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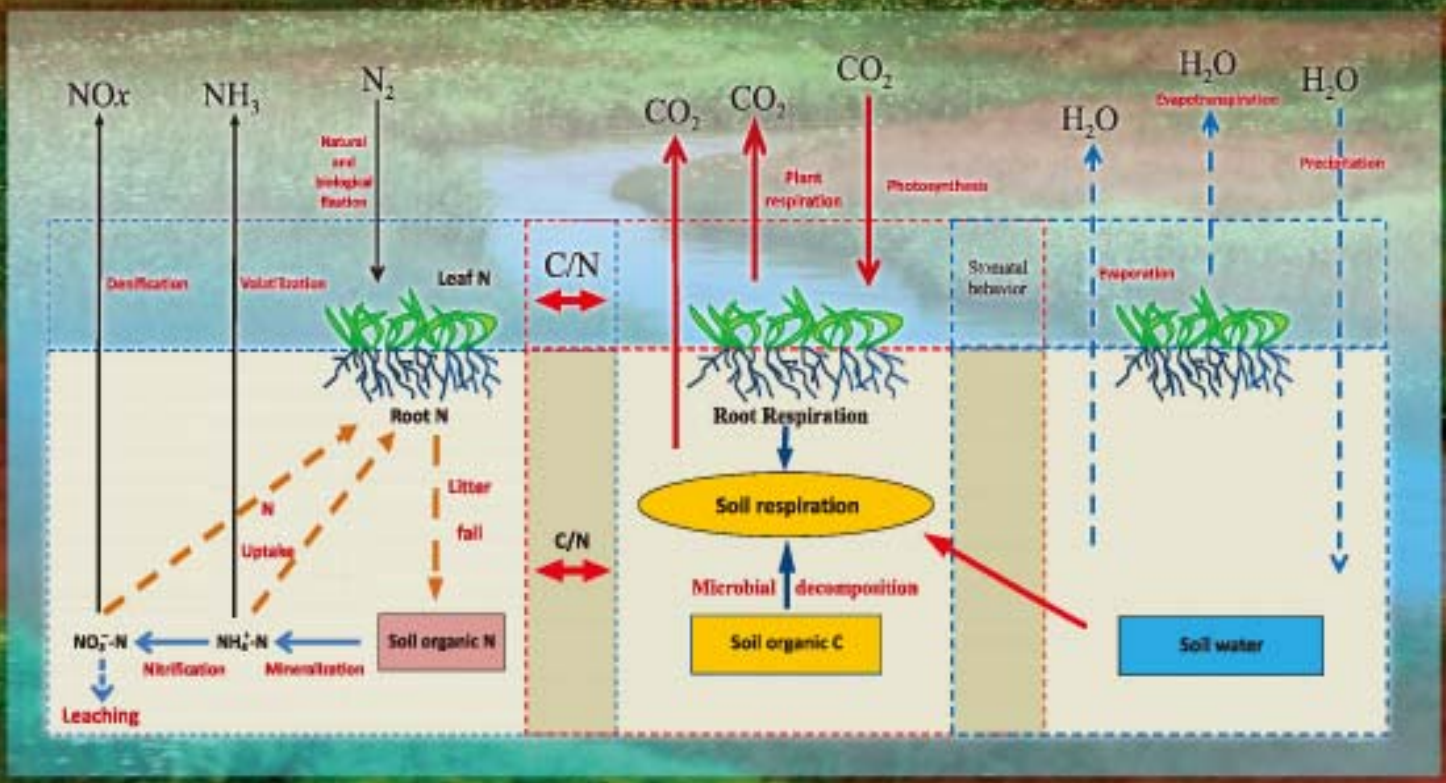

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T-2 toxin induces developmental toxicity and apoptosis in zebrafish embryos

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ABSTRACT

T-2 toxin is one of the most important trichothecene mycotoxins occurring in various agriculture products. The developmental toxicity of T-2 toxin and the exact mechanism of action at early life stages are not understood precisely. Zebrafish embryos were exposed to different concentrations of the toxin at 4–6 hours post fertilization (hpf) stage of development, and were observed for different developmental toxic effects at 24, 48, 72, and 144 hpf. Exposure to 0.20 μmol/L or higher concentrations of T-2 toxin significantly increased the mortality and malformation rate such as tail deformities, cardiovascular defects and behavioral changes in early developmental stages of zebrafish. T-2 toxin exposure resulted in significant increases in reactive oxygen species (ROS) production and cell apoptosis, mainly in the tail areas, as revealed by Acridine Orange staining at 24 hpf. In addition, T-2 toxin-induced severe tail deformities could be attenuated by co-exposure to reduced glutathione (GSH). T-2 toxin and GSH co-exposure induced a significant decrease of ROS production in the embryos. The overall results demonstrate that T-2 toxin is able to produce oxidative stress and induce apoptosis, which are involved in the developmental toxicity of T-2 toxin in zebrafish embryos.

