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Polystyrene microplastics induce blood-testis barrier disruption regulated by MAPK-Nrf2 signaling pathway in rats

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Abstract

As a persistent organic pollutant, microplastics (MPs) have been reported to induce sperm quantity decrease in male rats. However, the related mechanism remains obscure. Therefore, this study is intended to explore the effects of polystyrene microplastics (PS-MPs) on male reproduction and its related mechanism of blood-testis barrier (BTB) impairment. Thirty-two adult male Wistar rats were divided randomly into four groups fed with PS-MPs for 90 days at the dose of 0 mg/d (control group), 0.015 mg/d, 0.15 mg/d and 1.5 mg/d respectively. The present results have showed that PS-MPs exposure led to the damage of seminiferous tubule, resulted in apoptosis of spermatogenic cell and decreased the motility and concentration of sperm, while the abnormality of sperm was elevated. Meanwhile, PS-MPs could induce oxidative stress and activate p38 MAPK pathway and thus deplete the nuclear factor erythroid-2 related factor 2 (Nrf2). Noteworthily, the adverse effect of PS-MPs on BTB is only significant in 0.15 mg/d and 1.5 mg/d groups ,which demonstrated that high-dose PS-MPs exposure may lead to the destruction of BTB integrity and the apoptosis of spermatogenic cells through the activation of MAPK-Nrf2 pathway. The current study provided novelty evidence for elucidating the effects of PS-MPs on male reproductive toxicity and its potential mechanism.

Introduction

Plastic appeared over one hundred years ago and the trend of plastic products usage has swept across the world in the area of agriculture, industry and even our daily life owing to its convenience Therefore, a mass of plastic wastes have been dropped into environments and further decomposed into tiny particles which are called microplastics (MPs) with diameter less than 5 mm (Arthur et al., 2008; Boucher and Friot, 2017; Law and Thompson, 2014). MPs have been widely distributed, existing in marine, freshwater and land ecosystem (Santos et al., 2015; Dekiff et al., 2014; Wagner et al., 2014) and even in the polar regions (Lusher et al., 2015). Meanwhile, On the one hand, previously-published studies have detected MPs in food and air (Liebezeit and Liebezeit, 2013) and is difficult to degrade in the environment (Rios et al., 2007). On the other hand, other studies have proved that MPs can been absorbed by organism through ingestion and inhalation (Li et al., 2015; Van Cauwenberghe and Janssen, 2014) and even enter into circulatory system (Hussain et al., 2001) and accumulate in testis (Jin et al., 2020). Hence, owing to its pervasiveness of distribution and accumulation in organism, MPs has been listed as one of the persistent organic pollutants at the second UN Environmental Conference held in 2016, with potential hazards to human health.

Male infertility, a serious reproductive health problem for human, is getting into the spotlight for posing a threat to human reproductive safety with the deterioration of the environment nowadays. A study has shown that 8%-12% of the world's gestational age couples experienced childlessness (Inhorn and Patrizio, 2015), in which male reproductive problems accounted for 50% of these cases (Bisht et al., 2017). Meanwhile, the normal concentration and sperm count of young Chinese men have been found a drop over the past years (Huang et al., 2017). Environmental pollutions including those caused by plastics are considered as one of the key factors leading to the declining reproductive capacity of the male. Previous

studies have shown that exposure to polystyrene microplastics (PS-MPs) can reduce sperm count in oysters (Sussarellu et al., 2016) and can also lead to abnormal proliferation of germ cell in male Japanese medaka (Rochman et al., 2014). As for mammals, the decrease in mice sperm quality and quantity is closely associated with the exposure of PS-MPs (Xie et al., 2020). However, as a new persistent pollutant, there is a dearth of related information and researches on MPs specially its underlying toxic mechanism.

Multiple signaling pathways are involved with MPs, in which oxidative stress is viewed as a main and highlighting toxic mechanism as solidly supported by previous studies (Zhu et al., 2020; Hamed et al., 2020). Oxidative stress is a state of imbalance between antioxidant defenses and relative oxygen species (ROS) production, characterized with increasing level of malondialdehyde (MDA) and declining levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) (Wang et al., 2019). Meanwhile, it is demonstrated that the p38 mitogen-activated protein kinases (MAPK) pathway is activated with the enhancement of ROS and MDA levels and decline of glutathione (GSH) levels after exposure to PS-MPs in mice testis (Jin et al., 2020). Furthermore, previous studies have solidly certified the inseparable association between p38 pathway activation and blood-testis barrier (BTB) impairment. For example, BTB was disrupted by activating TGF-β3/p38 MAPK pathway in rats (Liu et al., 2018) and betaine has protective effects on BTB through downregulating the expressions of p38 MAPK phosphorylation (Jiang et al., 2019). However, it remains unclear whether BTB impairment can be induced under the effect of MPs through p38 MAPK pathway(Eerkes-Medrano et al., 2015) .

Normal spermatogenesis and male reproduction depend on the integrity of BTB ultrastructure. BTB, a physical barrier formed by of sertoli cells (SCs) between the blood vessels and testicular seminiferous tubules, is composed of the tight junctions (TJs), gap junctions (GJs), basal ectoplasmic specializations (ESs) and desmosomes (Mruk and Cheng, 2004). Besides its nutrition-providing fuction, BTB is an important physical barrier and immune privileged site which can prevent the entry of toxic and harmful contaminants and thus provide a comfortable microenvironment for spermatogenesis (Brouard et al., 2016). Whereas, normal spermatogenesis is of great significance for maintaining of male reproductive function. Therefore, the disruption of BTB normal structure can induce spermatogenesis disturbance and thus lead to reproductive disorder in male mammals. Previous studies have clarified the indispensable role of BTB in maintaining male fertility. For example, Bisphenol A (BPA) makes adverse effects on reproduction based on its disrupting the integrity of BTB in rare minnow (Tao et al., 2019), and silver nanoparticles (AgNPs) can reduce expression of tight junction proteins and then lead to negative changes of spermatogonial stem cells (Zhang et al., 2015). Meanwhile, automobile exhaust-derived $PM_{2.5}$ can also affect spermatogenesis by reducing the expression of BTB-related proteins (Liu et al., 2019). However, the literatures about the effect of MPs on BTB are still reported rarely. A recently-published study has mentioned that 0.5 μm PS-MPs absorbed by sertoli cells may exert potential destructive effects on BTB in mice (Jin et al., 2020). Nonetheless, its underlying molecular mechanism is far from being elucidated.

Based on these researches, our target was set to further investigate the effects of PS-MPs on male reproduction and to elucidate the underlying toxic mechanisms of BTB impairment, which will provide some experimental foundations for the treatment and prevention of male reproductive diseases.

Experimental Section

Preparation for experiment

PS-MPs with a diameter of 0.5 μm were purchased from Tianjin Baseline Chromtech Research Centre (Tianjin, China). These PS-MPs were chosen because small PS-MPs are easier to transfer in the body (Browne et al., 2008). Prior to using, PS-MPs' size, shape and composition were analyzed by Zetasizer (Nano ZS90; Malvern, United Kingdom), scanning electron microscopy (SEM, JEOL/EO, Japan), and Fourier transform infrared spectroscopy (FTIR, Frontier FT-IR Perkin Elmer Co., Ltd., USA). After analysis, its diameter and structure were proved to meet the experimental requirements.

