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RESEARCH ARTICLE

The effects of a short-term exposure to propiconazole in zebrafish (*Danio rerio*) embryos

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Abstract

Propiconazole (PCZ) is a widely used fungicide around the world and was frequently detected in surface waters, which would pose risk to aquatic organisms. Previous studies indicated that PCZ has high toxicity to different kinds of fish. However, most of the studies focus on the toxicity and mechanisms of PCZ to adult fish, the potential toxicity mechanism of PCZ to fish embryos is still poorly understood. The present study investigated the effects of PCZ on content of reactive oxygen species (ROS) and malondialdehyde (MDA); activities of superoxide dismutase (SOD), catalase (CAT), and Na^+-K^+-ATPase; and expression level of genes related to oxidative stress, cell apoptosis, and innate immune system in zebrafish embryos after 96-h exposure. The results showed that 5.0 mg/L PCZ induced oxidative damage in zebrafish embryos, as indicated by increased ROS and MDA content and alteration of antioxidative enzyme activity. The activity of Na^+-K^+-ATPase in zebrafish embryos was significantly inhibited after exposure to 0.5 mg/L PCZ. The expression levels of *bax*, *p53*, *casp-3*, *casp-9*, and *apaf-1* were significantly increased, indicating that cell apoptosis was caused in embryos by 5.0 mg/L PCZ. The expression level of interleukin-*1b* (*IL-1b*) and *IL-8* increased after exposure to 0.5 mg/L PCZ, but that of *IL-1b*, *IL-8*, and *cxcl-c1c* (*chemokine (C-X-C motif) ligand 18b*) decreased in 5.0-mg/L PCZ treatment group, indicating an immunotoxicity effect. Our results suggest that oxidative damage, cell apoptosis, and immunotoxicity would be induced in zebrafish embryos after short-term exposure to PCZ.

Keywords Propiconazole · *Danio rerio* · Zebrafish embryos · Oxidative stress · Cell apoptosis · Immunotoxicity

Introduction

Propiconazole (PCZ) is a triazole fungicide that has been broadly used in agriculture around the world (Egaas et al. 1999). There is concern about the wide application of PCZ and its possible detrimental effects on the health status of aquatic organisms that may arise from spray drift or surface run-off (Konwick et al. 2006). Several studies reported that PCZ would remain in the environment for a long time after being used, with a higher frequency of detection in surface waters, which may pose risk to aquatic species. For example, in inland surface waters of southern Sweden, PCZ was one of the most frequently detected pesticides with a detection frequency greater than 90% (Ahrens et al. 2018). The concentration of PCZ in the Mekong River delta in Vietnam was between 0.5 and 4.76 μg/L with a detection frequency of 39.2% (Chau et al. 2015). In banana plantation areas of Costa Rica, the detection frequency of PCZ in surface water samples was 43% (Castillo et al. 2009). Kahle et al. (2008) studied the occurrence of nine agricultural azole fungicides in wastewater treatment plants (WWTPs) in Switzerland and found that the concentration of PCZ was between 1 and 30 ng/L in WWTPs influents. Furthermore, the DT_{50} (time taken for 50% of the parent compound to dissipate from a given area) value of PCZ was 99–116 days (Edwards et al. 2016).

Previous studies of PCZ toxicity and its mechanisms in fish mainly focused on adult fish. The antioxidant defense system and Na^+-K^+-ATPase activity in brain of rainbow trout (*Oncorhynchus mykiss*) were disordered and inhibited by PCZ at sublethal concentrations (0.2, 50, 500 μg/L) after 30-day exposure (Li et al. 2010). Moreover, PCZ significantly inhibited the acetylcholinesterase (AChE) activity in nerve system and changed the