Introduction

T-2 toxin is a fungal metabolite that belongs to a group of type A trichothecenes, and it is produced by various *Fusarium* species. It can infect maize, wheat and oats during cultivation and/or storage (Creppy, 2002). Among trichothecenes, T-2 toxin is considered the most potent myelotoxin and haematotoxin. Both humans and animals suffer from several pathologies due to intoxication after consumption of food and feed that are contaminated with T-2 toxin (Joffe, 1978; Parent-Massin, 2004; Meissonnier et al., 2008). Oral, parenteral and cutaneous exposures to T-2 toxin induce lesions in hematopoietic, lymphoid and gastrointestinal tissues and suppress reproductive organ functions (Stanford et al., 1975; Williams, 1989; IARC, 1993; Sharma, 1993). It has been indicated that T-2 toxin readily passes through the placenta and is delivered to the fetal tissues, resulting in the induction of embryo/fetal death, fetal brain damage and fetal skeletal malformation (Lafarge-Frayssinet et al., 1990; Rousseaux and Schiefer, 1987). Sehata (2005) and coworkers demonstrated that the mechanism of T-2 toxin-induced maternal and fetal toxicities is due to oxidative stress, followed by activation of the MAPK pathway, finally inducing apoptotic cell death (Doi et al., 2008). Recently, members of our group described how T-2 toxin induced developmental toxicity in the *in vitro* embryo-toxicity test known as the embryonic stem cell test (EST) on differentiated murine embryonic stem cells. The mechanism is related to the apoptosis induced by the reactive oxygen species (ROS)-mediated mitochondrial pathway (Fang et al., 2012).

Although several studies have revealed the developmental toxicity potential of T-2 toxin and the possible mechanisms, there has been no definitive assessment of

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developmental toxicity of T-2 toxin, especially in the early life stages, and whether the known mechanisms such as oxidative stress and apoptosis are involved in T-2 toxin-induced developmental toxicity in the early life stages is still unclear. In recent years, the zebrafish embryo is becoming an important model in developmental toxicology.

Zebrafish embryos have some characteristics such as *in vitro* fertilization capability, rapid embryonic development and optical transparency, which make it easy to detect morphological endpoints or observe the development process in early life stages (Yang et al., 2009). On the other hand, the zebrafish genome is well characterized and dysmorphology phenotypes linked to genomic targets can potentially enable rapid evaluation of mechanisms of action for compound-induced teratogenicity (McGrath and Li, 2008). More important, zebrafish have also been demonstrated to share many common features with humans in development, anatomy, physiological responses, metabolism and chemical-induced organ/tissue responses, and many molecular pathways are evolutionarily conserved between zebrafish and humans (Goldsmith and Jobin, 2012; Lieschke and Currie, 2007). Based on the above-mentioned advantages and the similarity to the embryo development in vertebrates, the zebrafish embryo-based assay called the zebrafish embryotoxicity test (ZET) has been a useful model to evaluate the adverse effects of chemicals on embryo-fetal development in the early life stages. ZET could play a role in bridging the gap between *in vitro* cell-based models and *in vivo* mammalian models (Sukardi et al., 2011). Therefore, zebrafish embryos are considered an excellent model for the analysis of congenic human diseases and for detecting hazards for the developing fetus (Goldsmith, 2004; Xu and Zon, 2010).

According to the above-mentioned facts, by utilizing the zebrafish embryo as a model, the present study is therefore intended to evaluate the developmental toxicity of T-2 toxin and the potential mechanisms at early life stages in which the duration of T-2 toxin exposure spans the complete developmental period of a vertebrate embryo from the embryo-fetal phase to the juvenile phase (larvae).