Animals and experimental design

Thirty-two male Wistar rats (6 weeks old, weight approximately 180 grams) were purchased from Jinan Pengyue Animal Co., Ltd. (Shandong, China). The rats were fed adaptively under standard environmental conditions (20–25°C, 50%-70% humidity, 12 h dark and light cycle) with water (reverse osmosis pure water) and basal diet always available for one week. And then, they were weighed and arbitrarily divided into four groups with eight rats per group. Three groups were exposed to PS-MPs at does of 0.015 mg/d, 0.15 mg/d, and 1.5 mg/d, respectively (about 7.1838×10^{9} particles/L, 7.1838×10^{10} particles/L, 7.1838×10^{11} particles/L, respectively). Before each feeding, the PS-MPs were shaken by ultrasound for 20 minutes to fully suspend, and then deionized water was added to prepare different dose for animals to drink every day. The control group drank deionized water without PS-MPs. Exposure dose of PS-MPs used was based on the PS-MPs' content which presented in aquatic ecosystems (Lenz et al., 2016) and current toxicological studies (2×10^{-2} mg/L-1.5×10^{3} mg/L) (Avio et al., 2015; Lu et al., 2016). The entire housing period lasted 90 days and the body weight and health status of the rats were determined regularly throughout the treatment period. The research was supported by Binzhou Medical University Animal Ethics Committee and its internal standards and regulations were strictly followed in this research.

Sample collection, sperm quality and quantity analysis

Exposed by 90 days, all rats were sacrificed after anesthetizing with 4% chloral hydrate injected intraperitoneally, the blood was collected and remained to clot naturally, and then the serum was extracted and frozen at -80°C for future use. Some testis tissues were fixed in 4% paraformaldehyde while other part was stored at -80°C. And the epididymis of the rat was rapidly removed for abnormal rate and sperm count analysis. After isolating the rat's epididymis, it was cut open and placed in 2 mL dulbecco's modified eagle medium (DMED) of 37°C for culturing with scissors, and repeatedly blown to allow the sperm to leave the epididymal tubule to make a fresh sperm, and the sperm count was obtained

and morphological examination was performed. A small amount of sperm was sucked and placed on automatic sperm analyzer (Hamilton TOX IVOS, USA) to determine the sperm motility and density. Another 30 μL sperm suspension was dropped on a clean adhesive slide, pushed to the slide and then performed HE staining after drying naturally. 5 smears were made for each animal, and 12 fields were randomly collected for each slide at high power. The number of total and abnormal sperm in each field and group were counted, and the malformation rate was calculated by the number of abnormal sperm/the total sperm count.

Histological assessment in testis

Testis were dehydrated with alcohol at different concentrations, and embedded in paraffin. Then it was cut into thin sections (5μm), deparaffinized, hydrated, stained with hematoxylin and eosin, and mounted with neutral gum finally. Images were captured under a light microscope (Echo, USA).

Measurement of oxidative stress in testis

Testis tissues were homogenized, protein was extracted, and its concentration was quantified with bicinchoninic acid (BCA) protein assay (Solarbio, China). Superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and catalase (CAT) which belong to antioxidant defense systems and the levels of malondialdehyde (MDA) in testis were measured with special assay kits (Jiancheng, China). The absorbance values of each 96-well plate at specific wavelengths were measured using a multilabel microplate reader (Thermo Multiskan MK3, USA).

Bax and Bcl-2 expressions in testis

After deparaffinization and rehydration, the testicular sections were carried out antigen retrieval and treated with phosphate buffered saline (PBS) for 5 min three times. Then after blocking with 10% goat serum the sections were incubated with Bcl-2 (1:200, Protenintech, China. Catalog #26593-1-AP) and Bax (1:150, Protenintech, China. Catalog #50599-2-1g) and kept overnight at − 4℃. Then, the sections were incubated with enzyme-labeled secondary antibody for 60 min at room temperature, stained with diaminobenzidine (DAB) (Shanghai gene tech co., ltd., Shanghai, China), counterstained with hematoxylin, dehydrated, cleared, mounted finally and were observed under the light microscope (Echo, USA), and images were taken. Twelve fields were randomly collected from each section, immunopositivity and cumulative optical density values of cells in each field were determined and statistically analyzed (Image-Pro Plus, USA).

Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining in testis

Tissues were fixed, dehydrated and cut into sections at 14 μm with a cryostat (leica, Germany) with a − 20 ℃ incubator. Tissues were then stained with TUNEL fluorescent dye (Beyotime, China) for 60 min at 37℃ in the dark. Slides were finally mounted with anti-quencher and visualized by fluorescence microscopy (Echo, USA). Ten fields were randomly collected from each section, and the fluorescence intensity was statistically analyzed (Image-Pro Plus, USA).

Western blot analysis

Total protein extracts from testis tissue (Jiancheng, China) and concentration were measured by a bicinchoninic acid (BCA) protein assay (Solaibao, China). Equivalent cleaved protein (50 μg) were then electrophoresed in SDS-polyacrylamide gels (10%), and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% bovine serum albumin (BSA) (Beyotime, China) dissolved in Tris-buffered saline (TBS) at room temperature for 1h, the membrane was bound with Occludin (1:500, Proteintech, China, Catalog #27260-1-AP), Claudin-11 (1:300, Proteintech, China, Catalog #12152-1-AP), N-Cadherin (1:2000, Proteintech, China, Catalog #22018-1-AP), Connexin-43 (1:500, Boster, China, Catalog #BA1727), p38 (1:1000, Abcam, Catalog #ab27986), p-p38 (1:1000, Abcam, Catalog #ab47363), Nrf2 (1:1000, Beyotime, China, Catalog #AF7623). β-actin (1:2000, Bioss, China, Catalog #bs-0061R) was also measured as an internal control. The membranes were then treated with TBST, incubated with horseradish peroxidase-labeled goat anti-rabbit IgG secondary antibody (1:5000, Bioss, China) at indoor temperature for 1h. After washing with TBST for three times, antibody-bound proteins were detected with Electro-Chemi-Luminescence (ECL) chemiluminescence reagent (Bioss, China). Densitometric values of protein bands were analyzed using Image Lab Software (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Statistical analysis

The SPSS 23.0 (SPSS Inc., USA) software was used to statistical analysis. Differences were tested using one-way analysis of variance (ANOVA), and Kolmogorov-Smirnov and Levene tests were used for all data, respectively. $p < 0.05$ was considered as statistical significance. Datum are presented as mean ± standard deviation (SD).

Results

Characteristics of PS-MPs

The characteristics of PS-MPs are shown in Fig. 1. Scan electron microscopy (SEM) images showed that PS-MPs were all spherical structures with diameter of 0.5 μm and did not show obvious aggregation (Fig. 1A). The hydrodynamic sizes of PS-MPs in ultrapure water were approximately 500 nm (Fig. 1B). FTIR analysis displayed that the chemical composition of microplastics was polystyrene (Fig. 1C).