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behavior of freshwater fish (*Clarias batrachus*) (Singh 2014). PCZ markedly lowered the AChE and monoamine oxidase (MAO) activities in the brain, while it increased the levels of lipid peroxidation (LPO) and protein carbonyls (PC), oxidative stress biomarker of liver, kidney, and gills in *Channa punctata* Bloch (Tabassum et al. 2016). After exposure to sublethal dose of PCZ at 50 and 500 μg/L, the ROS (reactive oxygen species) contents were increased in gill of rainbow trout, while the activities of superoxide dismutase (SOD), catalase (CAT), and Na^+-K^+-ATPase were significantly decreased comparing to the control group (Li et al. 2011). Skolness et al. (2013) reported that PCZ (500 and 1000 μg/L) could increase gonad weight of female fish and up-regulate genes of *CYP19* (aromatase), *CYP17* (hydroxylase/lyase), and *CYP11A* (cholesterol side-chain-cleavage). Valadas et al. (2019) reported that PCZ would induce abnormal behavior at 1700 and 8500 ng/L and increase the activities of SOD and CAT in the brain at 425, 850, and 1700 ng/L in adult zebrafish. So far, studies on the toxic effects of PCZ in fish embryos are still very limited. Teng et al. (2019) reported that PCZ (2.5 and 4.5 mg/L) significantly inhibited zebrafish embryonic development, increased the expression levels of genes associated with lipid metabolism, but decreased the content of fatty acids in larvae. Souders et al. (2019) suggested that PCZ (10 and 100 μM) induced hypopigmentation and disrupted mitochondrial bioenergetics in zebrafish embryos. Kumar et al. (2019) found that PCZ exposure up to 5 days postfertilization would significantly increase the Casp3/7 (caspase-3/7) activity at 1 μg/L, the transcripts of *CYP51* (*cytochrome P450 proteins* 51), *GST* (*glutathione S-transferase*), *p53* (*tumor protein p53*) at 1000 μg/L, the MDA content at 1 and 1000 μg/L, and the transcripts of *BAX* (*BCL-2-associated X protein*) at 0.3, 1, and 1000 μg/L in embryo-larval zebrafish. However, to our knowledge, no studies have investigated the effect of PCZ on zebrafish embryos in oxidative stress, apoptosis, and immunotoxicity after a short-term exposure.

The purpose of this study was to further investigate the potential toxic mechanisms of PCZ in zebrafish embryos after short-term exposure. The activities of antioxidant enzymes (SOD, glutathione peroxidase (GPx), and CAT) and Na^+-K^+-ATPase, the content of ROS and MDA, and the expression levels of genes related to oxidative stress (*sod1*, *sod2*, *cat*, and *gpx*), cell apoptosis (*bax*, *bcl-2* (*BCL2 apoptosis regulator a*), *p53*, *casp-3*, *casp-9*, and *apaf-1* (*apoptotic protease activating factor 1*)) and innate immune response (*IL-8* (*interleukin-8*), *IL-1b* (*interleukin-1b*), *cxcl-c1c* (*chemokine (C-X-C motif) ligand 18b*), and *ifn* (*interferon phi 1*)) in the embryos were determined after 96-h exposure to PCZ. The results would provide more information about toxic mechanisms of PCZ on zebrafish embryonic development.

Materials and methods

Chemicals

PCZ (95% purity; CAS-No.:60207-90-1) was provided by Beijing Huarong Biological Hormone Plant Co., Ltd. (Beijing, China). Stock solution of 50,000 mg/L PCZ was prepared in acetone containing 1% Tween-80 and stored at 4 °C. Test solutions were diluted from stock solution using reconstituted water, which was prepared based on ISO-7346-3 protocol (ISO 1996).

Zebrafish cultivation and embryo collection

Parental adult zebrafish (wild-type AB) strain was obtained from Beijing Hongdagaofeng Aquarium Department. The parental zebrafish were cultivated at 27 ± 1 °C in a recirculation equipment (Esen Corp., Beijing, China) with a 14 h/10 h light/dark cycle. The procedure of embryo production and collection was based on previous studies (Li et al. 2018a; Zhao et al. 2019).

Exposures for enzyme activity and gene expression analyses

In order to investigate the effects of a short-term exposure to PCZ on enzyme activity and gene expression in zebrafish embryos, 300 embryos were randomly selected and exposed to a series of test solutions (control, 0.5, 2.0, and 5.0 mg/L) for 96 h. The concentrations of 0.5 and 2.0 mg/L propiconazole are doses lower than acute toxic doses, while 5.0 mg/L was the one that can induce obvious malformation after exposure for 96 h based on a pre-experiment. Each treatment group consisted of three replicates (with 100 embryos in each replicate). The exposure solutions were renewed and dead embryos were cleared every 24 h. There was no difference between blank control (reconstituted water) and solvent control (0.01% acetone and 0.001% Tween-80 dissolved in reconstituted water, *v*/*v*) in preliminary tests; so, the solvent control was used as control for statistical analysis in this study. The conditions of exposure were 27 ± 1 °C under a 14:10-h light/dark cycle.

