·生物技术与方法·

基于氧化应激与细胞凋亡探究双酚 A 慢性暴露致小鼠肾毒性作用机制

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摘　要：双酚 A (bisphenol A, BPA)被广泛应用于生产环氧树脂和聚碳酸酯塑料等制品，在强酸、强碱或高温条件下，BPA 被释放出来，然后渗入环境中。在大多数生物液体中都检测到了不同浓度的 BPA，BPA 的存在已被证明与许多慢性疾病密切相关，包括慢性肾病(chronic kidney disease, CKD)。然而，关于 BPA 的有害作用及其对 CKD 的不良影响知之甚少。为了探讨 BPA 对动物肾毒性的作用机制，本研究通过向饮水中加入 0.01、0.1 和 1 mg/L 的 BPA，暴露于雌性小鼠 4 周后，交配和怀孕的雌性小鼠持续接触 BPA，直到断奶；F1 代 3 周龄雄性仔鼠继续口服相同剂量的 BPA，持续 10 周。结果表明，0.1 mg/L 和 1mg/L BPA 处理组小鼠的肾脏损伤严重，血清中肾脏功能指标尿素氮(urea nitrogen, UN)、肌酐(creatinine, CR)和尿酸(uric acid, UA)的含量均发生显著升高(P<0.05)；肾脏组织形态结构被损害；肾脏抗氧化相关基因在 mRNA 和蛋白水平上的表达显著降低(P<0.05)，包括谷胱甘肽硫转移酶(glutathione-S-transferase, GST)、超氧化物歧化酶(superoxide dismutase, SOD)和过氧化氢酶(catalase, CAT)；硫代巴比妥酸反应物(thiobarbituric acid reactive substances, TBARS)的含量和凋亡指数(Caspase-3 和 Bax/Bcl-1 的比值)相关基因和蛋白的表达显著增强。以上研究结果证实，氧化应激和细胞凋亡在 BPA 慢性暴露诱导的动物肾毒性中起着重要作用。

关键词：双酚 A；细胞凋亡；慢性暴露；肾毒性；氧化应激

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Mechanism of nephrotoxicity induced by chronic exposure of bisphenol A in mice based on oxidative stress and cell apoptosis

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Abstract: Bisphenol A (BPA) is widely used to produce epoxy resin and polycarbonate plastic products. In severe cases, these plastics may release BPA, which then infiltrates into the environment. Various concentrations of BPA have been found in most biological fluid. Its presence has been well shown to be closely related to many chronic diseases, including chronic kidney disease (CKD). However, little is known regarding the adverse effects of BPA exposure and its succedent cellular events on CKD. Hence, in the current *in vivo* study, we aimed to assess the effects of chronic exposure to BPA on animal nephrotoxicity through investigating oxidative stress and apoptosis. Upon exposure to BPA at 0.01, 0.1, and 1 mg/L via drinking water for four weeks, the mated and pregnant females were continuously exposed to BPA until weaning. Subsequently, three weeks old F1-male neonates were also orally challenged with the same three doses of BPA for ten weeks. The results showed that the kidneys of 0.1 and 1 mg/L BPA-treated mice were seriously damaged; the contents of serum renal function indexes and lipid peroxidation products were significantly increased, including urea nitrogen, creatinine, uric acid, and thiobarbituric acid reactive substances; the morphological structure of mouse kidneys was impaired; the expressions of antioxidant-related genes at mRNA and protein levels from mouse kidneys were markedly diminished, including glutathione-S-transferase, superoxide dismutase, and catalase; the expressions of genes and proteins related to apoptosis index (ratio of Bax/Bcl-1 and Caspase-3) were significantly enhanced. The data manifested that cumulative oxidative stress and apoptosis might play an essential role in the animal nephrotoxicity induced by chronic exposure to BPA.

Keywords: bisphenol A; cell apoptosis; chronic exposure; nephrotoxicity; oxidative stress

Bisphenol A (BPA) has a weak estrogenic activity known for its ability to interact with estrogen receptors (ERs), which is considered as one of the environmental endocrine-disrupting chemicals[1-2]. BPA is a synthetic compound that is widely used as a critical starting material of epoxy resins and polycarbonate plastics among various consumer products, including food and beverage containers and medical and dental devices[1,3-5]. Harsh circumstances, including exposure to acidity, alkalinity, or high-temperature conditions, may lead to BPA releasing, which subsequently pollutes food, water, and the domestic environments[5-6]. Humans and animals are ineluctably exposed to BPA through drinking water and food

contaminated by BPA, as well as skin contact and inhalation of dust[5-7].

The ubiquity of BPA in the environment has attracted people's attention because BPA has been found in the maternal amniotic fluid, sweat, blood, breast milk, serum, placenta tissue, and urine of humans and other organisms[8-13]. BPA exposure can pass through the placenta at a critical stage of development, negatively transform hormone levels, and engender abnormalities in human and animal embryonic cells and tissues[2,14-15]. BPA exposure in adulthood also makes developing reproductive tissues susceptible to diseases/ abnormalities, which will be passed on to future generations[15-18].