Changes of sperm quality and quantity caused by PS-MPs

In the sperm smear, we found normal sperm morphology in control group and 0.015 mg/d group (Fig. 2A a and b), however, more abnormal sperms with defects in the head and tail were observed, especially in 0.15 mg/d and 1.5 mg/d groups (Fig. 2A c and d). Meanwhile, the sperm abnormity rate was significantly evaluated in 1.5 mg/d group compared with control group ($p < 0.05$), but no difference was shown in 0.015 mg/d and 0.15 mg/d groups ($p > 0.05$) (Fig. 2B). The sperm concentration and motility in 1.5 mg/d

group decreased compared with control group ($p < 0.05$), but there was no significant difference both in 0.015 mg/d and 0.15 mg/d groups when compared to control group ($p > 0.05$) (Fig. 2C and D).

Testis histopathology affected by PS-MPs

In control group, the spermatogenic cells were arranged regularly and tightly, the structure of it in 0.015 mg/d group was similar as control group (Fig. 3a and b). However, in 0.15 mg/d and 1.5 mg/d groups, the seminiferous tubules showed pathological changes including significant depletion and shedding of spermatogenic cells and intraepithelial vacuolization (Fig. 3c and d1). The thick arrow indicates that the diameter of seminiferous tubules decreased and the lumen of it disappeared (Fig. 3d2).

PS-MPs aggravate oxidative stress

To determine whether exposure to microplastics induce oxidative damage, we observed CAT, GSH-PX, MDA, and SOD levels in rat testis (Fig. 4). Compared with control group, the MDA levels showed a significant upregulation ($p < 0.05$) (Fig. 4C), and the CAT, GSH-PS, and SOD levels decreased both in 0.15 mg/d and 1.5 mg/d groups ($p < 0.05$) (Fig. 4A, B and D), but there was no significant difference in 0.015 mg/d group when compared to control group ($p > 0.05$) (Fig. 4). These results indicated that exposure to PS-MPs triggered oxidative stress in rat testis.

The effects on Bax and Bcl-2 expressions in testis by PS-MPs

As shown in Fig. 5A, the immunopositive products of Bax and Bcl-2 were located in the cytoplasm of spermatogenic cells and appeared tan. Compared with control group, the Integrated Optical Density (IOD) of Bax immunopositive products increased, while that of Bcl-2 significantly decreased in 0.15 mg/d and 1.5 mg/d groups ($p < 0.05$), but no significant difference in 0.015 mg/d group compared to control group ($p > 0.05$) (Fig. 5B and C).

The effects on TUNEL staining in testis by PS-MPs

The results of TUNEL staining indicated that the mean fluorescence intensity increased significantly in 0.15 mg/d and 1.5 mg/d groups compared with control group. There were no obvious differences in 0.015 mg/d group compared to control group ($p > 0.05$) (Fig. 6).

The effects on protein expressions of P38 MAPK signaling pathway by PS-MPs

The protein expressions of p38 MAPK signaling pathway and Nrf2 are shown in (Fig. 7A). The level of phosphorylation p38 was significantly elevated and the expression of Nrf2 was reduced in 0.15 mg/d and

1.5 mg/d groups compared with control group ($p < 0.05$), while no significant difference was observed in 0.015 mg/d group ($p > 0.05$) (Fig. 7B).

The effects on protein expressions of BTB by PS-MPs

The expressions of BTB related proteins, such as Occluding, Claudin-11, N-Cadherin, Connexin-43 are shown in (Fig. 8A). PS-MPs induced decrease of the four proteins compared to control group, especially at 0.15 mg/d and 1.5 mg/d groups ($p < 0.05$), while there was no obvious difference in 0.015 mg/d group (Fig. 8B).

Discussion

As a persistent organic pollutant, MPs has posed a threat to human fertility safety. Though related literature is limited, there are some recently published studies proving that MPs have adverse effects on reproductive system via varied pathways including the down-regulation of GnRH, Vtg, and Chg genes in marine medaka (Wang et al., 2019) and p38 MAPK pathway in mice (Xie et al., 2020). Our present results found that the levels of sperm motility and concentration were remarkably reduced and the abnormality of sperm was elevated after exposure to PS-MPs. As for the histological evidence, spermatogenic epithelium showed vacuoles, spermatogenic cells shed and tubules were abnormal shown by the results of HE staining. These negative changes further indicated that PS-MPs exposure induced reproductive toxicity in male rats.

Multiple pathways are involved with BTB impairment such as TGF-b3/p38 MAPK pathway and decreasing testosterone (Liu et al., 2018). To identify whether p38 pathway is one of the mechanisms of reproductive toxicity caused by PS-MPs, we detected the levels of oxidative stress and the protein expressions of p38 MAPK signaling pathway. Oxidative stress is an imbalance characterized with depleted antioxidant defenses system and overwhelmed relative oxygen species (ROS) production in which mitochondria has been regarded as an intracellular source of ROS formation (Cadenas and Davies, 2000). When the balance is disturbed and ROS production is overwhelming, oxidative stress appears and trigger numerous damage such as DNA damage(Rima, 2017), cell apoptosis (Chen et al., 2019) and even defective sperm (Bisht ., 2017). Recently, oxidative stress is demonstrated as a basic toxicological mechanism of microplastics (Hamed et al., 2020; Zhu et al., 2020; Xie et al., 2020). As clarified above, oxidative stress triggered by PS-MPs was apparently involved with the increased level of MDA and decreased levels of GSH-PX, CAT, and SOD detected in testis, which suggested that PS-MPs induced oxidative stress in the testis of male rats.

Persistent and severe oxidative stress has been confirmed to trigger diverse signaling pathways (Simon et al., 2000) in which the MAPK signaling pathways are involved MAPK pathways are a big family composed of p38, Jun NH2-terminal kinase (JNK), and also extracellular signal-regulated kinase (ERK). As a member of multiple redox-sensitive signal transduction factors, MAPK has a close association with spermatozoa maturation found by previous literatures (Almog et al., 2008; Duan et al., 2016). Particularly

the p38 MAPK pathway can downregulate the BTB-related proteins and thus cause BTB destruction in rats (Liu et al., 2018). Similarly, our western blotting results further proved the activation of p38 MAPK pathway triggered by oxidative stress with expression of p-p38 significantly upregulated in 0.15 mg/d and 1.5 mg/d groups.

Nrf2, a transcription factor, has been proved to combind to the promoter regions of its downstream target genes to stimulate the expression of antioxidant response elements and thus exert a cytoprotective effect (Miura et al., 2019; Song et al., 2019). As the downstream signaling pathway of MAPK (Zhao et al., 2016), MAPK-stimulated Nrf2 pathway is supposed to get involved in BTB disruption (Liu et al., 2020). The expression of Nrf2 showed an obvious downtrend ($p < 0.05$) in 0.15 mg/d and 1.5 mg/d groups and no significant differences in 0.015 mg/d group compared with control group revealed by our results. It suggested that the p38-Nrf2 pathway is triggered by oxidative stress with p38 phosphorylation and Nrf2 depletion under a high dose of PS-MPs exposure.