1 Materials and methods

1.1 Chemicals and test media

T-2 toxin and the oxidant-sensitive probe 2′,7′-dichlorofluorescein diacetate (DCF-DA) used to assess ROS concentration and tricaine used to anesthetize the embryos were obtained from Sigma-Aldrich (Louis, MO, USA), and Acridine Orange (AO) and reduced L-glutathione from Amresco (Solon, OH, USA). All other chemicals and reagents utilized in this study were of analytical grade.

Fish water, similar to the reconstituted water described in OECD 203, annex 2 (measured ranges: pH 7.5–8.0, conductivity 632–676 μS/cm^2, hardness 217–235 mg/L $CaCO_3$, oxygen 92%–98%) was used as the medium for all solutions during the experiments. The pH was adjusted to 6.8–8.0 and oxygen levels of the solutions were always higher than 80%.

1.2 Maintenance of zebrafish and collection of embryos

The wild-type (AB strain) zebrafish were obtained from the North Center of National Zebrafish Resources of China and maintained at (28 ± 0.5)°C in a 14-hr light/10-hr dark cycle in an automatic zebrafish housing system (ESEN, China) in charcoal-filtered tap water supplemented with a salt solution at a pH and conductivity range of 6.8–7.2 and 450–520 μS, respectively. The water was continuously aerated and renewal of the water occurred in a semi-static manner (complete renewal of solutions after 24 hr). The fish were fed with live brine shrimp twice daily. The health condition of the fish was regularly checked (daily except weekends). The care and husbandry of zebrafish used in this study was in conformity with the guidelines (ILAR, 1996) that regulate the humane care and use of laboratory animals for research purposes.

Prior to spawning, males and females were housed separately for a minimum of 5 days. The day before eggs were required, males and females were placed in breeding tanks with a 1:1 or 2:1 (male:female) ratio. The breeding tanks were equipped with a spawning tray, which consists of a fine net with an appropriate mesh size for eggs to fall through. The fish were left undisturbed overnight using a separator to hold the male and female fish separately in the middle of the tank. Spawning was induced in the morning after removing the separator, and eggs could be collected after 15 min.

1.3 Embryo toxicity tests

At 2–4 hpf (hours post fertilization), embryos were examined under a dissecting microscope (SEX10, Olympus, Japan), and those that had developed normally and had reached the blastula stage were selected for the subsequent experiments. At approximately the 4–6 hpf stage, 12 fertilized eggs were transferred individually to wells of a 24-well plate (one embryo per well) containing 2 mL solution of different concentrations of T-2 toxin (0, 0.05, 0.10, 0.20, 0.25, 0.30, 0.40 and 0.80 μmol/L). Three replicates were run for each concentration. The exposure was static and continuous throughout 144 hpf, and solutions were not renewed during the overall experiment. The range of concentrations was selected based on earlier dose range-finding studies that identified the concentration that would induce the presence of a 0 and 100% effect level (for both malformation and mortality). It was important to note that the mentioned concentrations do not reflect the real dose in the embryos. Bioavailability studies should be performed to evaluate this further.

1.4 Evaluation of developmental and teratogenic effects

Evaluation of developmental and teratogenic effects was performed according to Selderslaghs et al. (2009). Briefly, at 24, 48, 72 and 144 hpf, embryotoxicity and morphological characteristics were evaluated using an inverted microscope (CKX 41, Olympus, Japan) or dissecting microscope (SEX10, Olympus, Japan). The embryos were evaluated for the presence and morphological development of somites, tail detachment and otoliths, eyes, heartbeat and blood circulation. After hatching, larvae were evaluated for skeletal deformities, body position and their ability to swim (after stimulation if necessary). The percentage of embryotoxicity was calculated as the ratio of dead embryos and/or larvae over the number of embryos (generally 12 fertilized eggs) at the start of exposure. On the other hand, the percentage of malformation at 24, 48, 72 and 144 hpf was calculated as the ratio of malformed embryos and/or larvae over the number of embryos that were alive at 24 hpf. As for the effect of GSH on T-2 toxin-induced malformations, the percentage of severe tail deformities in the presence of 0.30 μmol/L T-2 toxin and increasing concentrations of GSH co-exposures was calculated as the ratio of malformed embryos over 12 fertilized eggs at 24 hpf.