Determination of enzyme activities and contents of MDA and ROS

After 96-h exposure, 50 embryos from each replicate of each treatment were washed and homogenized in cold phosphate buffer saline (PBS) (pH 7.4) and centrifuged at 12,000×*g* for 10 min at 4 °C. The supernatants in PBS were used to determine the activities of SOD, CAT, GPx, and contents of MDA and ROS based on a previous study (Zhao et al. 2019), using

SOD, CAT, GPx, MDA, and ROS assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China), respectively.

Fifty zebrafish embryos were washed and homogenized with cold stroke-physiological saline solution. The procedure of centrifugation was the same as that mentioned above, and the supernatant was used to measure the Na^+-K^+-ATPase activity, using Na^+-K^+-ATPase assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) by following the manufacturer's instruction.

The total protein content was determined using a Bradford Protein Assay Kit (Beyotime Institute of Biotechnology, China) following the manufacturer's instructions.

Gene expression analyses

After 96-h exposure, 30 embryos were collected for gene transcriptional level analysis. Total RNA was extracted with Trizol Reagent (Tiangen Biotech, Beijing, China) following the manufacturer's instruction. The total RNA content and quality were determined by a NanoDrop 2000 spectrophotometer (DS-11, DeNovix Inc. USA). First-strand complementary DNA (cDNA) was synthesized using Quant RTase Kit (Tiangen Biotech, Beijing, China) basing on the manufacturer's instruction. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed by the ABI 7500 q-PCR system (Applied Biosystem, Foster City, CA) using the SYBR Green PCR Master Mix Reagent Kit (Tiangen Biotech, Beijing, China) following the manufacturer's instruction. The thermal cycle was set at 95 °C for 15 min, followed by 40 cycles at 95 °C for 10 s, annealing at 60 °C for 20 s, and extension at 72 °C for 32 s.

The expression levels of genes related to oxidative stress (*sod1*, *sod2*, *cat*, and *gpx*), cell apoptosis (*bax*, *bcl-2*, *p53*, *casp-3*, *casp-9*, and *apaf-1*), and innate immune response (*IL-1b*, *IL-8*, *cxcl-c1c*, and *ifn*) were determined in zebrafish embryos after exposure to PCZ for 96 h. The primers of these genes are shown in the Table 1. *Beta actin* (*β-actin*) was selected as housekeeping gene (Cao et al. 2016; Mu et al. 2016). The expression levels of target genes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Chemical analysis

Ten milliliters of test solutions from different treatment groups was collected in centrifuge tubes, and then, 10 mL acetonitrile and 5.0 g NaCl were added. PCZ was extracted by vortexing in a vortex mixer for 1 min and centrifuged at 5000*g* for 3 min. After this, 1 mL of supernatants was filtered with a 0.22-μm organic membrane. PCZ was separated by an HP-5 column (0.18 mm × 20 m × 0.18 μm) (Agilent Technologies, USA) and analyzed using gas chromatography (GC) with electron capture detection (ECD) (Agilent 7820A, USA). The GC was operated under splitless mode, with a flow rate of nitrogen gas at 1.0 mL/min. The temperatures of injector port and detector were set at 250 °C and 300 °C, respectively. Oven programming was as follows: 100 °C (initial) to 270 °C (30 °C/min) and held for a further 7 min.

Data analysis

The data of physiological endpoint, enzyme activities, and gene expression levels were checked for normality and homogeneity of variances with Shapiro-Wilk's test and Levene's test, respectively, and then assessed by one-way analysis of variance (ANOVA) followed by the Dunnett's post hoc test to calculate significant differences between treatment groups and control, using SPSS 22.0 software (IBM SPSS Statistics, Chicago, IL, USA). All data were reported as means ± standard deviation (SD).

Results

Chemical analysis

The concentrations of PCZ in test solutions were maintained within ± 20% of the nominal concentration from 0 to 24 h of exposure solution (Table 2). Therefore, the nominal concentration could represent the actual content of PCZ in this study based on the OECD guidelines (OECD 2013).

PCZ-induced oxidative stress in zebrafish embryos

The contents of ROS and MDA and activities of GPx and CAT were significantly increased in zebrafish embryos after exposure to 5.0 mg/L PCZ for 96 h. The activity of SOD in embryos was not obviously changed in all treatment groups. The expression levels of genes related to antioxidant system (*sod1*, *sod2*, and *cat*) were not significantly changed comparing with that of control group, except that of *gpx* markedly decreased in 5.0 mg/L PCZ group (Fig. 1 A–F).