BTB has an indispensable function in maintaining spermatogenesis through protecting sperms from toxicological substance and providing appropriate microenvironment. Multiple literatures have illustrated that during the epithelial cycle, BTB reconstructs to facilitate spermiation and spermiogenesis (Alves et al., 2013; Xia et al., 2009). Therefore, normal spermatogenesis depends on BTB structural integrity. As for the structure, BTB is a significant barrier consisting of TJs, GJs, ESs and desmosomes. TJs are connective structures with selective permeability in which Occluding (a BTB-related proteins) is a vital component (Mruk and Cheng, 2010) and elucidated as the initiator of BTB shaping (Gerber et al., 2016). Connexin-43, considered as crucial component of gap junctions, plays a role in keeping BTB homeostasis (Li et al., 2009). Claudin-11 and N-Cadherin are also expressed on the sertoli cells membrane as indispensable components. The levels of BTB-related proteins mentioned above can be markers of BTB integrity (Hasegawa and Saga, 2012; Liu et al., 2019). As for our results, a downward trend of the levels of BTB-related proteins was shown in our results with the dose of PS-MPs increasing, which indicated that PS-MPs induced the disruption of BTB. Moreover, accumulating literatures demonstrated that the disruption of BTB integrity may induce the apoptosis of spermatogenic cells (Chihara et al., 2013; Hasegawa and Saga, 2012; Morales et al., 2007). Our results of TUNEL staining and Bax/Bcl-2 immunohistochemistry indicated the increasing level of spermatogenic cells apoptosis was induced by PS-MPs exposure. To conclude, all these results indicated that BTB impairment induced by PS-MPs exposure may lead to apoptosis of spermatogenic cells through ROS-triggered p38-Nrf2 signaling pathway.

Conclusions

The present results preliminarily show that PS-MPs at sufficiently high dose can induce disruption of BTB integrity and thus apoptosis of spermatogenic cells via p38 MAPK- Nrf2 pathway triggered by oxidative stress, which leads to the decline of sperm quantity and quality. This study provides experimental support for the adverse effect of high-dose PS-MPs on male reproductive toxicity. However, some limitations are

supposed to be taken into consideration that inhibitors are needed to add to further clarify the mechanism. Thus, the follow-up experiments are required.

Declarations

Authors' contributions Shengda Li: Conceptualization, Investigation, Writing-Original draft preparation. Qimeng Wang: Conceptualization, Investigation, Writing-Original draft preparation. Hui Yu: Conceptualization, Investigation, Writing-Original draft preparation. Long Yang: Investigation. Data curation. Yiqing Sun: Investigation. Data curation. Ning Xu: Investigation, Methodology. Nana Wang: Investigation, Methodology. Zhimin Lei: Software, Validation. Junyu Hou: Software, Validation Yinchuan Jin: Formal analysis. Hongqin Zhang: Formal analysis. Lianqin Li: Formal analysis. Feibo Xu: Investigation. Lianshuang Zhang: Conceptualization, Investigation, Writing-Reviewing and Editing.

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Data availability All data generated or analysed during this study are included in this published article.

Compliance with ethical standards

Conflict of interests All the authors declare no conflict of interest.

Ethical approval The research was supported by Binzhou Medical University Animal Ethics Committee and its internal standards and regulations were strictly followed in this research.

Consent to participate Not applicable.

Consent to publish Not applicable

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Figures

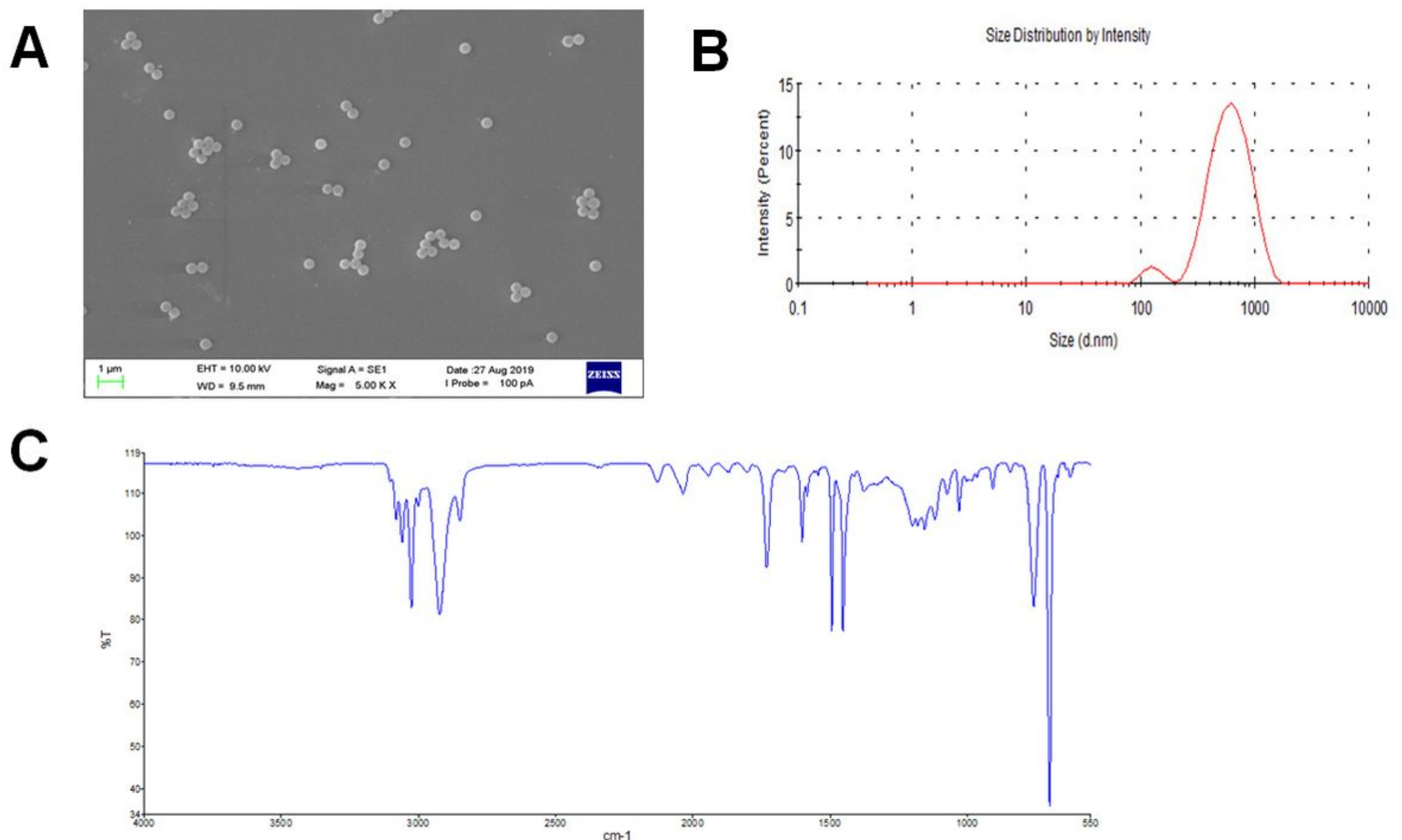


Figure 1

Characterization of the PS-MPs used in this experiment. (A) SEM images of 500 nm PS-MPs (×5000). (B) Size distribution by intensity. (C) FTIR spectroscopy of 500 nm PS-MPs.