An increasing number of human and animal studies have shown the relevant, irreversible, and permanent effects between BPA and animal health anomalies on different organ systems, such as carcinogenesis (neuroblastoma, breast, and prostate cancer), neuroendocrine disruption, immune system impairment, the decline of sperm quantity and quality indices system, changes of the endogenous cannabinoid system in liver and central nervous system, increased risk of inflammation, congenital disability, development disorder, as well as other chronic diseases, like reproductive, cardiovascular, metabolic, and neurological disorders[2,19-23]. Meanwhile, a good body of epidemiological studies has shown a close connection of increased urinary BPA levels and chronic kidney disease (CKD), low-grade albuminuria in both children and adults, and cardiovascular disease[24-29]. BPA exposure could also result in DNA damage on renal epithelial Marc-145 cells *in vitro*[5]. Some studies indicate that animals exposed to BPA revealed renal damages as shown by decreased membrane potential change, creatinine clearance, mitochondrial swelling, antioxidant glutathione, and superoxide dismutase, increased serum urea and creatinine, lipid peroxidation, reactive oxygen species (ROS) production, inflammatory markers, and oxidative stress, along with the presence of glomerular injuries and subsequent proteinuria[28-31]. These findings remind people that exposure to BPA in daily life might adversely affect the renal system and lead to lifelong cumulative renal injury. However, the in-depth mechanisms of BPA-induced renal injuries are not clear and remain to be clarified.

A healthy kidney is vital to the stability of normal metabolism and internal environment homeostasis[5]. Under pathological conditions, many factors accumulate in patients with CKD, which leads to uremia and increased mortality, as well as symptoms including sleep disorder, weakness, anorexia, vomiting, cardiovascular disease, neuropathy, and gradual loss of renal function. Accordingly, eliminating urinary toxins is always coupled with ameliorating clinical symptoms[24,32-35]. Hence, based on the above literature, the main aim of the current *in vivo* study was to appraise the impact of BPA on the concentrations of uric acid (UA), creatinine (CR), and urea nitrogen (UN), as well as to elucidate whether mechanisms of apoptosis and oxidative stress at the expression levels of gene and protein are involved in these damaging influences.

1 Materials and methods

1.1 Animal model establishment

A total 30 Kunming strain mice were purchased from the Shanxi Cancer Research Institute (license number: SCXK-Jin 2017-0003), including 20 female mice (breeders, 23−25 g b.w., aged four weeks) and 10 male mice (breeders, 31−33 g b.w., aged five weeks), and housed at (23±2) ℃ with 12-h light/dark cycle. All animals in the study were processed according to the Institutional Animal Care and Use Committee of Shanxi Agricultural University (registered number: SXAU-EAW-2021M0315002). To minimize breeding environment pollution of BPA, mice were housed in cages made of polypropylene. Standard chew pellets were supplied by Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd and water was provided in glass bottles. After seven days of the acclimatization period, the maternal generation was randomly allotted four groups (5/group). The

control group received 0.1% ethanol in drinking water, a concentration of ethanol used as a vehicle for BPA solution. BPA was dissolved in absolute ethanol, diluted, and administered to the remaining three BPA exposure groups via drinking water at a dose of 0.01, 0.1, and 1 mg/L for four consecutive weeks[36-37]. Then, two female and one male Kunming strain mice were housed in the same cage for mating. Pregnant female mice were continuously exposed to various doses of BPA (i.e., 0, 0.01, 0.1, and 1 mg/L, respectively) from gestational day 1 until postnatal day (PND) 21. Afterward, male mice of the first generation (F1) were randomly allotted into four groups (20/group) and treated persistently with the same maternal doses for ten weeks. After 90 days of BPA chronic exposure, the F1-male generation was sacrificed, and their kidneys were removed quickly, weighed, and frozen at −80 ℃ for further analysis. The drinking water, food consumption, body weight of male offspring mice were recorded. There were no changes in the renal organ coefficient and body weight gain including the intake of drinking water and food.

1.2 Histological observation of renal tissue

For histological analysis, renal tissues of male offspring mice (*n*=5 mice/group, 1/male offspring mouse/litter) were processed as previously detailed[15,38]. Kidneys of mice were fixed by 4% paraformaldehyde (Solarbio Technology Co. Ltd), dehydrated in a range of graded ethanol-water solutions, and then embedded in paraffin. The renal tissue blocks were sliced at a 5 μm thickness and stained with hematoxylin and eosin (HE, Solarbio Technology Co. Ltd). Slides were examined and imaged under Leica DM6 Blight microscopy[39].

1.3 UN, CR, and UA levels in the serum

Blood samples of male mice (*n*=10 mice/group, 2/male offspring mouse/litter) were centrifuged at 3 000 r/min and 20 ℃ for 10 min to obtain serum. Serum samples were kept in 200 μL eppendorf tubes at −80 ℃. UN, CR, and UA levels in the serum were measured using the TBA-120FR fully automatic analyzer.

1.4 Measurement of oxidative damage

After the exposure period, the kidneys of male mice (*n*=10 mice/group, 2/male offspring mouse/litter) were accurately weighed, homogenized in ice-cold PBS (10% tissue homogenate), and centrifuged at 12 000 r/min and 4 ℃ for 15 min. After that, the protein concentration of tissue homogenate supernatant extracted from the kidney was determined with a BCA protein concentration assay kit of Biosharp. Eventually, TBARS (an index of lipid peroxidation) were detected using a lipid peroxidation malondialdehyde (MDA) kit. Subsequently, the supernatant absorbance was read in a Thermo Fisher 1510 spectrophotometer at 532 nm[18]. Ultimately, the content of TBARS was reported in nmol/mg protein.