The resulting data, from at minimum 3 independent experiments (n = 3) each with 12 replicates (one embryo per well) per concentration were imported into Graphpad Prism (Graphpad Prism, version 2.01) to create concentration-response curves for mortality and malformation for each time point. These concentration-response curves were required to determine EC_{50} (teratogenic effects) and LC_{50} (lethal/embryotoxic effects) values. Based on LC_{50} and EC_{50} values, a teratogenic index (TI) was calculated.

1.5 Acridine Orange staining

Apoptosis was assessed using AO, a nucleic acid-selective metachromatic stain. At 24 hpf, after exposure to the concentrations of T-2 toxin (0, 0.05, 0.10, 0.20, and 0.30 μmol/L), 10 embryos from each group (n = 3) were washed twice in 30% Danieau's solution (58 mmol/L NaCl, 0.7 mmol/L KCl, 0.4 mmol/L $MgSO_4$, 0.6 mmol/L $Ca(NO_3)_2$ and 5 mmol/L HEPES, pH 7.4), transferred to 5 μg/mL AO dissolved in 30% Danieau's solution for 20 min at room temperature. Singular embryos were washed with 30% Danieau's solution three times for 5 min. Before examination, the embryos were anesthetized with 1× (0.016%) Tricaine for 3 min. Apoptotic cells were identified with a fluorescence microscope (Olympus BX61, Japan).

1.6 ROS measurement

The generation of ROS in the embryos exposed to T-2 toxin (0, 0.05, 0.10, 0.20, and 0.30 μmol/L) at 24 hpf was measured using dichlorofluorescein-diacetate (DCF-DA). Twenty embryos were washed with cold PBS (pH 7.4) twice and then homogenized in cold buffer (0.32 mmol/L sucrose, 20 mmol/L HEPES, 1 mmol/L $MgCl_2$, and 0.5 mmol/L phenylmethyl sulfonylfluoride (PMSF), pH 7.4). The homogenate was centrifuged at 15,000 r/min at 4°C for 20 min, and the supernatant was transferred to new tubes for further analysis.

Twenty microliters of the homogenate was added to a 96-well plate and incubated at room temperature for 5 min, after which 100 μL PBS (pH 7.4) and 4 μL DCF-DA stock solution (dissolved in absolute alcohol, 10 mg/mL) were added to each well. The plate was incubated at 37°C for 30 min. The fluorescence intensity was measured using a SpectraMax M5 multi-mode microplate reader (Molecular Device, USA) with excitation and emission at 485 and 530 nm, respectively. The ROS concentration was expressed in arbitrary units (mg DCF/protein).

1.7 Statistical analysis

The homogeneity of variances was checked with Levene's test, and the differences were evaluated by one-way ANOVA followed by Tukey's test (a post hoc test: LSD) using SPSS 11.5 (SPSS, Chicago, USA). The value $p < 0.05$ was used as the criterion for statistical significance. All values were expressed as the mean ± standard error.

2 Results

2.1 Mortality and malformations of T-2 toxin in zebrafish embryos

Zebrafish embryos were observed for developmental toxic effects as produced by various concentrations of T-2 toxin at developmental stages of 24, 48, 72, and 144 hpf. Embryonic mortality rate was 0% among control embryos. Groups treated with 0.05 and 0.10 μmol/L of T-2 toxin at all observation time points also showed no mortality, while embryos treated with 0.80 μmol/L of T-2 toxin induced 100% mortality within the first 24 hr of exposure. Embryos treated with 0.20, 0.25, 0.30, and 0.40 μmol/L of T-2 toxin experienced mortality rates of 0%, 5.5%, 30.5%, and 83.3%, respectively, at 24 hpf. Furthermore, T-2 toxin exposure caused mortality in a time- and concentration-dependent manner (**Fig. 1**).

The embryos of the control group developed normally in embryo medium, as did embryos treated with 0.05 and 0.10 μmol/L of T-2 toxin. Abnormalities in tail formation were observed in embryos exposed to 0.20 μmol/L or higher concentrations of T-2 toxin at 24 hpf (**Fig. 2a**). At 48 and 72 hpf, cardiovascular defects were observed in the embryos treated with 0.20 μmol/L or higher concentrations of T-2 toxin (**Fig. 2b, c**). At 144 hpf side-wise position and lack of swimming behavior (**Fig. 2d**) were most prominent. The percentage of all the developmental mal-