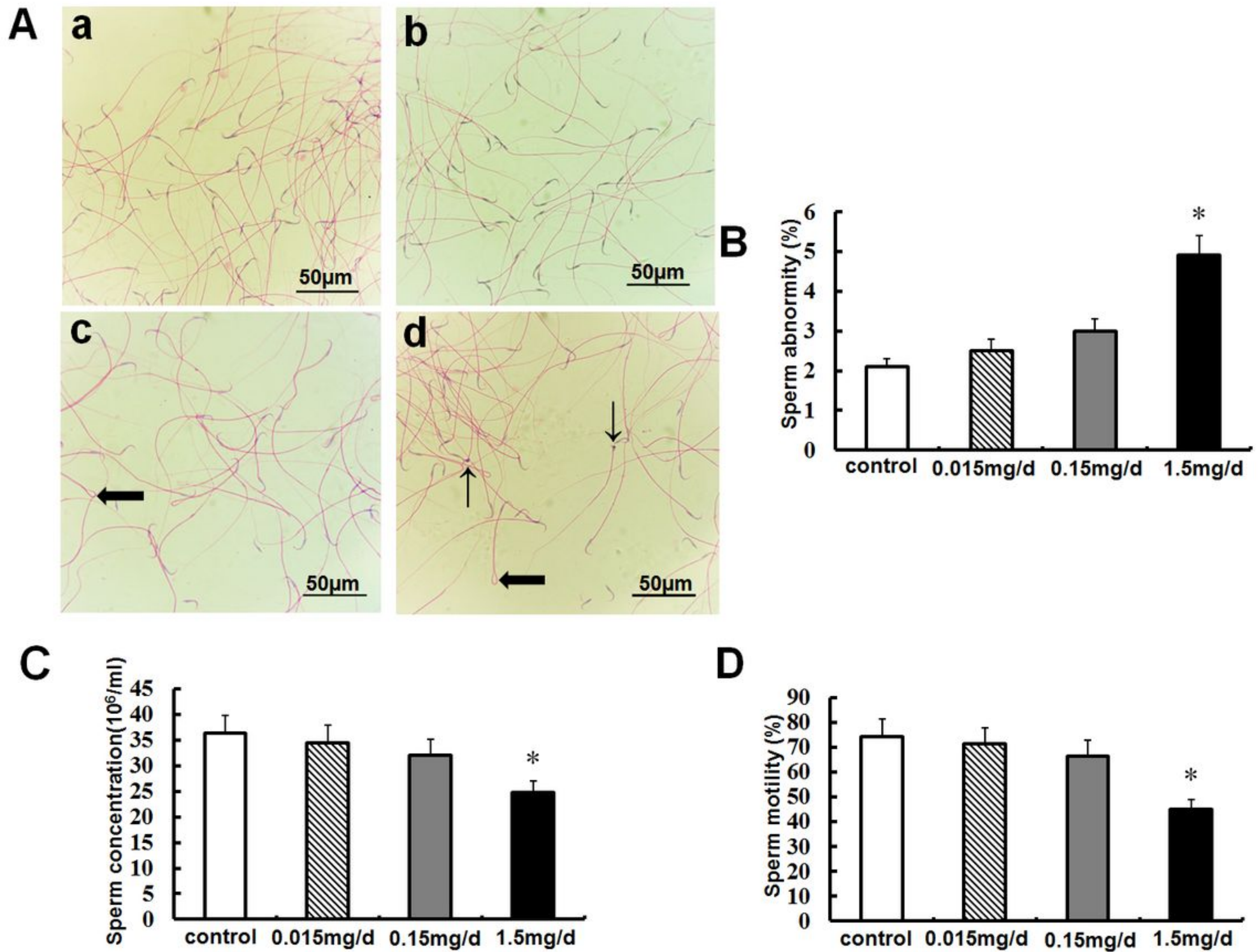


Figure 2

The effects on sperm quality and quantity in testis of rats by PS-MPs. (A) The observation of sperm smear (×400). (a) control group. (b) 0.015 mg/d group. (c) 0.15 mg/d group. (d) 1.5 mg/d group. Thin arrows and thick arrows denote the sperm with deformity in the head and tail respectively. (B) Determination of sperm abnormality. (C) Determination of sperm concentration. (D) Determination of sperm motility. * indicates significant difference from control group ($p < 0.05$). Data are expressed as mean ± SD.