1.5 Quantitative real-time PCR (qRT-PCR)

A total RNA of the left kidney (*n*=5 mice/group, 1/male offspring mouse/litter) was extracted by the Biosharp manufacturer's instructions of Trizol reagent, and then reversely transcribed into cDNA. On the CFX96^{TM} detection system (Bio-Rad), qRT-PCR was performed with Premix Ex *Taq*^{TM} Ⅱ (TaKaRa). Each renal sample was run in triplicate in the experiment. As an internal control, the mRNA expression level of β-actin was measured. Subsequently, mRNA expressions of the genes in Table 1 were normalized to β-actin expression level. All data were expressed as fold change versus the control (without BPA exposure). The primer sequences are listed in Table 1.

1.6 Western blotting analysis

After treatments with BPA, total protein was extracted from the kidney of male mice (*n*=5 mice/group, 1/male offspring mouse/litter) with RIPA tissue/cell total protein lysis buffer containing protease inhibitor (Solarbio Technology Co. Ltd). After incubation for 30 min on ice, all sample mixtures were centrifuged at 4 ℃ with 13 500 r/min for 12 min and their supernatants were collected. Then, the concentration of proteins extracted from the kidney was quantified with a

Table 1 Primer used for the quantitative real-time PCR (qRT-PCR) analysis

Primer names	Primer sequences (5′→3′)	Amplified regions	Products (bp)	GenBank accession No.
β-actin F	AGGGAAATCGTGCGTGAC	725−916	192	NM_007393.5
β-actin R	CATACCCAAGAAGGAAGGCT			
Bax F	TGAAGACAGGGGCCTTTTTG	182−321	140	NM_007527.3
Bax R	AATTCGCCGGAGACACTCG			
Bcl-2 F	TCCTTCCAGCCTGAGAGCAACC	1 558−1 733	176	NM_009741.5
Bcl-2 R	TCACGACGGTAGCGACGAGAG			
Caspase-3 F	GTGGAGGCTGACTTCCTGTATGC	786−966	181	NM_009810.3
Caspase-3 R	ACTCGAATTCCGTTGCCACCTTC			
SOD F	AGCAGAAGGCAAGCGGTGAAC	161−287	127	NM_011434.2
SOD R	TGAGGTCCTGCACTGGTACAGC			
CAT F	AGGTGTTGAACGAGGAGGAGAGG	1 461−1 621	161	NM_009804.2
CAT R	AGCGTTGTACTTGTCCAGAAGAGC			
GST F	AGCTGGAAGGAGGAGGTGGTTAC	131−274	144	NM_013541.1
GST R	GCGGCCAAGGTGTCTCAAGATG			

BCA protein concentration kit. Subsequently, the protein sample extracts were mixed with SDS-PAGE protein loading buffer (Beyotime), followed by boiling at 100 ℃ for 10 min. The extracted protein (12 μL and 50 μg) was separated by SDS polyacrylamide gel for 1.5 h (each sample was from the kidney of a mouse in lane), and then electrophoretically transferred onto nitrocellulose (NC) membrane (Boster) for 1.5 h[16]. The NC membrane was blocked, incubated with primary antibody at 4 ℃ overnight, and followed by secondary antibody (1:10 000, ImmunoWay Biotechnology Company). Finally, specific bands of renal proteins were visualized using an eECL detection kit (CWBIO) and quantified by Image J software. The primary antibodies (Bioss) included rabbit anti- Bax (1:1 000), SOD (1:500), Bcl-2 (1:500), Caspase-3 (1:500), GST (1:1 000), CAT (1:500), and β-actin (loading control, 1:500) polyclonal antibody, respectively.

1.7 Statistical analysis

Values in figures were presented as $\bar{x} \pm s$. Relevant statistical analyses of this study were operated in GraphPad Prism 8.0 software. To avoid "litter effects" of mice, the mean of the two values of male offspring mice (contents of UN, CR, UA, and TBARS) from the same litter mice was performed as a single value in the statistical analysis. The differences among four groups of experimental animals were estimated using one-way ANOVA with Tukey's test for the sake of multiple comparisons as the post hoc test. Ultimately, P-value<0.05 was determined to have statistical significance.

2 Results

2.1 Histopathological features of kidney in mice

Results of HE staining were presented in Figure 1. As shown in Figure 1, light microscopic examinations of the renal sections obtained from the control group showed typical architecture and histological features with intact glomeruli, renal tubules, and tubulointerstitium. By contrast, all mice treated with BPA exhibited different degrees of glomerular injuries with the increase of BPA concentration, which ranged from slight dilatation of renal tubules in the 0.01 mg/L BPA exposed group to glomerulosclerosis and atrophy in the 1 mg/L BPA exposed group.

