting endocrine homeostasis. MPs accumulate in murine testes and are internalized into the cytoplasm, further suppressing glutathione peroxidase 1 (GPX1) through ubiquitination-mediated degradation and miR-425-3p regulation. This oxidative stress triggers activation of the endoplasmic reticulum (ER) stress pathway (PERK-EIF2α-ATF4-CHOP), accelerating testosterone metabolism and ultimately reducing testosterone levels. Additionally, MPs impair testosterone homeostasis by interfering with the hypothalamic-pituitary-testicular (HPT) axis(Qu et al., 2024). Polystyrene microplastics (PS-MPs) disrupt Leydig cell functional homeostasis through impaired mitochondrial-ER interorganelle communication, ultimately disrupting testicular endocrine homeostasis through dual interference with steroidogenic biosynthesis and Sertoli cell paracrine signaling networks in the interstitial compartment(Grillo et al., 2024).

MPs may contribute to tissue injury through the regulation of multiple signaling pathways. MPs activate the TLR4/NF-κB pathway, promoting the release of inflammatory cytokines such as TNF-α and IL-6, thereby triggering chronic inflammation and damage in tissues like the testes and kidneys(Hou et al., 2022) (Meng et al., 2022). In diabetic models, MPs exacerbate the epithelial-mesenchymal transition (EMT) process by activating the TGF-β1/Smad2/3 pathway and induce pyroptosis via NLRP3 inflammasome signaling (Feng et al., 2024; S. Li et al., 2024). Additionally, MPs impair intestinal barrier function through the NF-κB/NLRP3/IL-1β/MCLK axis(Zeng et al., 2024). Emerging evidence implicates the Wnt/β-catenin pathway in MPs-induced fibrotic damage across multiple organs: MPs activate Wnt/β-catenin signaling to drive cardiac and hepatic fibrosis, while oxidative stress-mediated apoptosis exacerbates tissue toxicity(X. Li et al., 2024; Li et al., 2020). Inhalation of PS-MPs induces pulmonary fibrosis in mice via oxidative stress and Wnt/β-catenin pathway activation(Li et al., 2022). Chronic MPs exposure also severely compromises the hematopoietic system by disrupting the gut microbiota-hypoxanthine-Wnt axis, thereby suppressing the self-renewal capacity of hematopoietic stem cells(Jiang et al., 2024).

Plant-derived extracts demonstrate significant potential in mitigating MPs toxicity due to their natural antioxidant, anti-inflammatory, and detoxifying properties, primarily through the following mechanisms: 1. Antioxidant Defense System Activation: Polyphenols (e.g., tea polyphenols, curcumin) counteract MPs-induced ROS overproduction through dual-phase intervention - directly scavenging hydroxyl radicals and superoxide anions while orchestrating Nrf2/ARE pathway activation to enhance endogenous antioxidant enzyme biosynthesis. 2. Anti-inflammatory Action: Compounds such as resveratrol inhibit TLR4/NF-κB signaling, reducing the release of pro-inflammatory cytokines (e.g., TNF-α, IL-6). 3. Adsorption and Detoxification: The polyphenolic architecture

enables simultaneous physical adsorption of MPs, and effectively sequestering and detoxifying them. These multifaceted mechanisms highlight the critical role of plant extracts in counteracting MPs-induced cellular and systemic damage. Consequently, the strategic development of plant-based bioactives with multi-target detoxification capacities represents a transformative approach for pioneering sustainable biotechnological solutions against particulate pollutant toxicity.

Curcumin's anti-inflammatory and antioxidant properties, representing its most pharmacologically salient attributes. Curcumin exerts its effects through multi-target and multi-level actions, primarily by modulating key pathways such as NF-κB and Nrf2. It is reported that curcumin neutralizes ROS and reactive nitrogen species (RNS), including superoxide anions (O_2^-), hydroxyl radicals (·OH), and hydrogen peroxide (H_2O_2), via its phenolic hydroxyl structure(Truong et al., 2025). By activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, curcumin can activate endogenous antioxidant systems and enhances the transcription of antioxidant response elements (ARE), upregulating the expression of glutathione (GSH), superoxide dismutase (SOD), and heme oxygenase-1 (HO-1). Curcumin enhances mitochondrial antioxidant defenses, maintains mitochondrial homeostasis, and reduces oxidative stress-mediated cellular injury(Ebrahimi et al., 2023). Despite these established protective properties, investigations into curcumin's potential to counteract MPs-induced toxicity remain critically underexplored.

The murine model, with its high controllability, evolutionary conservation in reproductive biology relative to humans, and robust experimental toolkits, serves as an ideal platform for investigating the reproductive toxicity of microplastics. Research outcomes derived from this model not only elucidate molecular mechanisms but also directly inform human risk assessment and intervention strategy development, establishing an indispensable translational bridge in environmental medical research. In this study, we established a MP-induced injury model using murine testicular interstitial TM3 cells and mice as experimental subjects. Our findings demonstrate that MPs trigger oxidative stress and inflammatory damage in both TM3 cells and testicular tissues, concomitant with activation of the NLRP3 and WNT/β-catenin signaling pathway. Curcumin treatment effectively attenuated MPs-induced inflammatory pathology by concurrently suppressing NLRP3 inflammasome activation and blocking aberrant WNT/β-catenin signaling hyperactivation. This study confirms the protective role of curcumin against MPs-mediated testicular damage and provides a theoretical foundation for its future clinical translation.

Materials and Methods

Characterization of Microplastics

Polystyrene MPs were purchased from Tianjin Baseline ChromTech Research Center (Tianjin, China). The morphological features of MPs were analyzed using scanning electron microscopy (SEM; ZEISS Sigma 300, Germany). Elemental composition was determined via SEM-energy dispersive spectroscopy (SEM-EDS). MPs were dispersed in deionized water at a concentration of 5 μg/mL. After 10 minutes of sonication, the zeta potential and particle size distribution were measured using a Zetasizer Nano ZS system (Malvern Instruments, UK).

Cell Culture

TM3 cells, obtained from Biodee Biotechnology (Zhejiang, China), were cultured in DMEM/F12 medium supplemented with 5% horse serum (HS), 2.5% fetal bovine serum (FBS), and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified incubator with 5% CO_2. Upon reaching 70% confluence, cells were treated with MPs for subsequent experiments.