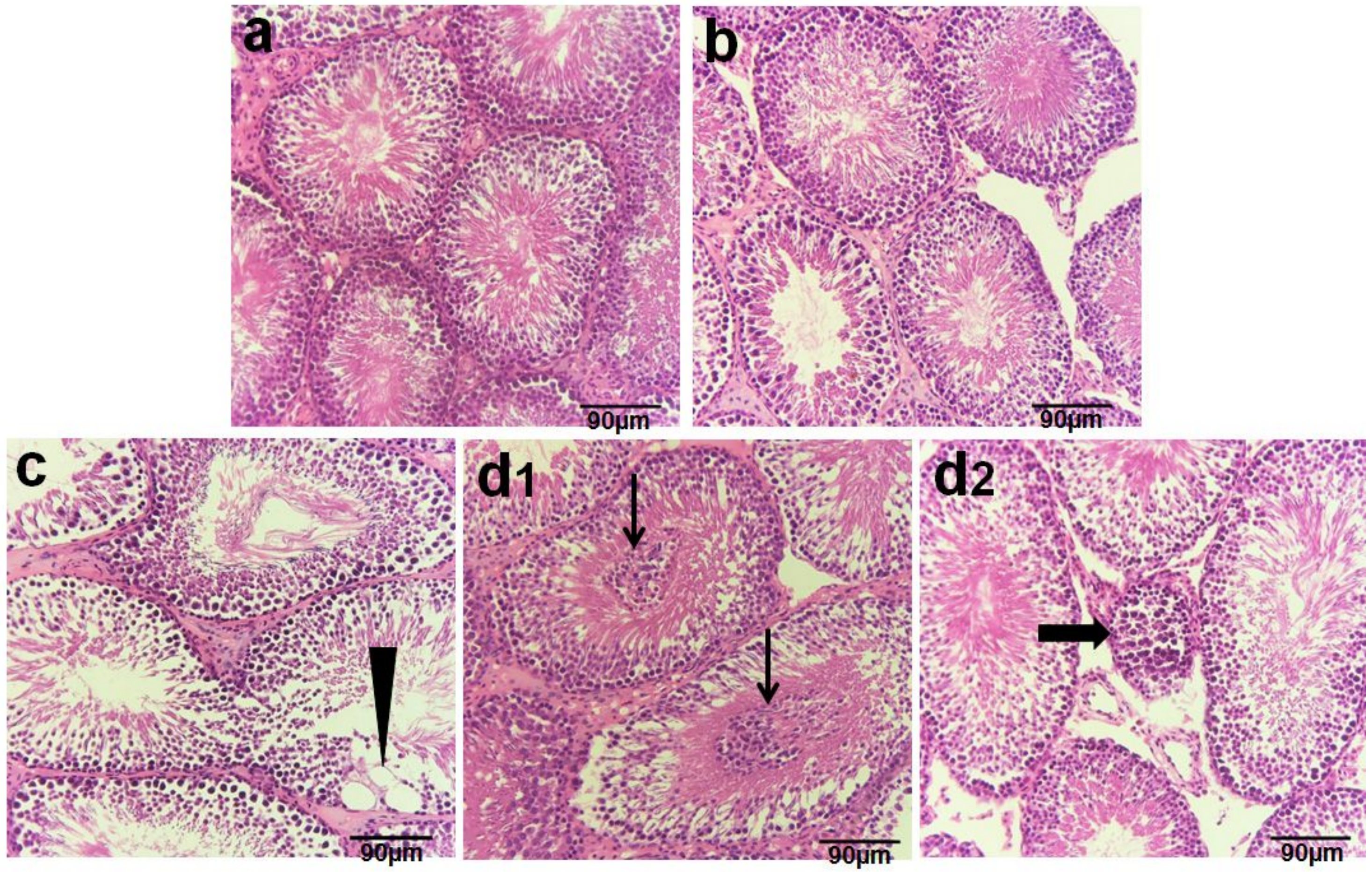


Figure 3

The effects on testicular histopathology in testis of rats by PS-MPs. (a) control group (×400). (b) 0.015 mg/d group (×400). (c) 0.15 mg/d group (×400). Triangular arrow denotes intraepithelial vacuolation. (d1, d2) 1.5 mg/d group (×400). Thin arrows denote detached spermatogenic cells, thick arrow denotes abnormal seminiferous tubules.

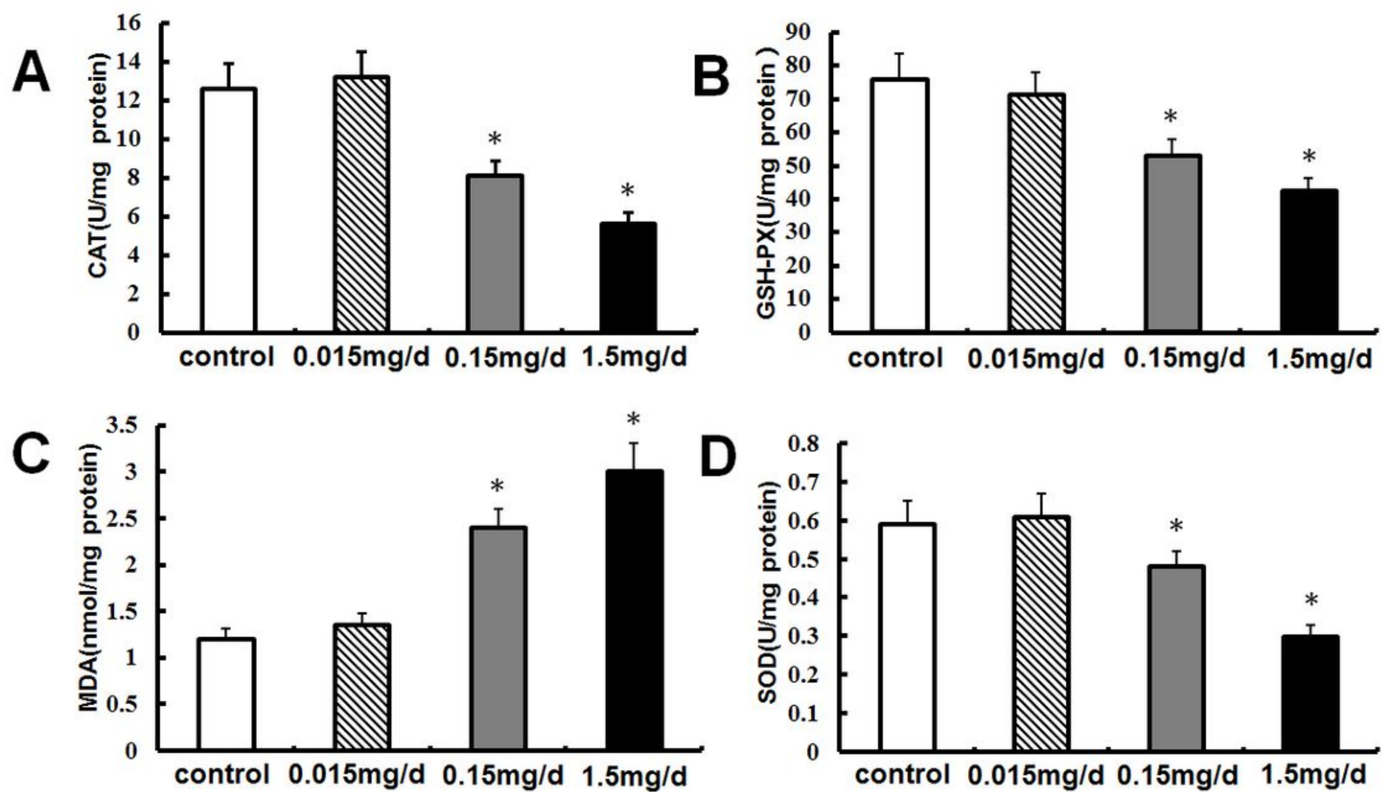
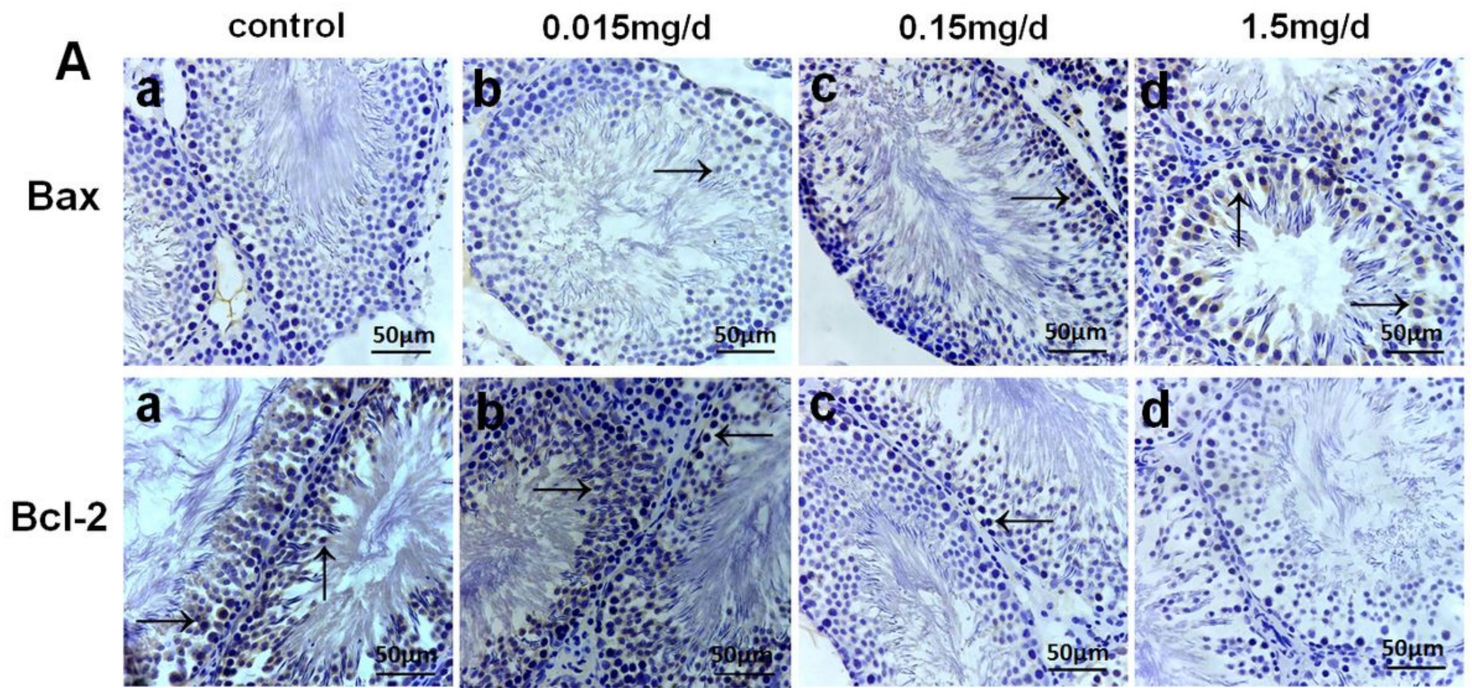
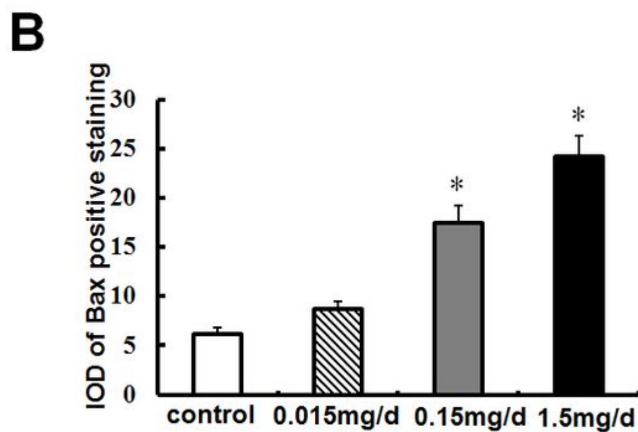