2.2 Effects of BPA on the contents of UN, CR, and UA

Figure 2 showed the influence of BPA exposure on related indexes of renal function in serum of Kunming mice. Compared to the control

group, the concentration of UN (F (3, 16)=6.485, *P*=0.004), CR (F (3, 16)=7.797, *P*=0.002), and UA (F (3, 16)=4.684, *P*=0.016) in mice treated with 0.1 and 1 mg/L BPA significantly increased. Chronic exposure of 0.01 mg/L BPA had no significant impacts on renal function in mice.

2.3 BPA induces renal lipid peroxidation

To examine renal oxidative stress damage, we detected lipid peroxidation products by measuring TBARS content in the renal tissues of male mice. As displayed in Figure 3 (F (3, 16)= 8.253, *P*=0.002), TBARS content in the kidney of

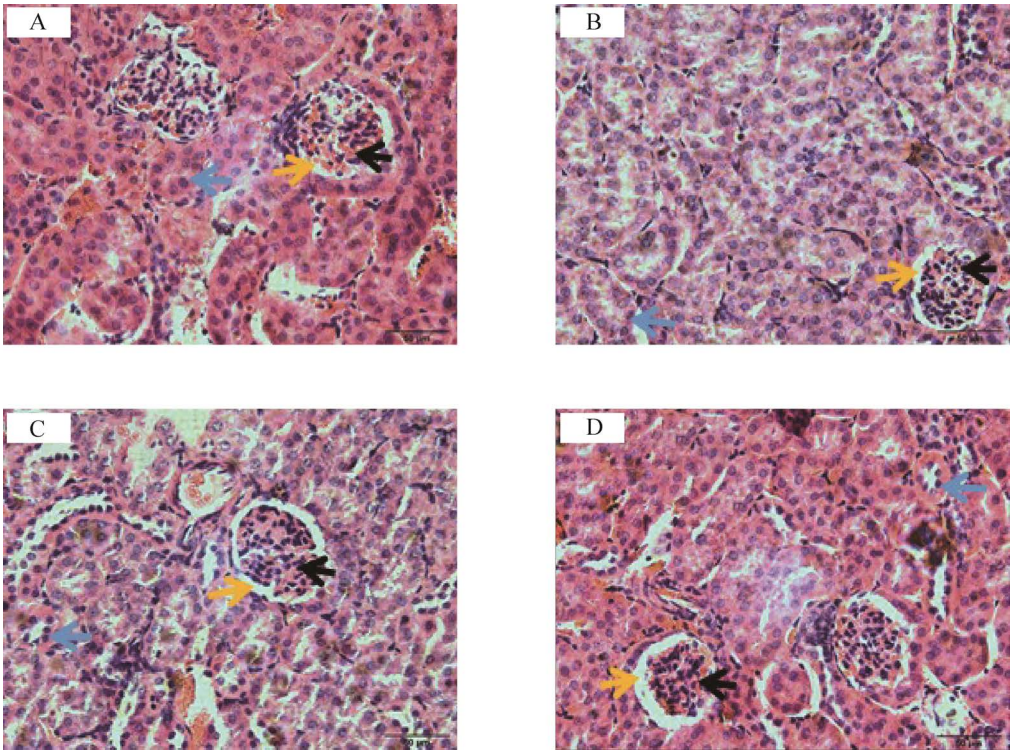

Figure 1 Photomicrographs of the renal tissues stained with HE following 0 (A), 0.01 (B), 0.1 (C) and 1 (D) mg/L BPA exposure (*n*=5 mice/group, 400×, bar=50 μm). The black arrow represents glomeruli. The blue arrow represents the renal tubule. The yellow arrow represents the renal capsule cavity.

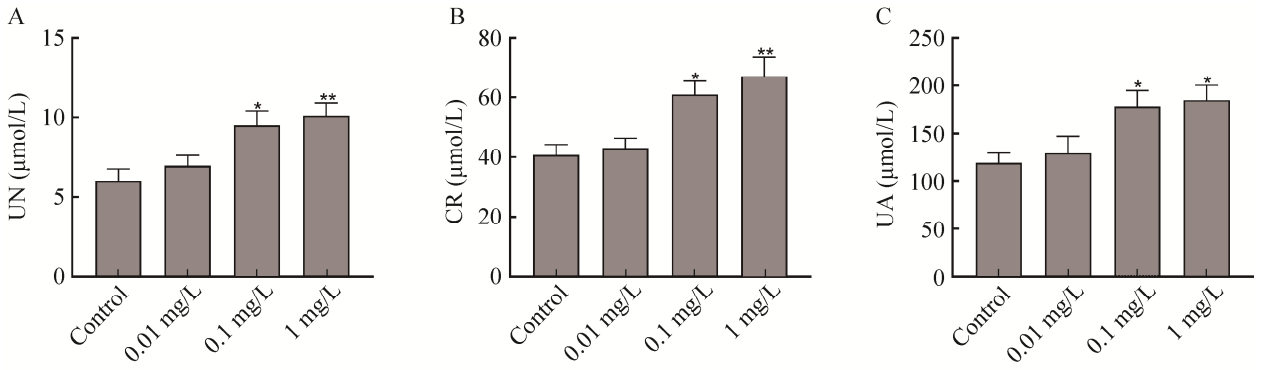

Figure 2 Effects of different doses of BPA on serum biochemical indexes of mice. * and ** mean *P*<0.05 and *P*<0.01, respectively ($\bar{x}\pm s$, *n*=10 mice/group).