PCZ decreased Na^+-K^+-ATPase activity

The Na^+-K^+-ATPase activity in zebrafish embryos was significantly decreased after exposure to 5.0 mg/L PCZ when compared with that of the control group (Fig. 2).

Effect of PCZ on expression of cell apoptosis genes

The expressions of *bax*, *p53*, *casp-3*, *casp-9*, and *apaf-1* were markedly up-regulated after exposure to 5.0 mg/L PCZ

Table 1 Primer pairs used in RT-qPCR. All sequences are shown 5′ → 3′

Category	Target gene	Forward primer	Reverse primer	Reference
Oxidative stress	*sod1*	GTCGTCTGGCTTGTGGAGTG	TGTCAGCGGGCTAGTGCTT	Zhu et al. (2015)
	sod2	CCGGACTATGTTAAGGCCATCT	ACACTCGGTTGCTCTCTTTTCTCT	Zhu et al. (2015)
	cat	AGGGCAACTGGGATCTTACA	TTTATGGGACCAGACCTTGG	Zhu et al. (2015)
	gpx	AGATGTCATTCCTGCACACG	AAGGAGAAGCTTCCTCAGCC	Zhu et al. (2015)
Cell apoptosis	*p53*	TTGTCCCATATGAAGCACCA	TGGAGCCCTTGGCCTTTTT	Deng et al. (2009)
	bcl-2	AACGCAGCTTTCTAACCGTG	TCTGCTGACCGTACATCTCCA	Mu et al. (2016)
	bax	ATGAGCTGGATGGAAATGC	CCTGTTCCCTGATCCAGTTAA	Mu et al. (2016)
	casp-3	CCGCTGCCCATCACTA	ATCCTTTCACGACCATCT	Zhu et al. (2015)
	casp-9	AAATACATAGCAAGGCAACC	CACAGGGAATCAAGAAAGG	Zhu et al. (2015)
	apaf-1	CTGTGATGGCTGCCGAGGTTGTT	GCCGCTGACTGGTGTCTGAACTT	Zhu et al. (2015)
Innate immune response	*IL-8*	GTCGCTGCATTGAAACAGAA	CTTAACCCATGGAGCAGAGG	Mu et al. (2016)
	IL-1b	TTGAAAGTGCGCTTCAGCA	CGGTCTCCTTCCTGAAGAACA	Mu et al. (2016)
	cxcl-c1c	CATCCGGCCAGCTCTGCTTGAAT	CCACTCTTGACCTCCTGTGCTCTCT	Mu et al. (2016)
	ifn	ATCATCCACCTTATGGATGCC	CCCATGCTTCAGCACTGTATT	Mu et al. (2016)
	β-actin	ATTGACTCAGGATGCGGAA	GAGGGCAAAGTGGTAAACG	Yin et al. (2018)

comparing with that of the control group. The mRNA levels of *bcl-2* showed no significant change when compared with that of the control group. However, the ratios of *bcl-2/bax* were significantly decreased by 5.0 mg/L PCZ, which were only 0.21-fold of the control group (Fig. 3 A, B).

Effect of PCZ on immune system genes

The expression levels of *IL-8* and *IL-1b* were significantly increased after exposure to 0.5 mg/L PCZ, which were 1.52- and 1.86-fold of the control group, respectively. However, after exposure to 5.0 mg/L PCZ, the transcriptional levels of *IL-8*, *IL-1b*, and *cxcl-c1c* were significantly decreased after exposure to 5.0 mg/L PCZ, which were only 0.30-, 0.33-, and 0.24-fold of the control group, respectively. The mRNA level of *ifn* was not obviously changed in all treatment groups (Fig. 4).

Discussion

Previous study indicated that PCZ significantly increased the malformation rate, decreased heartbeat number, hatching rate, and body length of embryos, as well as increased the expression levels of genes associated with lipid metabolism, and decreased the content of fatty acids in zebrafish embryos (Teng et al. 2019). In the present study, we further investigated the effect of PCZ on oxidative stress, cell apoptosis, and innate immune response of zebrafish embryos after a short-term exposure. The results showed that 5.0 mg/L PCZ caused oxidative damage (with markedly increased content of ROS and MDA and decreased expression level of *gpx*), cell apoptosis (with significant up-regulation of *bax*, *p53*, *casp-3*, *casp-9*, and *apaf-1*), and immunotoxicity (with marked down-regulation of *cxcl-c1c*, *IL-8*, and *IL-1b*) in zebrafish embryos.