Cell Viability Assay

TM3 cells were seeded into 96-well plates at a density of 1×10^4 cells/well and pre-cultured for 24 h at 37°C under 5% CO_2. Cells were then exposed to MPs at varying concentrations (0–50 μg/mL) and/or 1 mg/L curcumin for 24 h. Cell viability was assessed using the CCK-8 assay: 100 μL of DMEM/F12 containing 10% CCK-8 reagent was added to each well, followed by incubation at 37°C for 2–4 h. Absorbance was measured at 450 nm using a microplate reader. Results were calculated as percentage cell viability and expressed as mean ± standard deviation.

ROS Detection

TM3 cells cultured in 12-well plates were treated with 5 μg/mL MPs and/or 1 mg/L curcumin for 24 h. After treatment, cells were incubated with 10 μM 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) probe (Servicebio, Wuhan, China) in serum-free medium at 37°C in the dark for 30 min. Following two washes with phosphate-buffered saline (PBS), fluorescent images were captured using a fluorescence microscope (Leica DMi8, Wetzlar, Germany).

Mouse Model

Mice were provided by Hunan Slack Jingda Laboratory Animal Co., Ltd. (Changsha, China). All experimental mice in this study were housed in a Specific Pathogen Free (SPF)-grade barrier environment, with environmental parameters strictly monitored and recorded. The specific conditions are as follows: Temperature: 22 ± 1°C, Humidity: 50 ± 10%, Light cycle: 12 hours light/12 hours dark.

Healthy male C57 mice (8 weeks old) were randomly divided into four experimental groups (n = 12 per group): (i) Control (saline), (ii) MPs (20 mg/L), (iii) Curcumin (50 mg/kg), (iv) MPs (20 mg/L) + Curcumin (50 mg/kg). Mice in treatment groups received MPs or curcumin via drinking water for 90 days, while controls were administered equivalent volumes of saline.Animals exhibiting >15% weight loss, persistent diarrhea, severe lethargy, or stress-related behaviors (e.g., self-injury) were result in the exclusion of the animal from the experiment and euthanasia was be performed. After the experimental period, mice were euthanized after anesthesia induction with 4% isoflurane, and testicular tissues were collected for histopathological analysis and immunofluorescence. All protocols were approved by the Institutional Animal Ethics Committee of Xiangya Hospital(NO. 2023111825) and conducted in strict accordance with ethical guidelines for animal research. Data from 5 independent replicates are expressed as mean ± standard deviation. At the conclusion of the experiment, all surviving animals should be humanely euthanized in accordance with established institutional animal care and use committee (IACUC) protocols and relevant ethical guidelines.

Immunofluorescence

Testicular tissues from C57 mice were paraffin-embedded, and sections were deparaffinized with xylene and rehydrated through graded ethanol solutions. Antigen retrieval was performed by microwaving in citrate buffer (pH 6.0, 95°C, 10 min), followed by natural cooling to room temperature. Sections were blocked with 5% bovine serum albumin (BSA) for 30 min. Immunostaining was conducted using primary antibodies against NLRP3 (Proteintech, China; 1:200) and β-catenin (Proteintech, China; 1:200), incubated overnight at 4°C. After PBS washes, sections were incubated with Cy3-labeled anti-rabbit fluorescent secondary antibodies (Servicebio, China) in the dark, followed by nuclear counterstaining with DAPI for 5–10 min. Fluorescent images were acquired using a Zeiss LSM880 confocal microscope (Oberkochen, Germany). Quantitative analysis was performed using ImageJ software (NIH), with data from six biological replicates per group expressed as mean ± standard deviation (SD).

Quantitative Real-Time PCR

Total RNA was extracted from cells and testicular tissues using TRIzol reagent (Sigma, St. Louis, USA), followed by quantification and purity assessment. cDNA synthesis was performed using a reverse transcription kit (Servicebio, Wuhan, China). qPCR was carried out with SYBR Green (Servicebio, Wuhan, China) to measure mRNA expression levels of *NLRP3* , *IL-6* and β-catenin, using cDNA as the template. Relative gene expression was calculated via the $2^{-\Delta\Delta Ct}$ method, with data presented as mean ± standard deviation. The primers used in the study are listed in Table 1.

Table 1 Primers use d for quantitative real-time PCR

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	AGGTCGGAGTCAACGGATTT	ATCGCCCCACTTGATTTTGG
NLRP3	TGGCCTTACGTCAGTCTGTT	CAACACCTGAAGCTTGCAGT
IL-6	AGTCCTGATCCAGTTCCTGC	CTACATTTGCCGAAGAGCCC
β-catenin（CTNNB1）	AGGATGCCTTGGGTATGGAC	AGTATCAAACCAGGCCAGCT

Histological Analysis

Testicular tissues from C57 mice were fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned at 4 μm thickness. After deparaffinization, sections were stained with hematoxylin-eosin (HE) to evaluate seminiferous tubule structural integrity. Imaging was performed using an optical microscope (Nikon, Japan).

Cytokine Quantification

After treating TM3 cells with MPs (5 mg/L) and/or curcumin for 24 h, supernatants were collected. Testicular tissues were weighed, homogenized in PBS, and centrifuged (12,000×g, 10 min) to obtain tissue supernatants. Concentrations of IL-6, TNF-α, and IL-1β in cell supernatants and tissue homogenates were measured using ELISA kits. Data are expressed as mean ± SD, and intergroup comparisons were analyzed by one-way ANOVA (GraphPad Prism 9.0).

Statistical Analysis

Data are presented as mean ±SD. The Shapiro-Wilk test was used to assess whether the data conform to a normal distribution. When the normality assumption is satisfied, analysis of variance (ANOVA) is further performed to compare differences between groups. Group comparisons were performed using one-way ANOVA (Tukey's multiple comparison test) or independent-sample t-test (for two groups). All experiments were performed in at least three independent replicates. Statistical analyses were conducted using GraphPad Prism 9.0, with significance set at $P < 0.05$.