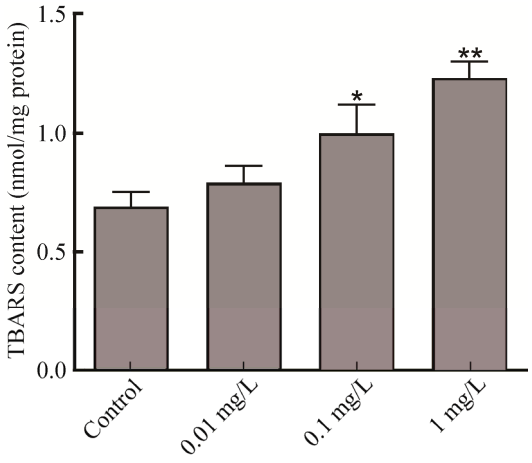

Figure 3 Effect of BPA on TBARS content in kidney tissues of male mice treated with 0, 0.01, 0.1, and 1 mg/L BPA ($\bar{x}\pm s$, n=10 mice/group). * and ** mean P<0.05 and P<0.01, respectively.

male mice exposed to BPA (0.1 and 1 mg/L) was significantly increased from (0.69±0.06) nmol/mg in the control group to (1.00±0.12) nmol/mg and (1.23±0.07) nmol/mg in the medium and high doses.

2.4 BPA decreases the antioxidant capacity of the kidney

To appraise the antioxidant capacity of the kidney under BPA exposure, we examined the mRNA expression levels of SOD, glutathione-S-transferase (GST), and catalase (CAT). As shown in Figure 4A−C, the mRNA expression levels of *SOD* (F (3, 56)=4.988, P=0.004), *GST* (F (3, 56)=4.872, P=0.004), and *CAT* (F (3, 56)= 3.655, P=0.018) were lower in the kidney of male mice isolated from BPA (0.1 and 1 mg/L)-exposure groups than those isolated from the

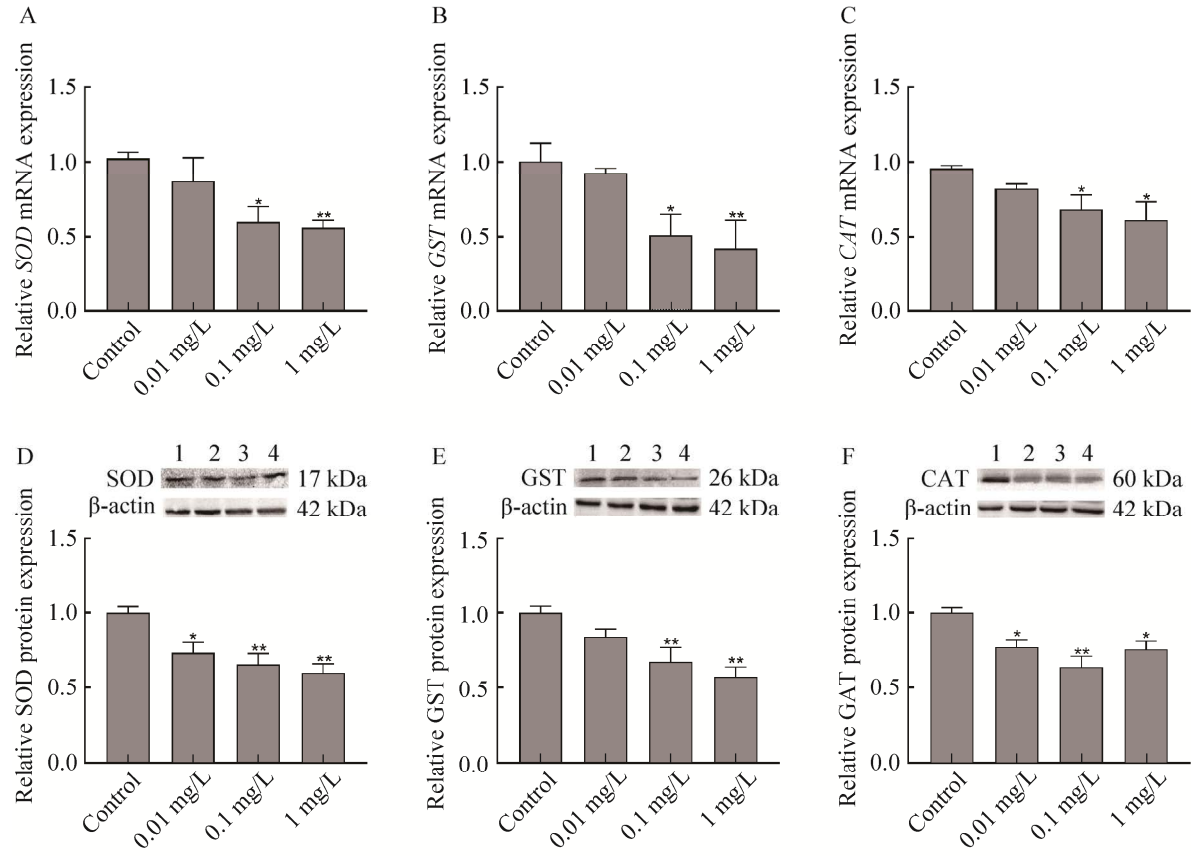

Figure 4 The changes in expressions of antioxidant-related genes and proteins in kidney of male mice treated with 0, 0.01, 0.1, and 1 mg/L BPA ($\bar{x}\pm s$, n=5 mice/group). A−C: Gene expressions (qRT-PCR) of *SOD*, *GST*, and *CAT*. D−F: Protein expressions of SOD, GST, and CAT. Western blotting: 1, 2, 3, and 4 correspond to control, 0.01, 0.1, and 1 mg/L BPA. * and ** mean P<0.05 and P<0.01, respectively.