Table 2 Measured concentrations of PCZ in different test solutions

Nominal concentration (mg/L)	Measured concentration (mg/L)		Deviation (%)	
	0 h	24 h	0 h	24 h
Control	ND	ND	–	–
0.5	0.45 ± 0.021	0.49 ± 0.026	− 10.55	− 1.9
2	1.70 ± 0.093	1.79 ± 0.018	− 14.81	− 10.51
5	4.73 ± 0.13	4.91 ± 0.034	− 0.53	− 1.84

ND not detected

Deviation (%) = actual concentration − nominal concentration) / nominal concentration × 100%

"–" not available

Data for measured concentrations were presented as mean ± standard deviation

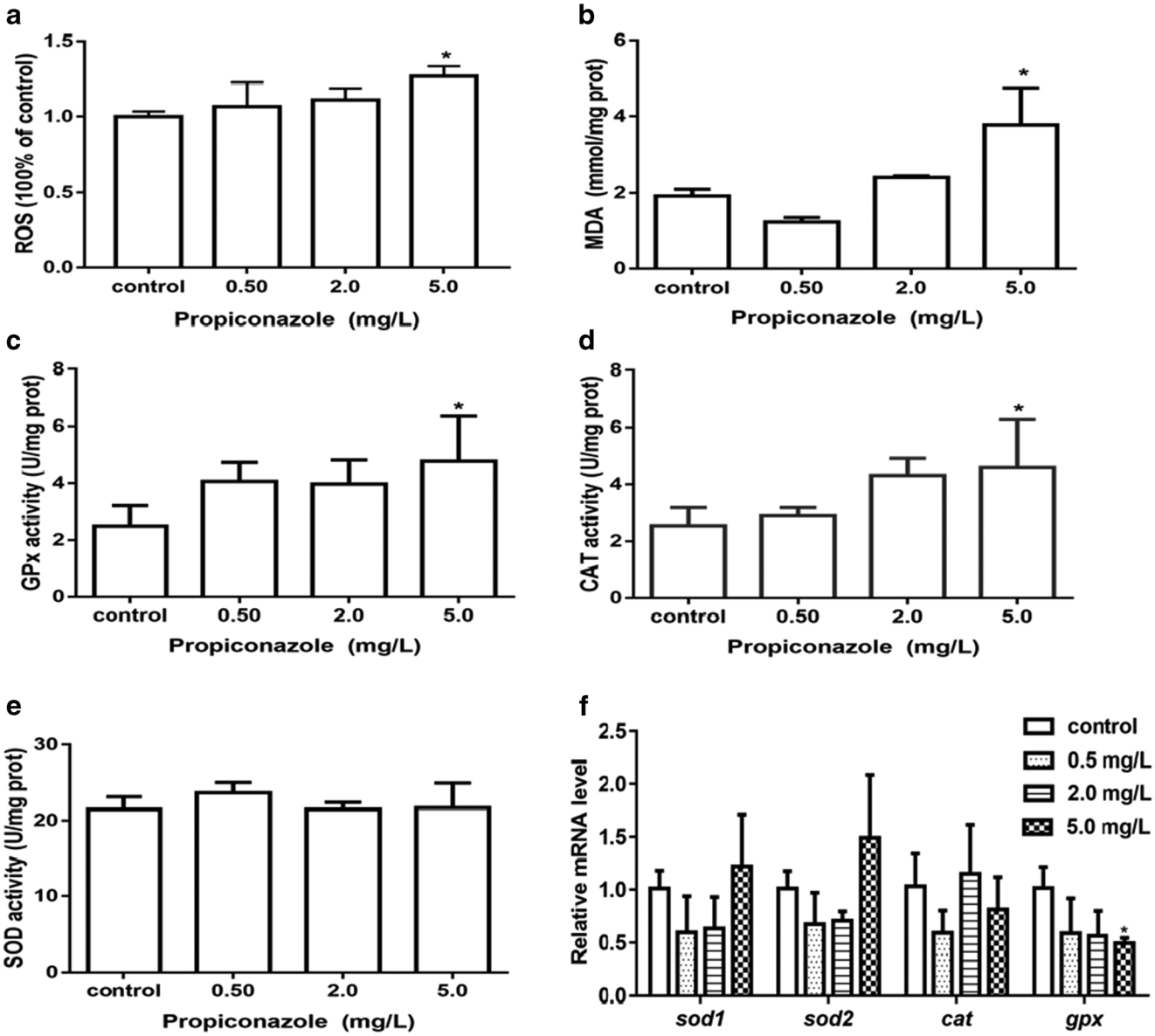

Fig. 1 ROS and MDA contents, antioxidant enzyme activities, and relative mRNA levels of genes related to antioxidant system in embryos after exposure to PCZ. **A** ROS content. **B** MDA content. **C**–**E** Activities of GPx, CAT, and SOD, respectively. **F** Expression levels of *sod1*, *sod2*, *cat*, and *gpx*. Asterisks denote significant differences comparing with the control group (determined by Dunnett's post hoc comparison, $*p < 0.05$). Error bars indicate standard deviation

Pollutants as pesticides (insecticides, herbicides, and fungicides) would induce oxidative stress in fish (Lushchak 2016), which was caused by high levels of ROS and led to damage of lipids, proteins, and DNA (Schieber and Chandel Navdeep 2014). In the present study, the levels of ROS and MDA were markedly increased after exposure to 5.0 mg/L PCZ, indicating that PCZ caused oxidative stress in zebrafish embryos. This result was similar as those in other fish. For example, PCZ increased the ROS content, altered homeostasis of SOD, CAT, GST, and protein peroxidation in early life