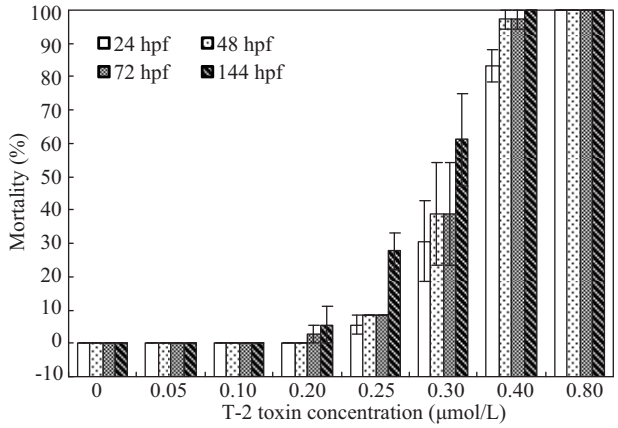

Fig. 1 Dose-response of mortality in zebrafish embryos exposed to different concentrations of T-2 toxin at 24, 48, 72, and 144 hours post fertilization (hpf). The values were presented as the mean ± SEM.

formations following exposure to T-2 toxin significantly increased in a dose-dependent manner (**Fig. 3**). Based on the percentage of individuals affected (malformation for any of the observed characteristics) for each concentration, concentration-response curves were created for each time point. LC_{50} (for embryotoxic effects or lethality) and EC_{50} (for specific teratogenic effects) values were derived from the concentration-response curves for all time points evaluated (**Fig. 4**). TI values were calculated as the ratio LC_{50}/EC_{50} (**Table 1**). The data showed T-2 toxin induced teratogenic effects on the zebrafish embryos.

Table 1 LC_{50}, EC_{50} (mean values of 3-independent experiments) and TI values as derived from the concentration-response curves for T-2 toxin

	LC_{50} (μmol/L)	EC_{50} (μmol/L)	TI (LC_{50}/EC_{50})
24 hpf	0.33	0.18	1.83
48 hpf	0.31	0.18	1.72
72 hpf	0.31	0.18	1.72
144 hpf	0.28	0.18	1.56

LC_{50}: concentration having caused the death of 50% tessted compared to control; EC_{50}: concentration having 50% of effect compared to control; TI (teratogenic index) was calculated as the ratio LC_{50}/EC_{50}.