Figure 4

The effects on oxidative stress level in testis of rats by PS-MPs. The activities of CAT (A), GSH-PX (B), SOD (D) and the level of MDA (C). * indicates significant difference from control group ($p < 0.05$). Data are expressed as mean ± SD.





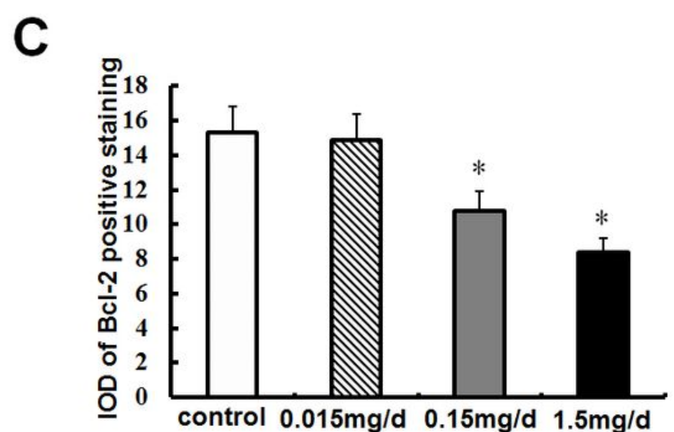


Figure 5

The effects on Bax and Bcl-2 expressions in testis of rats by PS-MPs. (A) Immunohistochemical staining (×400). (a) control group. (b) 0.015 mg/d group. (c) 0.15 mg/d group. (d) 1.5 mg/d group. Arrows denote immunopositive cells. (B) Histogram of Integrated Optical Density for Bax Positive Staining. (C) Histogram of Integrated Optical Density for Bcl-2 Positive Staining. * indicates significant difference from control group ($p < 0.05$). Data are expressed as mean ± SD.

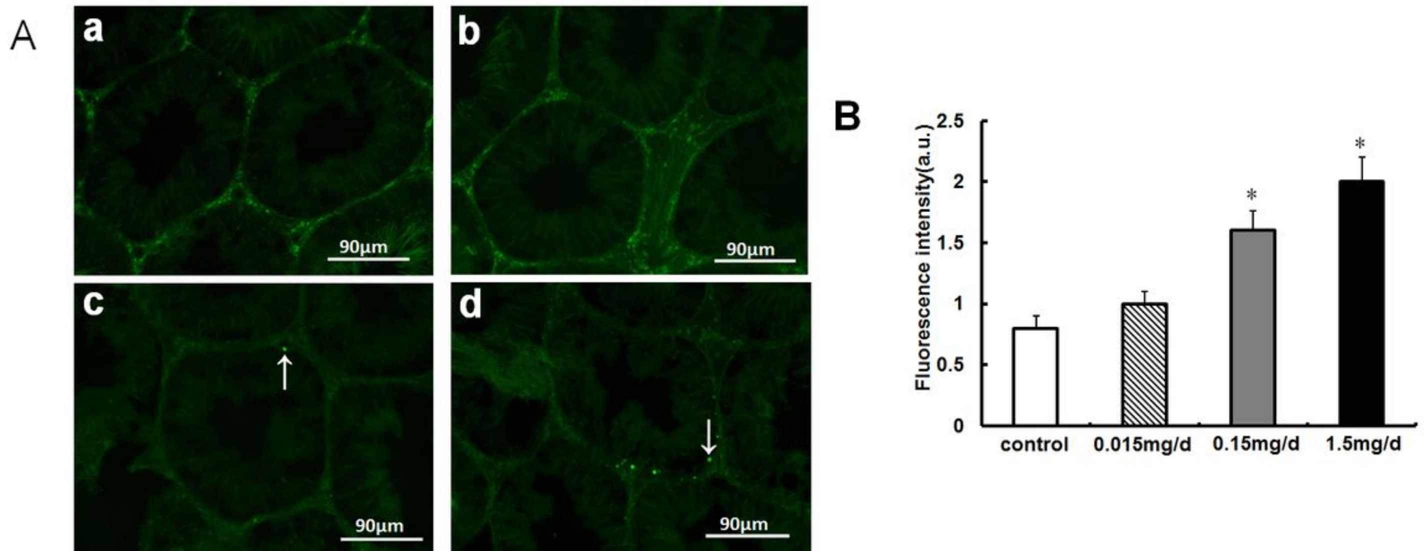


Figure 6

The effects on TUNEL staining in testis of rats by PS-MPs. (A) The TUNEL staining of testis and clearly observed under LSCM (×200). (a) control group. (b) 0.015 mg/d group. (c) 0.15 mg/d group. (d) 1.5 mg/d group. Arrows denote TUNEL-positive apoptotic cells. (B) The fluorescence intensity analyzed by Leica QWin image analysis software. * indicates significant difference from control group ($p < 0.05$). Data are expressed as mean ± SD.