stage of medaka fish (Tu et al. 2016). The activities of antioxidant enzymes (GSH, SOD, CAT, GPx) were increased significantly in liver of juvenile rainbow trout after exposure to PCZ (Li et al. 2013). In addition, many triazole fungicides (triadimenol, myclobutanil, cyproconazole, difenoconazole, epoxiconazole, etc.) also induced oxidative stress in zebrafish embryos (Chu et al. 2016; Huang et al. 2016; Mu et al. 2016; Zhu et al. 2014). CAT is primarily located in the cytosol and peroxisomes and converts H_2O_2 to H_2O and O_2 (Hu et al. 2017), and GPx can remove H_2O_2 at high levels (Ng et al. 2007). These antioxidant enzymes form the first line of alleviating oxidative stress in organisms (Rodriguez et al. 2004). The result of the present study showed that the activities of GPx and CAT were significantly increased by 5 mg/L PCZ, indicating that the enzymes were triggered to alleviate oxidative stress in zebrafish embryos. However, the high content of

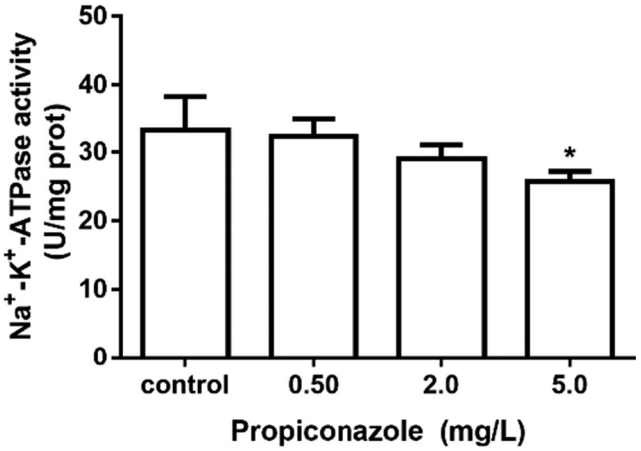

Fig. 2 Na^+-K^+ ATPase activity in embryos of zebrafish after exposure to PCZ. Asterisks denote significant differences comparing with the control group (determined by Dunnett's post hoc comparison, $*p < 0.05$). Error bars indicate standard deviation

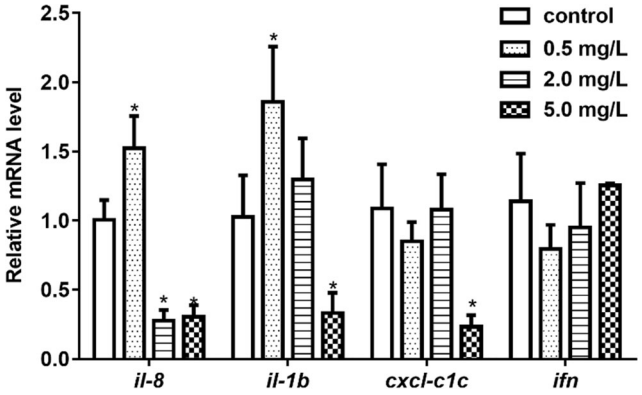

Fig. 4 Relative mRNA levels of immune-related genes (*IL-8*, *IL-1b*, *cxcl-c1c*, and *ifn*) in zebrafish embryos after exposure to PCZ for 96 h. Asterisk denotes a significant difference between the control and treatment groups (determined by Dunnett's post hoc comparison, $*p < 0.05$). Error bars indicate standard deviation