Results

Microplastics Induce Oxidative Stress and Inflammatory Injury in TM3 Cells

The morphological characteristics of MPs were first identified via SEM (Fig. 1A),

and SEM-EDS analysis confirmed that the primary components of MPs were carbon (C) and oxygen (O) (Fig. 1B). Zeta potential and particle size distribution analyses demonstrated the high stability of MPs (Fig. 1C, D). Treatment of TM3 cells with 5 μg/mL MPs revealed cellular internalization of MPs (Fig. 2A). MPs exposure induced a concentration-dependent decline in TM3 cell viability (Fig. 2B). Furthermore, MPs triggered inflammatory responses in TM3 cells, significantly upregulating mRNA expression of *TNF-α* and *IL-6* to 2.11 ± 0.23-fold and 2.68 ± 0.17-fold of the control group, respectively (Fig. 2C, D). ELISA confirmed elevated secretion of TNF-α and IL-6 in MPs-treated TM3 cell supernatants (Fig. 2E, F). Additionally, quantitative analysis using DCFH-DA fluorescence staining revealed a significant elevation in intracellular ROS levels upon MPs exposure, with oxidative stress parameters reaching 2.63 ± 0.19-fold higher values compared to untreated controls(Fig. 2G, H). These findings collectively demonstrate that MPs induce oxidative stress and inflammatory injury in TM3 cells.

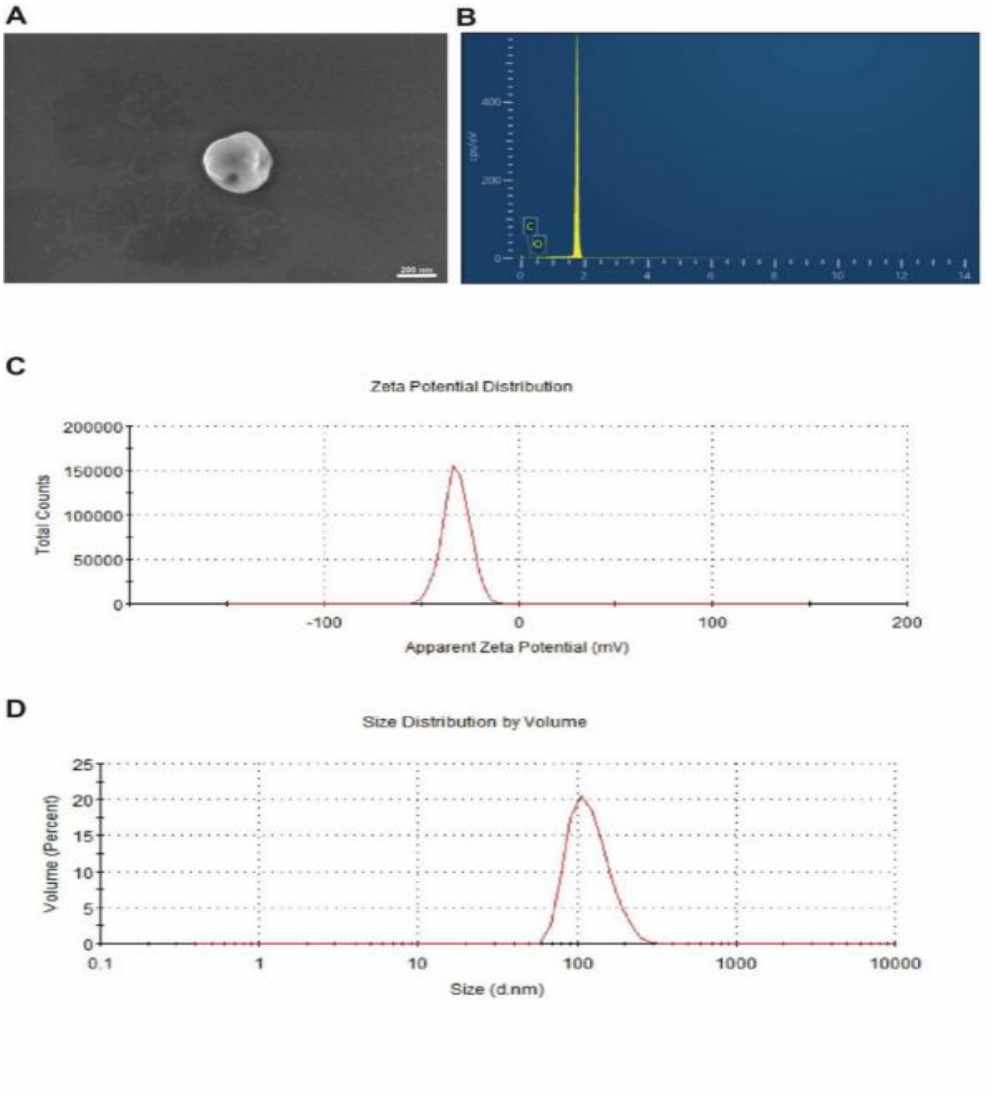

Figure 1 Characterization of Microplastics.
(A) Morphology of MPs under SEM.
(B) SEM-EDS analysis of MPs, indicating carbon (C) and oxygen (O) as the predominant. elements.
(C) Zeta potential analysis of MPs, showing a surface charge of -31.8 mV.
(D) Particle size distribution of MPs, with an average diameter of 131.5 nm.