control group. Furthermore, the Western blotting analysis results exhibited that the protein expressions of SOD (F (3, 16)=8.231, *P*=0.002), GST (F (3, 16)=7.588, *P*=0.002), and CAT (F (3, 16)=7.766, *P*=0.002) were lower in the kidney of male mice from BPA-treatment groups than those from the control group (Figure 4D−F).

2.5 BPA induces apoptosis in the kidney of male mice

We analyzed the possible role of apoptosis in BPA-induced renal abnormalities. The mRNA expression levels of *Caspase*-3 (F (3, 56)=5.364, *P*=0.003) and *Bax* (F (3, 56)=4.340, *P*=0.008) were sharply risen in the 0.1 and 1 mg/L BPA- exposed groups than those in the control group (Figure 5A−B). The *Bcl-2* mRNA expression levels were significantly decreased in the 0.1 and 1 mg/L BPA-treated groups (Figure 5C, *Bcl-2* (F (3, 56)= 3.731, *P*=0.016). Additionally, the ratio of Bax/Bcl-2 mRNA expressions was dose- dependently higher in all BPA-exposed groups than that of the control group (Figure 5D, *Bax/Bcl-2* (F (3, 56)=6.371, *P*<0.001). Except for the Bax in the low dose group, the protein expressions of Caspase-3 (F (3, 16)=7.583, *P*=0.002) and Bax (F (3, 16)=6.182, *P*=0.005) were significantly up-regulated in the BPA-treated groups compared with those in the control group (Figure 6A−B). Unexpectedly, the

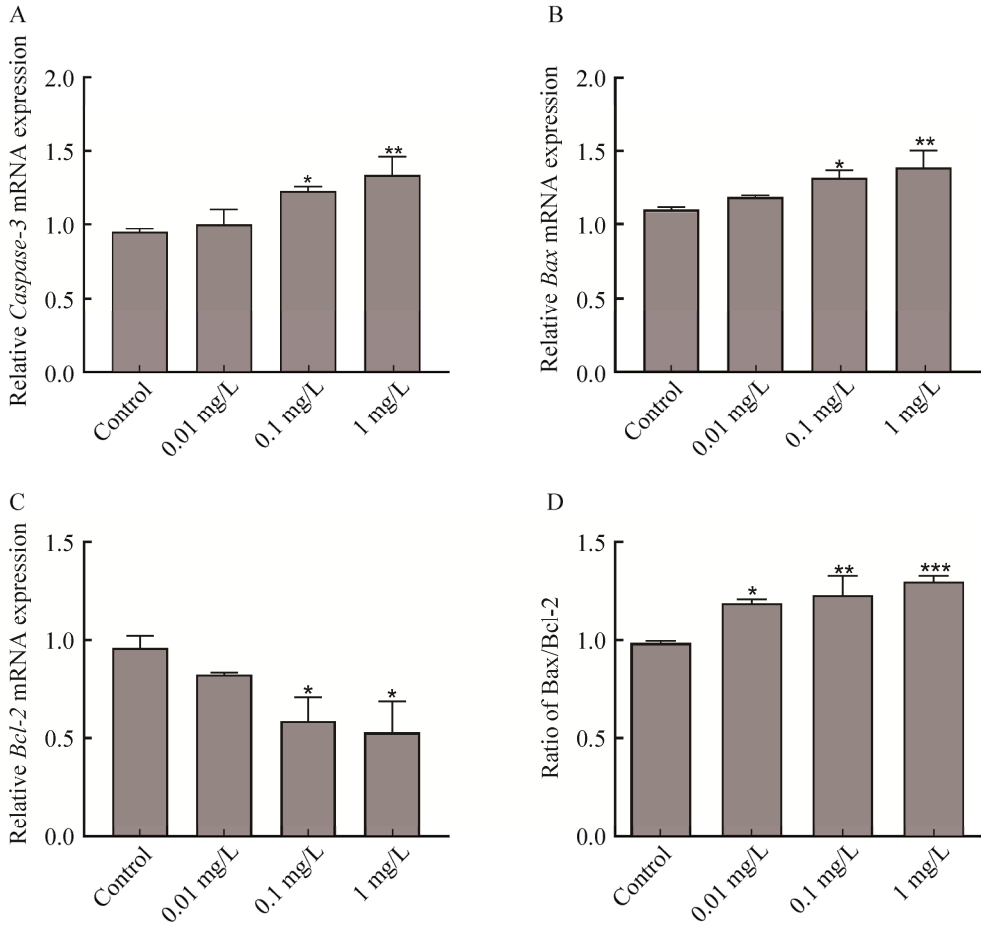

Figure 5 The changes in expressions of apoptosis-associated genes in kidney of male mice treated with 0, 0.01, 0.1, and 1 mg/L BPA ($\bar{x}\pm s$, n=5 mice/group). A−C: Gene expressions (qRT-PCR) of *Caspase-3*, *Bax*, and *Bcl-2*. D: The ratio of *Bax*/*Bcl-2* in gene expressions. *, **, and *** mean *P*<0.05, *P*<0.01, and *P*<0.001, respectively.