ROS and MDA in zebrafish embryos observed in 5.0-mg/L PCZ group suggested that the increased activity of CAT and GPx could not entirely remove ROS caused by PCZ. It has to be mentioned that although GPx and CAT were significantly induced by PCZ at 5.0 mg/L, the expression levels of *gpx* and *cat* were not induced in the same treatment group. Several authors have also reported this kind of disparity between changes of gene expression level and alterations of enzyme activity (Velki et al. 2017; Zheng et al. 2016). The mismatch between mRNA levels and enzyme activities may be caused by two reasons: first, the transcriptome might not always be reflected at proteome levels, and there is a time-lag effect between transcription and translation (Nam et al. 2005); second, the enzyme activity might be modulated by post-translational events including post-translational modifications and regulation of enzyme activity (Glanemann et al. 2003).

The Na^+-K^+-ATPase plays a crucial role in generating and maintaining transmembrane ionic gradients, which is vitally important for cellular function (Kaplan 2002; Mobasheri et al. 2000), and is essential for zebrafish embryonic organ development as well (Blasiole et al. 2006; Shu et al. 2003). The activity and signaling of Na^+-K^+-ATPase could be regulated by oxidative stress in organism (Shah et al. 2016). In this study, the Na^+-K^+-ATPase activity of zebrafish embryos was significantly decreased after exposure to 5.0 mg/L PCZ. We speculated that the oxidative stress induced by PCZ may impact the Na^+-K^+-ATPase activity in zebrafish embryos. This result was in consistence with that of previous studies, which indicated that Na^+-K^+-ATPase activity was significantly decreased in gills and brain of adult fish after exposure to PCZ (Li et al. 2010; Li et al. 2011; Tabassum et al. 2016).

Excess ROS content could activate cell apoptosis signaling pathways (Redza-Dutordoir and Averill-Bates 2016; Sinha et al. 2013). Cell apoptosis is a cell suicide mechanism that enables to eliminate any unnecessary or unwanted cells (Pfeffer and Singh 2018). The p53 protein is a transcription factor that could prevent tumor development through

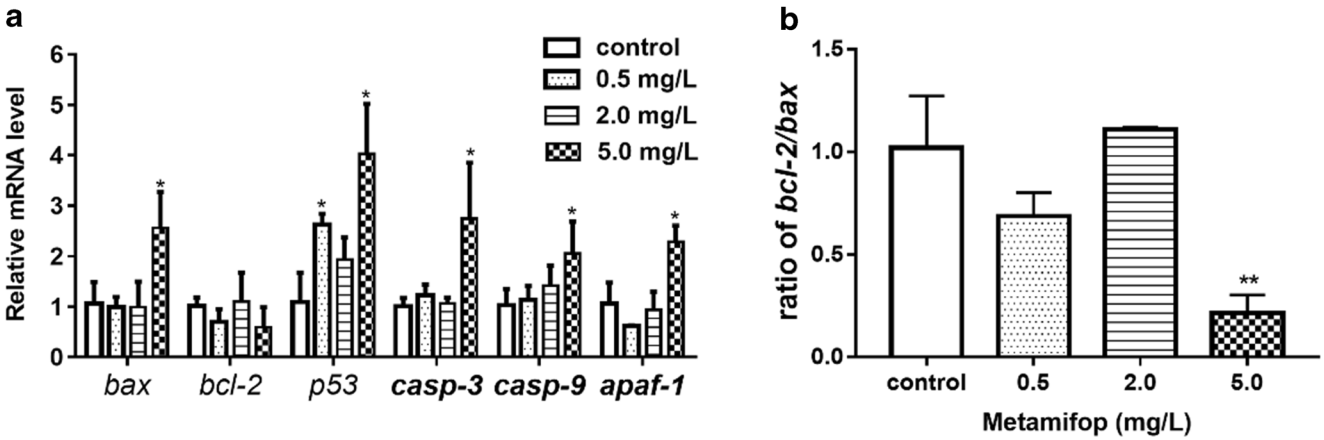

Fig. 3 Effect of PCZ on expression of cell apoptosis genes. **A** Expression levels of genes related to cell apoptosis in zebrafish embryos after exposure to PCZ. **B** The ratio of *bcl-2*/*bax*. Asterisk denotes a significant difference between the control and the treatment groups (determined by Dunnett's post hoc comparison, $*p < 0.05$, $**p < 0.01$). Error bars indicate standard deviation