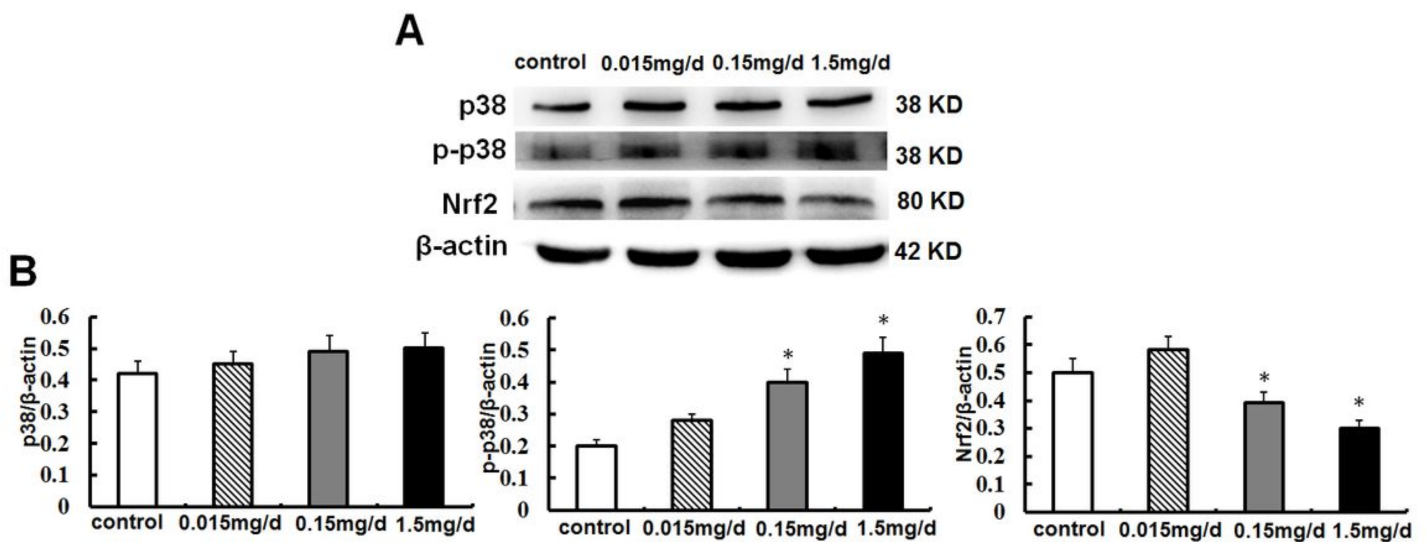


Figure 7

The effects on protein expressions of p38 MAPK signaling pathway by PS-MPs. (A) The protein expressions of p38, p-p38 and Nrf2. (B) The relative densitometric analysis of these proteins. * indicates significant difference from control group ($p < 0.05$). Data are expressed as mean ± SD.

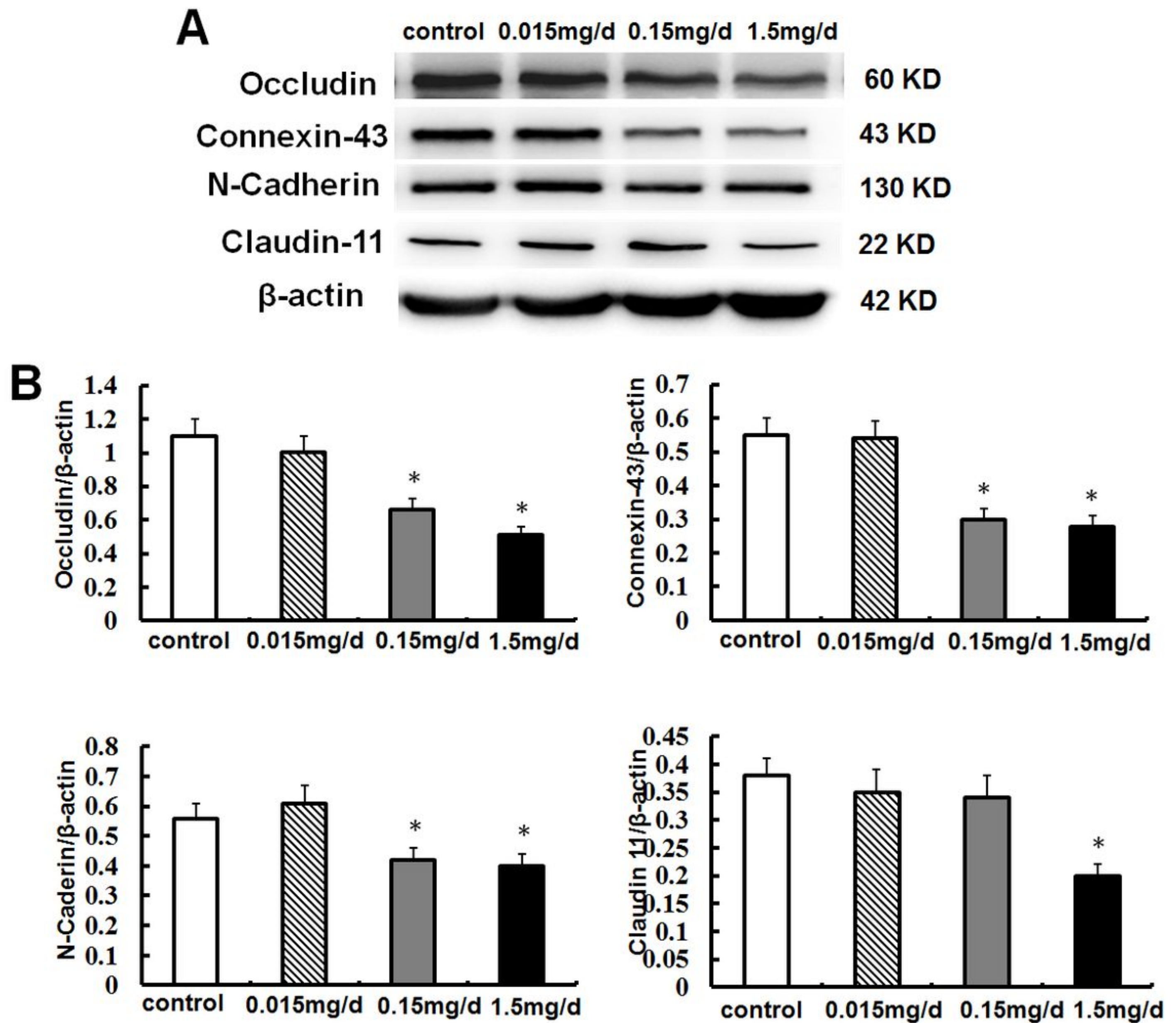


Figure 8

The effects on BTB-associated protein expressions in testis of rats by PS-MPs. (A) The expressions of Occludin, Connexin-43, N-Cadherin and Claudin-11. (B) The relative densitometric analysis of these proteins. * indicates significant difference from control group ($p < 0.05$). Data are expressed as mean ± SD.