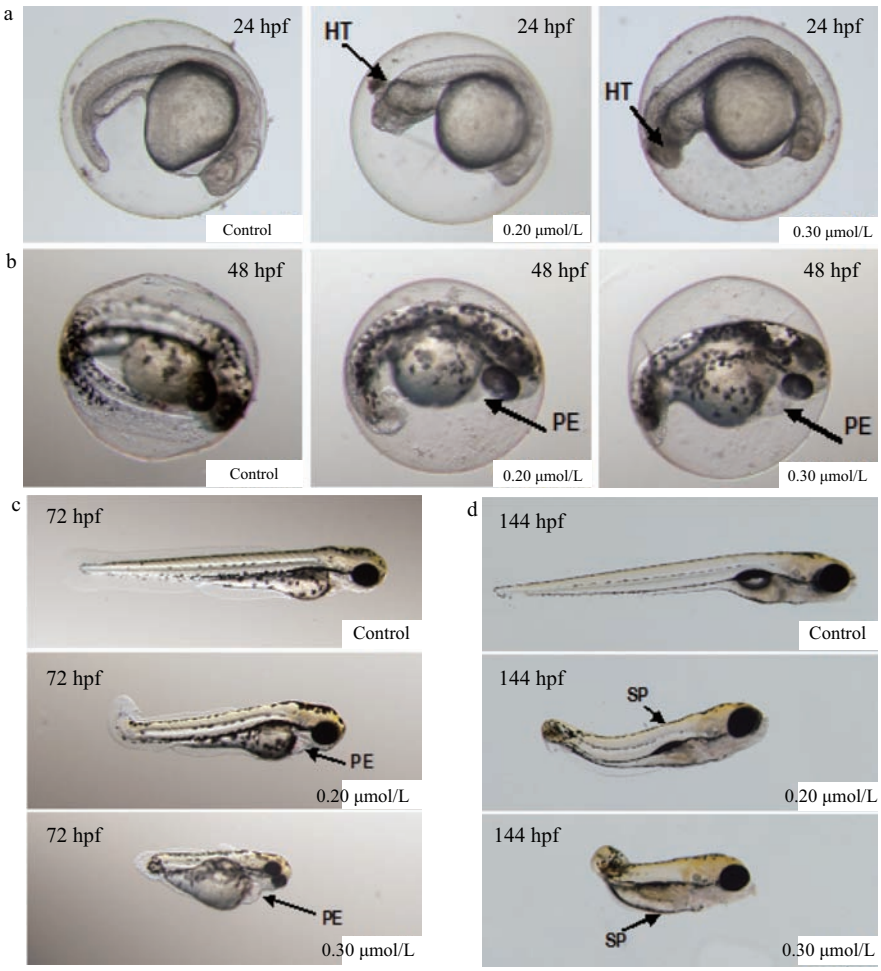

Fig. 2 Representative photographs of malformations caused by 0.20 and 0.30 μmol/L of T-2 toxin at (a) 24 hpf; (b) 48 hpf; (c) 72 hpf, and (d) 144 hpf. Malformations are indicated by arrows. HT: hook-like tail; PE: pericardial edema; SP: side-wise position.

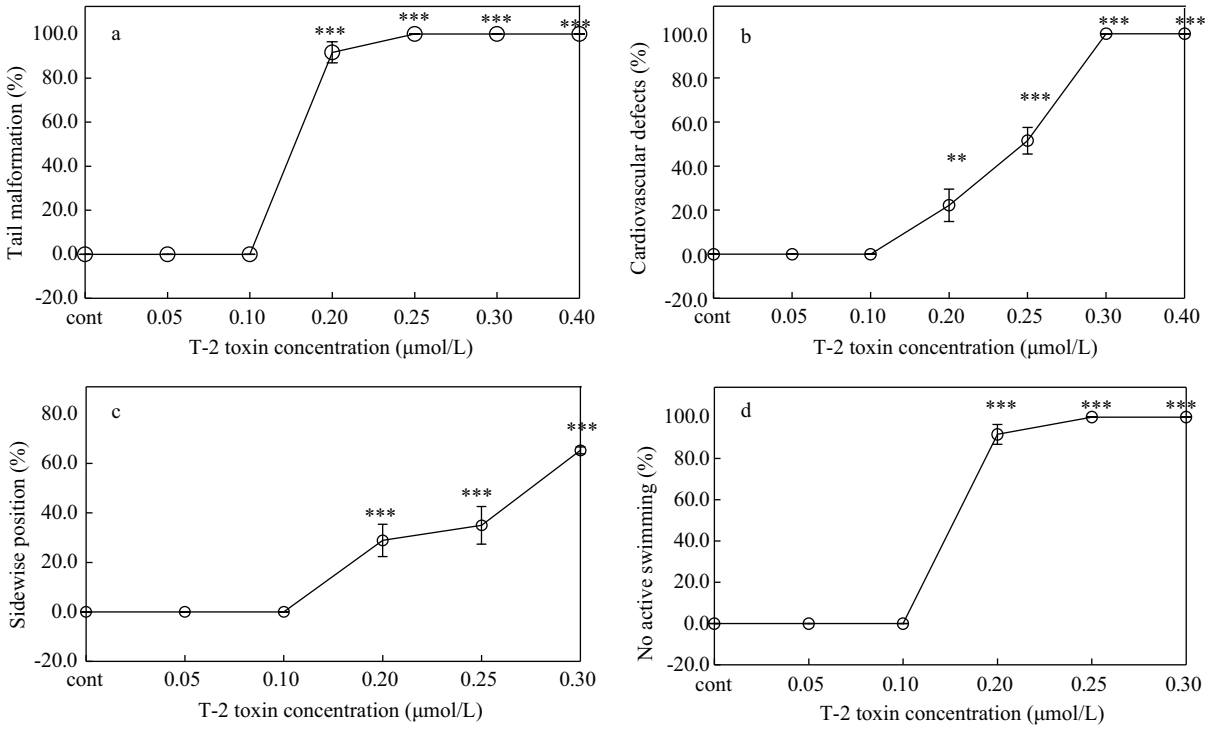

Fig. 3 Cumulative malformation rate in zebrafish embryos exposed to different concentrations of T-2 toxin. (a) tail deformities at 24 hpf; (b) cardiovascular defects at 48 hpf; (c) side-wise position at 144 hpf; (d) no active swimming at 144 hpf. Values that were significantly different from the control are indicated by asterisks (one-way ANOVA, followed by a *post hoc* test, LSD: $^{**}p < 0.01$; $^{***}p < 0.001$). Values are presented as mean ± standard error.