induction of cell cycle arrest and cell death by apoptosis (Bykov and Wiman 2003). Apaf1 is the molecular core of the apoptosome, which is the executioner of mitochondria-dependent apoptosis (Ferraro et al. 2003). Caspase-9 remains bound to the apoptosome where it could activate executioner caspases such as caspase-3 and -7 (Acehan et al. 2002; Rodriguez and Lazebnik 1999). The BCL-2-associated X protein (BAX) is a cardinal proapoptotic member of the BCL-2 family, which regulates the critical balance between cellular life and death (Walensky and Gavathiotis 2011). In the present study, significant up-regulations of genes related to cell apoptosis (*bax*, *p53*, *casp-3*, *casp-9*, and *apaf-1*) were observed in zebrafish embryos in 5.0-mg/L PCZ group, indicating that cell apoptosis was probably induced after PCZ exposure. In Kumar et al. (2019) study, the transcription level of *casp-9* was not markedly changed after exposure to PCZ at 0.3, 1, and 1000 μg/L; the possible reason might be that the PCZ exposure levels were much lower than those of the present study. The ratio of *bcl-2*/*bax* determines the survival or death of cells following an apoptotic stimulus (Korsmeyer et al. 1995). In the present study, the ratio of *bcl-2*/*bax* of zebrafish embryos was markedly decreased in 5.0-mg/L PCZ treatment group, which further confirm that cell apoptosis might be caused by PCZ.

Many studies have indicated that pesticides disturbed the immune response of fish embryos (Cao et al. 2019; Jiang et al. 2016; Jin et al. 2013; Li et al. 2018a). The innate immune system is a fundamental defense mechanism of fish (Magnadóttir 2006). Cytokines are important regulators of immune system and host defense network in fish (Savan and Sakai 2006). Cytokines (IL-1b, IL-2, IL-6 etc.) and chemokines (IL-8, CXCL-clc) are secreted proteins that can regulate the nature of immune responses (Steinke and Borish 2006). The result of the present study showed that the transcription levels of *IL-8* and *IL-1b* were markedly increased in the 0.5-mg/L PCZ group; however, the transcription levels of *IL-1b*, *IL-8*, and *cxcl-c1c* were significantly decreased in 5.0-mg/L PCZ group, indicating that innate immune responses might be disturbed in zebrafish embryos. Some previous studies also showed that the transcription levels of immune system-associated genes were decreased in low-dose treatment groups, but increased in high-dose treatment groups. For example, the transcription levels of *TNF-a* in zebrafish embryos up-regulated markedly after exposure to 2.0 μg/L pyraoxystrobin for 96 hpf, but decreased significantly in 4.0-μg/L treatment group (Li et al. 2018b), and the transcription of *cxcl-c1c* in zebrafish embryos increased significantly after exposure to 0.1 μg/L azoxystrobin for 72 hpf, but decreased in 1.0- and 10-μg/L treatment group (Jiang et al. 2018). The potential mechanism of such alteration of immune-related genes in zebrafish embryos after exposure to different concentration of PCZ is unknown. Further study on this mechanism seems necessary.

Conclusion

In summary, the results of the present study indicated that PCZ induced high levels of ROS and MDA, altered the activities of antioxdative enzyme (SOD and CAT), and decreased Na^+-K^+-ATP activity in zebrafish embryos; meanwhile, alterted transcription of oxidative stress, cell apoptosis, and immune response-related genes were induced in the zebrafish embryos after exposure to PCZ. These results provide more insights into the mechanisms of PCZ toxicity, which suggest that in addition to toxic mechanisms being reported previously (Kumar et al. 2019; Souders et al. 2019; Teng et al. 2019), oxidative damage, cell apoptosis, and innate immune response might be involved in the acute toxicity of PCZ to zebrafish embryos.

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