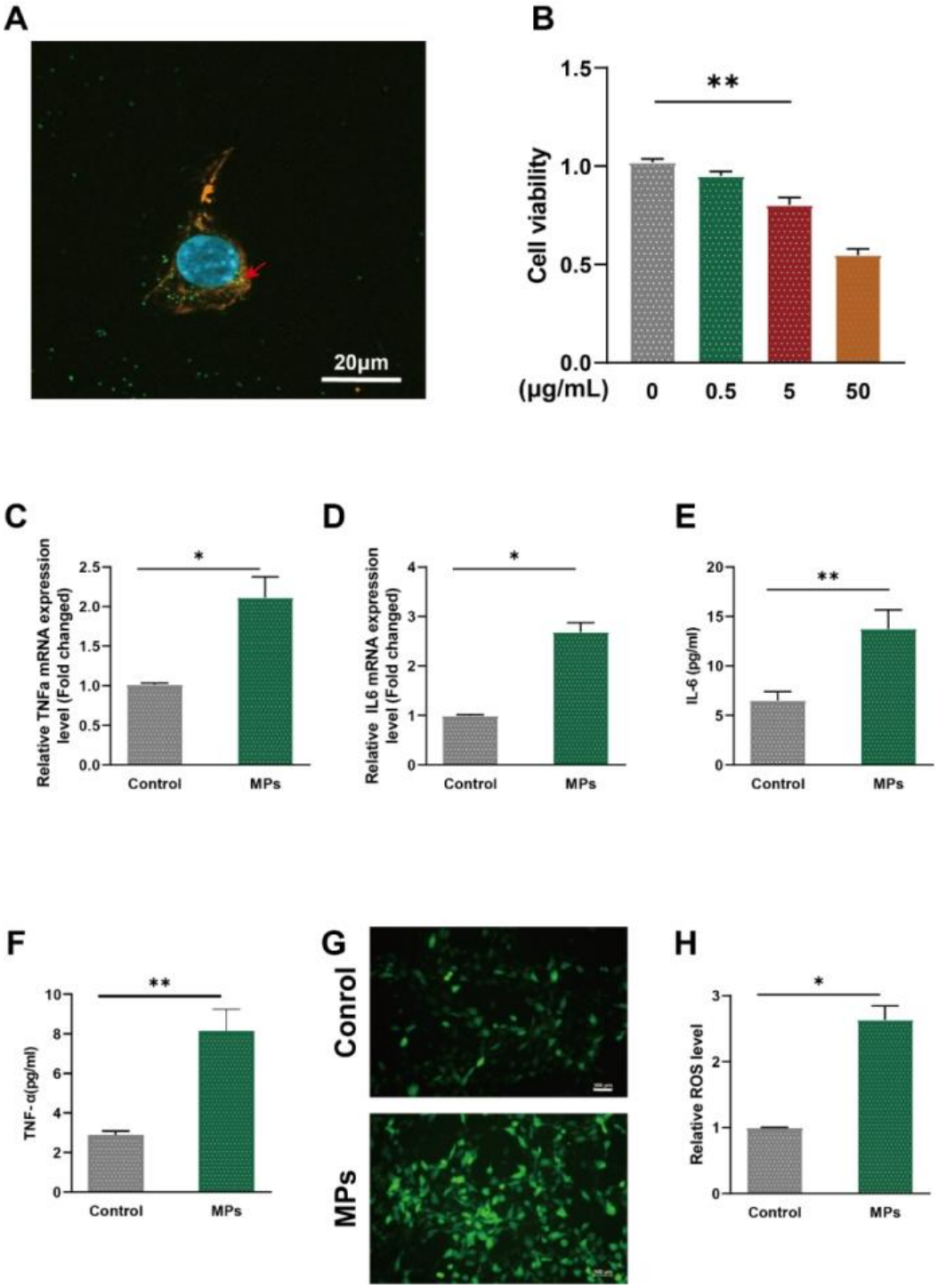

Figure 2 Microplastics Induce Oxidative Stress and Inflammatory Injury in TM3 Cells. (A) Internalization of PS-MPs visualized via confocal microscopy: MPs (green) and F-actin (yellow). (B) Viability of TM3 cells treated with varying concentrations of MPs (n = 5). (C, D) qPCR analysis of *TNF-α* and *IL-6* mRNA expression levels. The control group was normalized to 1.0. (E, F) ELISA quantification of TNF-α and IL-6 levels in TM3 cell supernatants (n = 5). (G, H) Intracellular ROS levels in TM3 cells post-MPs treatment, measured using DCFDA (n = 5). Asterisks (*) indicate significant intergroup differences (*P < 0.05*). Data are expressed as mean ± SD.

Microplastics Activate the NLRP3 and WNT/β-Catenin Signaling Pathway in TM3 Cells

As a core component of the inflammasome, NLRP3 plays a pivotal role in innate immune responses and inflammatory cascades. We further investigated NLRP3 expression following MPs exposure. TM3 cells treated with MPs exhibited a significant induction of NLRP3 inflammasome activation, as evidenced by qPCR analysis

demonstrating a 3.01 ± 0.36-fold upregulation in NLRP3 mRNA expression compared to untreated controls(Fig. 3A). Concurrently, MPs activated the WNT/β-catenin signaling pathway in TM3 cells (Fig. 3B). Immunofluorescence assays also confirmed MPs-induced activation of β-catenin (Fig. 3C, D) and NLRP3 (Fig. 3E, F) at the protein level, with expression levels elevated to 1.99 ± 0.11-fold and 2.37 ± 0.15-fold of the control, respectively. These results demonstrate that MPs exposure activates the NLRP3 and WNT/β-catenin signaling pathway in TM3 cells.