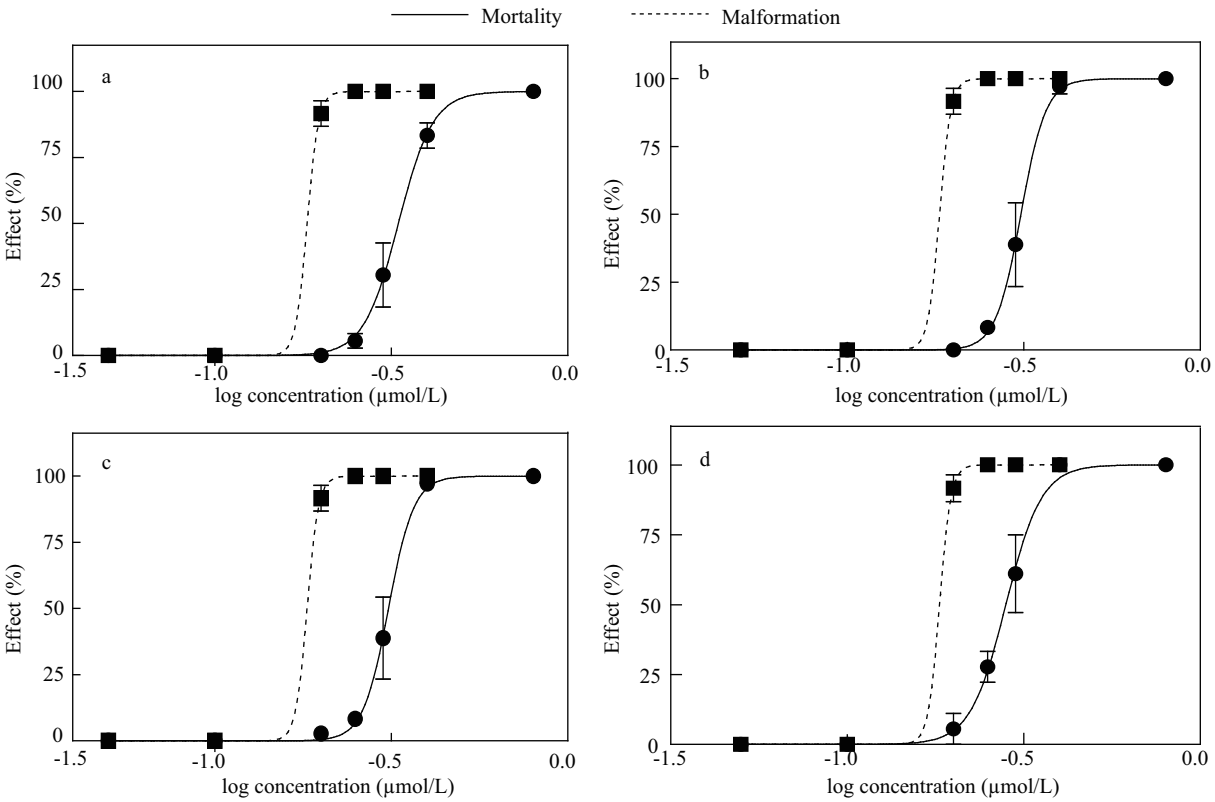

Fig. 4 Concentration-response curves of malformation and mortality for T-2 toxin (n = 3) at (a) 24 hpf; (b) 48 hpf; (c) 72 hpf and (d) 144 hpf. Values are presented as mean ± standard error.

2.2 Apoptosis analysis

There were no obvious apoptotic cells observed in the control embryos or the 0.05 μmol/L T-2 toxin-treated group, whereas considerable numbers of apoptotic cells appeared, mainly around the tail area, in 0.10, 0.15, 0.20, and 0.30 μmol/L T-2 toxin-treated groups. In addition, the apoptotic cells increased dose-dependently (**Fig. 5**).

2.3 Effect of T-2 toxin on production of ROS in zebrafish embryos

The percent of ROS levels treated with different concentrations of T-2 toxin in zebrafish embryos at 24 hpf compared with the control are shown in **Fig. 6**. No significant difference in the level of ROS production was observed in the embryos treated with 0.05 μmol/L of T-2 toxin when compared with the controls. However, ROS levels in the embryos treated with the other concentrations of T-2 toxin were significantly higher than the controls.