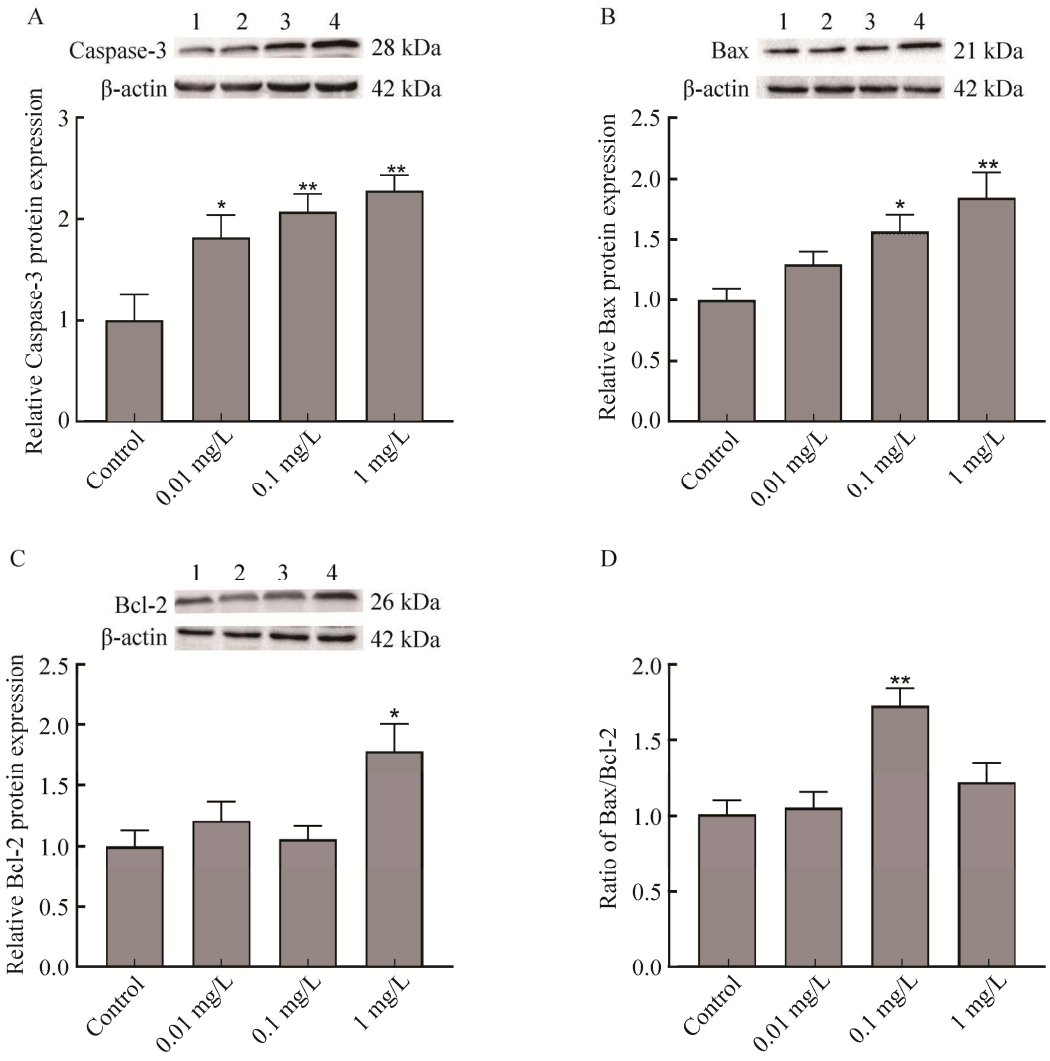

Figure 6 The changes in expressions of apoptosis-associated proteins in kidney of male mice treated with 0, 0.01, 0.1, and 1 mg/L BPA ($\bar{x}\pm s$, n=5 mice/group). A−C: Protein expressions of Caspase-3, Bax, and Bcl-2. D: The ratio of Bax/Bcl-2 in protein expressions. Western blotting: 1, 2, 3, and 4 correspond to control, 0.01, 0.1, and 1 mg/L BPA. * and ** mean P<0.05 and P<0.01, respectively.

Bcl-2 protein expression levels were memorably increased in the 1 mg/L BPA-treated group (Figure 6C, F (3, 16)=5.025, P=0.012). However, the ratio of Bax/Bcl-2 protein expressions was significantly elevated in the 0.1 mg/L BPA-exposed group (Figure 6D, Bax/Bcl-2 (F (3, 16)= 8.253, P=0.002).

3 Discussion

It is generally believed that BPA is one of the toxic chemicals with the highest production in different consumer goods at present[4,40]. As evident from many studies, BPA may give rise to toxicity in humans and animals' nervous system, immune system, cardiologic, reproductive, metabolic, and renal functions, due to excessive daily exposure[23,31,40-41]. The toxicity of BPA exposure in renal dysfunction, histopathological alterations, mitochondrial oxidative stress, and apoptosis is getting progressively clear[28,31]. Whereas, there is relatively little evidence about the toxic effects of BPA on renal system.