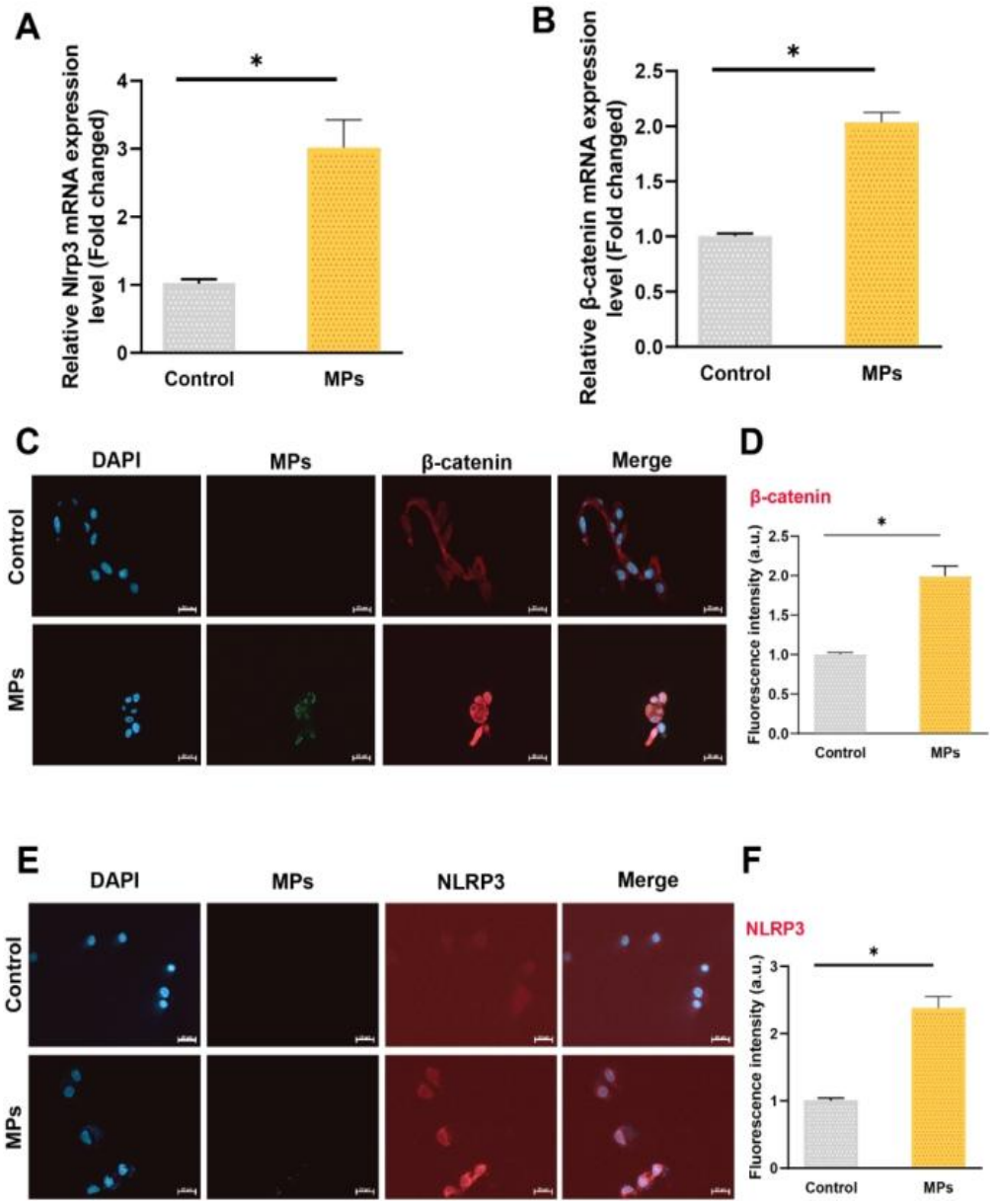

Figure 3. Microplastics Activate the NLRP3 and WNT/β-Catenin Signaling Pathway. (A, B) qPCR analysis of *NLRP3* and β-catenin mRNA expression levels. The control group was normalized to 1.0 (n = 5). (C, D) Immunofluorescence detection of β-catenin protein expression in MPs-treated TM3 cells (n = 5). (E, F) Immunofluorescence detection of NLRP3 protein expression in MPs-treated TM3 cells (n = 5). Asterisks (*) indicate significant intergroup differences (*P* < *0.05*). Data are expressed as mean ± SD.

Microplastics Trigger NLRP3-WNT/β-Catenin Crosstalk-Mediated Testicular Inflammatory Pathology in Murine Models

A chronic oral exposure model was established in C57BL/6J mice through administration of MPs via drinking water for 90 days. MPs exposure induced structural abnormalities in testicular

tissues, control group testes exhibited well-organized seminiferous tubules with morphologically intact spermatogonia, spermatocytes, and clearly visible spermatozoa. In contrast, the MPs group displayed chromatin condensation in spermatogonia and spermatocytes, widened intercellular gaps, reduced germ cell counts, and decreased sperm numbers (Fig. 4A). ELISA analysis revealed elevated IL-6 production in MPs-treated testicular tissues (Fig. 4B). qPCR confirmed significant upregulation of *NLRP3* and β-catenin mRNA levels in the MPs group (Fig. 4C, D). Immunofluorescence staining demonstrated increased β-catenin expression, particularly localized in spermatogonia (Fig. 4E, F), alongside markedly elevated NLRP3 protein levels in MPs-exposed testes (Fig. 4G, H). Thus, these results indicate that MPs induce testicular injury *in vivo*, likely through activation of the NLRP3 and WNT/β-catenin signaling pathway.

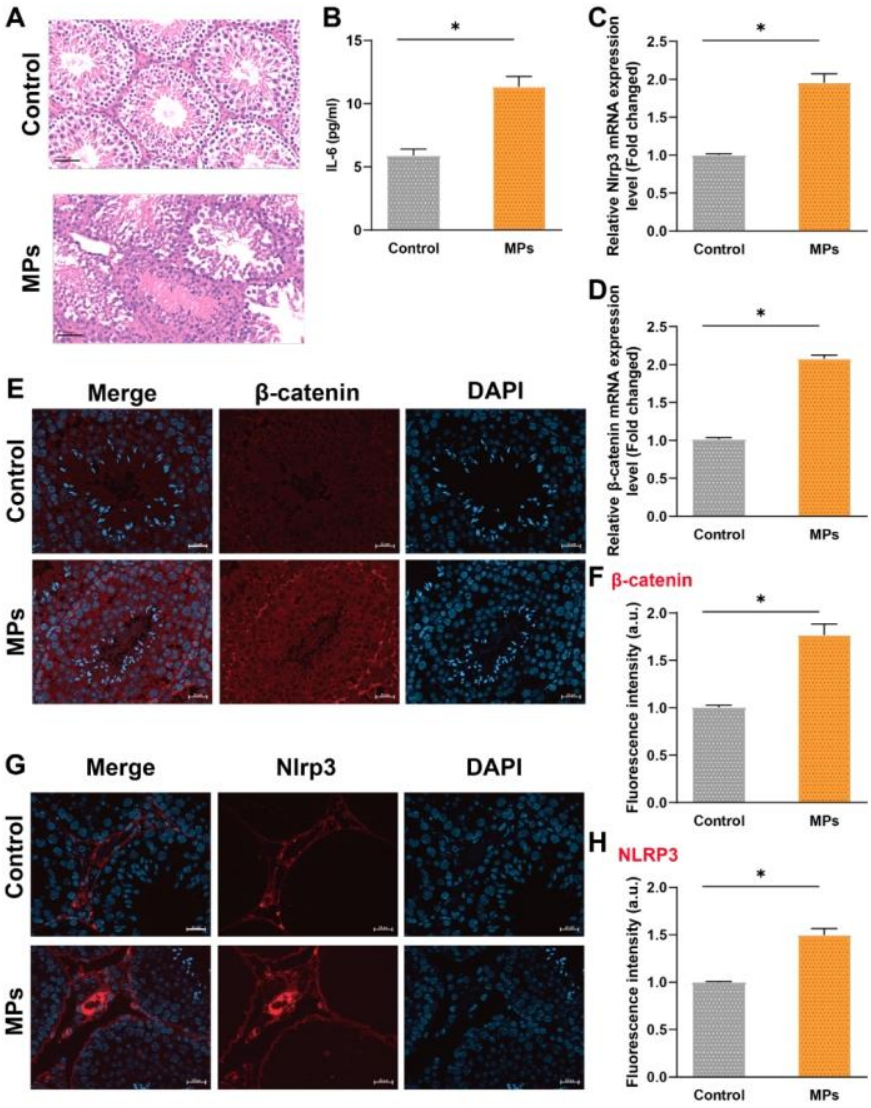

Figure 4 Microplastics Trigger NLRP3-WNT/β-Catenin Crosstalk-Mediated Testicular Inflammatory Pathology in Murine Models.

(A) HE staining of testicular tissues from C57 mice after MPs intervention.

(B) ELISA quantification of IL-6 levels in testicular tissues (n = 5).

(C, D) qPCR analysis of *NLRP3* and β-catenin mRNA levels in testicular tissues (n = 5).

(E, F) Immunofluorescence analysis of β-catenin expression in testicular tissues following MPs treatment (n = 5).

(G, H) Immunofluorescence analysis of NLRP3 expression in testicular tissues following MPs treatment (n = 5). Asterisks (*) indicate significant intergroup differences (*P* < 0.05). Data are expressed as mean ± SD.

Curcumin Suppresses Microplastic-Induced Inflammatory Damage in TM3 Cells via Dual Modulation of NLRP3 Inflammasome and WNT/β-Catenin Crosstalk

Prior studies have established the anti-inflammatory and antioxidant properties of curcumin. Rescue experiments employing curcumin were performed to evaluate its cytoprotective potential against MPs-induced cytotoxicity in TM3 cells. Functional analysis revealed that pharmacological intervention with curcumin ameliorated MPs-mediated cellular damage, with concomitant restoration of cellular homeostasis compared to MPs-treated groups (Fig. 5A). ELISA confirmed that curcumin reduced MPs-triggered TNF-α and IL-6 secretion (Fig. 5B, C). DCFH-DA-based ROS detection revealed that curcumin significantly mitigated MPs-induced oxidative stress, decreasing ROS levels to 0.72 ± 0.04-fold of the MPs group (Fig. 5D, E). Furthermore, curcumin suppressed MPs-induced upregulation of *NLRP3* and β-catenin mRNA expression (Fig. 5F, G). These results collectively indicate that curcumin effectively counteracts MPs-induced inflammatory injury and NLRP3-WNT/β-catenin pathway activation in TM3 cells.

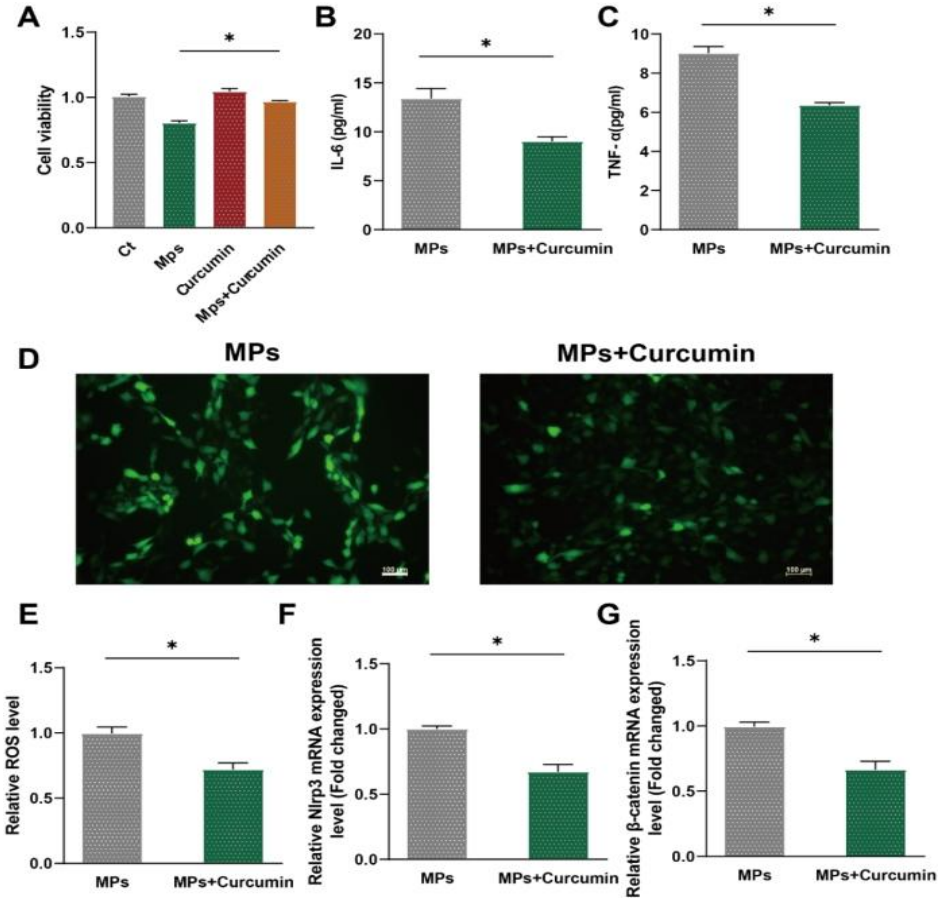

Figure 5 Curcumin Suppresses Microplastic-Induced Inflammatory Damage in TM3 Cells via Dual Modulation of NLRP3 Inflammasome and WNT/β-Catenin Crosstalk.

(A) Cell viability following curcumin intervention (n = 5).

(B, C) ELISA quantification of IL-6 and TNF-α levels in TM3 cell supernatants after curcumin treatment (n = 5).

(D, E) Intracellular ROS levels after curcumin intervention, measured using DCFH-DA assay Fluorescence-based detection of intracellular ROS levels using DCFH-DA assay was employed following curcumin pharmacological modulation(n = 5).