Results of this study presented that

BPA-induced renal injury was dose-dependent. In the present study, we found impairment in the kidney by morphologic analysis at the light microscopic level. We also revealed that BPA-induced renal toxicity was associated with renal oxidative stress and apoptosis in F1 male mice by qRT-PCR and Western blotting methods via analyzing the expressions of SOD, GST, CAT, Caspase-3, Bax, and Bcl-2 at gene and protein levels, TBARS content, and UN, CR, and UA concentrations.

The principal findings in the current *in vivo* study revealed that BPA significantly promoted renal pathology of male mice as demonstrated by histological alterations. Specifically, BPA with different concentrations could affect the secretion of sex hormones in male offspring mice. Over the years, we have frequently reported that the imbalances of testosterone and estradiol will result in abnormal spermatogenesis and induce reproductive toxicity[42-44]. In the current study, BPA-induced lung inflammation and aggravation of atherosclerosis of mice have been reported previously using this dosing paradigm[36-37]. Our data showed that the concentration of UN, CR, and UA was markedly increased in 0.1 and 1 mg/L BPA treatment animals compared to the control group. Therefore, these results demonstrated that BPA could affect the biochemical function of mice kidney and cause disease.

BPA-induced oxidative stress was monitored by measuring TBARS content and gene and protein expression levels of SOD, GST, and CAT antioxidants. Increasing TBARS (MDA) content is considered a biomarker of oxidative stress[45-47]. Consistent with our previous reports of oxidative stress induced by BPA in Marc-145 cells, *in vitro*[5], increased TBARS level in the kidney of male offspring mice indicates elevated cellular oxidative stress as a result of ROS generation and depletion of antioxidants. Significant declines in SOD, GST, and CAT genes and proteins expression levels observed in this study might be due to inhibiting enzyme activity induced by BPA. The inhibition of BPA on SOD, GST, and CAT would probably damage the antioxidant defenses of renal cells and make them more vulnerable to oxidative attacks. In conclusion, we found that BPA induces renal oxidative stress by elevating the production of TBTRS (MDA) and inhibiting antioxidant capacity.

Cell apoptosis is a more impressible marker for renal histopathology[48]. Caspase-3 belongs to the pro-apoptotic caspase family protein. Bax and Bcl-2 are Bcl-2 family proteins, which play a crucial role in apoptosis as a regulator of the integrity of the outer mitochondrial membrane. Bcl-2 is the anti-apoptotic protein that postpones cytochrome c release entering cytosol from mitochondria. Bax is the pro-apoptotic protein that impedes the cytoprotective effect of Bcl-2 by stimulating cytochrome c release entering cytosol from mitochondria[6,49-50]. This study detected the elevated mRNA and protein expressions of Caspase-3, an apoptotic marker of the Fas/FasL signaling pathway. Furthermore, the mRNA and protein expressions of Bax were significantly increased, and mRNA expression levels of the Bcl-2 were reduced in the kidneys of male mice exposed to BPA. The ratio of Bax/Bcl-2 determines the promotion or inhibition of apoptosis because it impinges cell sensitivity to apoptosis[51]. Hence, the past decade has witnessed a steady accumulation of observations that mitochondrial impairment, mitochondria-facilitated oxidative stress, and mitochondria-mediated cell death have fundamental roles in renal injury[52-53]. The effects of BPA on pro-apoptotic and anti-apoptotic protein expressions (Bax and Bcl-2) were previously reported[48], which verifies the current study results. Similarly, we also uncovered the Bcl-2 protein expression levels were prominently increased in the 1 mg/L BPA-treated group (the specific remains need to be further ascertained). However, the ratio of the Bax/Bcl-2 was risen in renal tissues of male mice exposed to 0.01, 0.1, and 1 mg/L BPA via drinking water, especially in the 0.1 mg/L BPA-treated group. Thus, the elevated Bax and Caspase-3 might release cytochrome c from

mitochondria mediated by BPA and the subsequent activation of Caspase-3. Additionally, significant apoptosis and DNA damage induction with BPA dose-dependent were detected in renal Marc-145 cells[5]. Importantly, these results indicated that BPA induced renal cellular apoptosis in mice, possibly through Fas/FasL signaling pathway and mitochondrial apoptotic pathway.

4 Conclusion

In summary, our findings demonstrated that BPA could considerably increase the contents of serum renal function indexes (UN, CR, and UA), damage glomerular structure in the kidney of male mice *in vivo*. BPA exposure groups showed the strongest nephrotoxicity, primarily through elevating TBARS content, increasing expressions of Caspase-3, Bax, and ratio of Bax/Bcl-2 from gene and protein levels, and reducing expressions of SOD, GST, and CAT at gene and protein levels. BPA could lead to lack of balance between oxidation and antioxidation, pro-apoptotic and anti-apoptotic protein, leading to renal oxidative stress, causing irreparable damage and/or death. To sum up, these findings suggest that oxidative stress and apoptosis may play an essential role in BPA-induced male nephrotoxicity.

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