(F, G) qPCR analysis of *NLRP3* and β-catenin mRNA expression levels in TM3 cells treated with curcumin (n = 5).

Asterisks (*) indicate significant intergroup differences (P < 0.05). Data are expressed as mean ± SD.

Curcumin Suppresses Microplastic-Induced Testicular Inflammatory Pathology in Mice via Dual Modulation of NLRP3 Inflammasome and WNT/β-Catenin Crosstalk

To investigate the protective effects of curcumin against MPs-induced testicular injury, we examined its role in the MPs-exposed murine model. C57 mice co-administered with curcumin and MPs (20 mg/L) were evaluated. HE staining revealed that curcumin treatment ameliorated MPs-induced structural damage in testicular tissues (Fig. 6A). ELISA analysis demonstrated reduced IL-6 production in curcumin-treated testicular tissues compared to the MPs group (Fig. 6B). qPCR confirmed that curcumin suppressed MPs-induced upregulation of *NLRP3* and β-catenin mRNA expression, decreasing levels to 0.68 ± 0.04-fold and 0.71 ± 0.04-fold of the MPs group, respectively (Fig. 6C, D). Immunofluorescence staining further showed that curcumin attenuated MPs-induced β-catenin expression (Fig. 6E, F) and inhibited NLRP3 protein expression in testicular tissues (Fig. 6G, H). These results demonstrate that curcumin effectively mitigates MPs-induced inflammatory injury and NLRP3-WNT/β-catenin pathway activation in the chronic MPs exposure mice model.

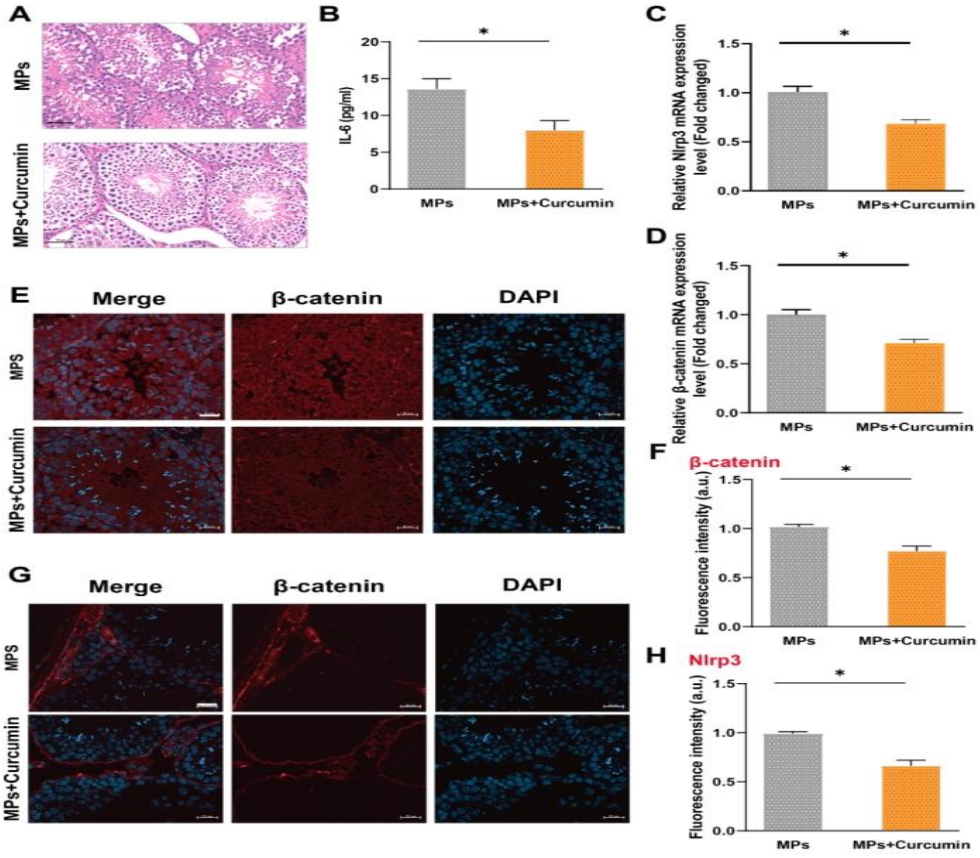

Fig. 6. Curcumin Suppresses Microplastic-Induced Testicular Inflammatory Pathology in Mice via Dual Modulation of NLRP3 Inflammasome and WNT/β-Catenin Crosstalk.
(A) HE staining of testicular tissues from MPs-exposed mice with or without curcumin treatment.
(B) ELISA quantification of IL-6 levels in testicular tissue homogenates from MPs-exposed mice with or without curcumin treatment (n = 5).
(C, D) qPCR analysis of *NLRP3* and β-catenin mRNA expression levels in MPs-exposed mice testicular tissues following curcumin intervention (n = 5).
(E, F) Immunofluorescence analysis of β-catenin expression in in MPs-exposed mice testicular tissues after curcumin treatment (n = 5).
(G, H) Immunofluorescence analysis of NLRP3 expression in testicular tissues after curcumin treatment (n = 5).
Asterisks (*) indicate significant intergroup differences (*P* < 0.05). Data are presented as mean ± SD.

Discussion

As emerging environmental contaminants, MPs exhibit ubiquitous distribution across global ecosystems. From deep-sea sediments to atmospheric dust, and from bottled water to food chains, secondary microplastic particles (<5 μm) have breached multiple biological barriers, leading to bioaccumulation in humans. Notably, MPs have been detected in human semen samples(Q. Zhao et al., 2023). Furthermore, MPs can compromise the BTB and induce reproductive dysfunction in murine models(Li et al., 2021). The spatiotemporal correlation between declining global male fertility and environmental pollutant exposure has become a critical public health concern. However, the mechanisms underlying MPs-induced testicular injury remain poorly elucidated. In this study, we established *in vitro* (TM3 cells) and *in vivo* (murine) MPs-induced injury models. MPs exposure triggered TNF-α and IL-6 production, confirming oxidative stress and inflammatory injury in both cellular and animal systems. These findings align with prior evidence of MPs-driven redox imbalance and cytokine dysregulation, reinforcing the role of oxidative-inflammatory crosstalk in MPs-mediated reproductive toxicity. Furthermore, we demonstrated curcumin's protective efficacy in both MPs-induced TM3 cell and murine testicular injury models. Curcumin effectively attenuated MPs-induced inflammatory pathology by concurrently suppressing NLRP3 inflammasome activation and inhibiting canonical WNT/β-catenin signaling hyperactivation. These findings elucidate curcumin's role in alleviating MPs-mediated testicular toxicity, with its pleiotropic effects and low toxicity offering unique advantages for combating MPs-related disorders. This study provides a theoretical foundation for the clinical translation of curcumin as a therapeutic agent against environmental pollutant-induced reproductive damage.

MPs induce tissue and cellular injury primarily through inflammation and oxidative stress. Polyethylene microplastics (PE-MPs) reduce the activity of antioxidant enzymes, including catalase (CAT), glutathione reductase (GSR), SOD, HO-1, and glutathione peroxidase (GPx), while markedly increasing malondialdehyde (MDA) and ROS levels(Kadac-Czapska et al., 2024). Additionally, PE-MPs suppress steroidogenic enzyme expression and Bcl-2 (anti-apoptotic protein), while elevating Caspase-3 and Bax (pro-apoptotic) levels, thereby accelerating apoptosis(Zhu et al., 2023). PS-MPs exposure significantly decreases follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels, concomitant with elevated inflammatory indices and histopathological damage in testes. Kaempferide has been shown to mitigate polyethylene microplastic-induced testicular toxicity by modulating steroidogenic enzymes, apoptosis markers, and inflammatory mediators, while restoring testicular histoarchitecture(Ijaz et al., 2024).

MPs induce tissue injury through multiple signaling pathways. Polystyrene nanoplastics (PS-NPs) activate the NF-κB/TLR4/GSDMD pathway in mice, promoting ROS generation and subsequent hepatic damage(Chen et al., 2024). PS-MPs suppress steroidogenic acute regulatory protein (StAR) expression by inhibiting the AC/cAMP/PKA pathway, thereby downregulating testosterone synthesis and exacerbating testicular injury(Jin et al., 2022). Additionally, PS-MPs trigger testicular cell apoptosis and necrosis via ROS/MAPK/HIF1α pathway activation(Wang et al., 2022). Pyroptosis plays a critical role in regulating PS-MPs-induced vascular endothelial injury. PS-MPs trigger pyroptosis signaling pathways in HUVECs, and inhibition of NLRP3-mediated pyroptosis effectively protects HUVECs from PS-MPs-induced damage(Huo et al., 2024). Our study reveals a previously unrecognized crosstalk between NLRP3 inflammasome activation and canonical WNT/β-catenin signaling as the key mechanistic driver of MPs -induced testicular toxicity. Both *in vitro* and *in vivo* experiments demonstrated significant upregulation of NLRP3 and β-catenin expression following MPs exposure, further validating the pivotal role of this pathway in MPs-induced tissue damage.

Curcumin, a natural polyphenolic compound, has garnered extensive research interest for its therapeutic potential in neurodegenerative diseases, metabolic disorders, cancer, and inflammatory conditions, as well as its protective effects against environmental pollutants such as heavy metals(Liu et al., 2023) (Nguyen & Kim, 2022). Prior studies indicate that particulate matter (PM), a critical environmental pollutant,

promotes respiratory diseases by inducing inflammatory responses. For instance, inhaled PM triggers immune activation in alveolar macrophages and airway epithelial cells. Curcumin mitigates PM-induced pulmonary inflammation by modulating MAPK/NF-κB signaling and suppressing pro-inflammatory mediator production. It also attenuates ROS generation via the Nrf2/HO-1 axis, thereby counteracting pollutant-driven oxidative stress and lung injury (Lee et al., 2023).

This study revealed that aberrant activation of the NLRP3 inflammasome and WNT/β-catenin signaling pathway constitutes a central mechanism underlying MPs-induced testicular injury. By concurrently suppressing NLRP3-mediated inflammatory cascades and β-catenin-driven transcriptional dysregulation, curcumin emerges as a promising dual-target therapeutic agent against microplastic-induced male reproductive toxicity. However, the short-term murine exposure model employed here does not fully replicate chronic low-dose MPs exposure patterns typical in humans. Future studies should establish cross-species chronic exposure systems to better mimic real-world scenarios. Given the dose-dependent nature of MPs toxicity, further investigations into broader concentration ranges are warranted to delineate threshold effects and non-linear responses in testicular damage. This study did not systematically evaluate toxicity disparities among different polymer types (e.g., PET, PP) or synergistic interactions with co-pollutants (e.g., bisphenol A). Developing multi-factor exposure models will enhance risk assessment accuracy.

Conclusion

This study elucidates that MPs-induce testicular injury through synergistic activation of the NLRP3 inflammasome and dysregulated WNT/β-catenin signaling. Curcumin counteracts MPs toxicity by dual-target inhibition: suppressing NLRP3-mediated inflammatory cascades and normalizing WNT pathway hyperactivity, thereby restoring Sertoli cell function. These findings unveil a novel NLRP3-WNT crosstalk mechanism in MPs-induced reproductive damage and validate curcumin's multi-target therapeutic potential.

Additional Information and Declarations

Author Contributions

Bingru Luo and Li Li: investigation, conceptualization, supervision, writing.

Lili Huang: investigation, methodology, visualization.

Li Deng: conceptualization, investigation, visualization, formal analysis, methodology, writing – review and editing, funding acquisition.

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Institutional Review Board Statement

This study was approved by the Institutional Animal Ethics Committee of Xiangya Hospital.

Informed Consent Statement

Not applicable.

Data Availability Statement

The datasets used in the present study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

NLRP3	NOD-like receptor family pyrin domain-containing protein 3
BTB	blood-testis barrier
ROS	reactive oxygen species
FSH	follicle-stimulating hormone
LH	luteinizing hormone

HPT	hypothalamic-pituitary-testicular
EMT	epithelial-mesenchymal transition
Nrf2	nuclear factor erythroid 2-related factor 2
ARE	antioxidant response elements
PE-MPs	Polyethylene microplastics
SOD	superoxide dismutase
HO-1	heme oxygenase-1
GPx	glutathione peroxidase
MDA	malondialdehyde