2.4 Effect of GSH on T-2 toxin-induced malformations and oxidative stress in zebrafish embryos

GSH is a known antioxidant and free-radical scavenger; thus, it is important for detoxification of ROS associated with chemical exposure. Since the embryotoxicity of T-2 toxin is considered to be associated with ROS overproduction and the resulting oxidative stress, we then determined if the developmental toxicity and teratogenicity caused by T-2 toxin at 24 hpf could be alleviated by this antioxidant.

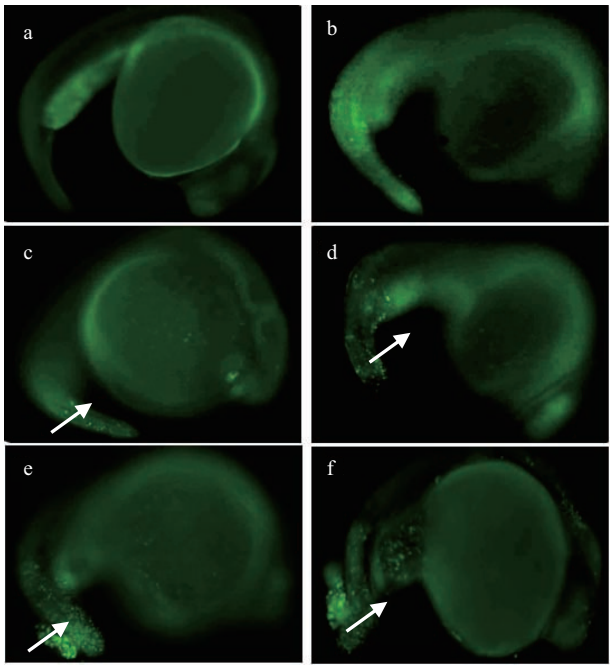

Fig. 5 Zebrafish embryos exposed to T-2 toxin at 24 hpf were stained with Acridine Orange (AO). Apoptotic cells stained with AO appeared mainly in the tail region. Apoptotic cells and abnormalities are indicated by arrows. (a) control; (b) 0.05 μmol/L; (c) 0.10 μmol/L; (d) 0.15 μmol/L; (e) 0.20 μmol/L; (f) 0.30 μmol/L.

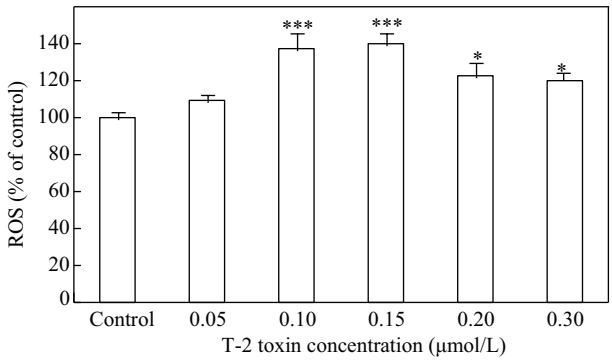

Fig. 6 Effects of different concentrations of T-2 toxin on reactive oxygen species (ROS) production at 24 hpf. The values are presented as the mean ± SEM. Values that were significantly different from the control are indicated by asterisks (one-way ANOVA, followed by a *post hoc* test, LSD: $^{*}p < 0.05$; $^{***}p < 0.001$).

Representative tail deformities of 24 hpf embryos exposed to 0.30 μmol/L T-2 toxin and after co-exposure with 200 μmol/L GSH are shown in **Fig. 7**. Severe tail deformities were defined as the presence of hook-like tail (**Fig. 7d**) and the absence of complete tails (**Fig. 7e**). It was established that the percent of the embryos exhibiting severe tail deformities induced by T-2 toxin was noticeably diminished when co-exposed to 200 μmol/L GSH (**Fig. 8a**), while slight tail deformities (**Fig. 7b, c**) were still observed. In addition, there was no difference in the percent of the severe tail deformities treated with the other concentrations of GSH.

We examined the effectiveness of GSH on ROS generation. In the ROS assay, GSH co-exposure (200 μmol/L) with 0.30 μmol/L T-2 toxin induced a significant decrease of ROS production in the embryos compared with T-2 toxin treatment alone, whereas hyper-generation of ROS in zebrafish embryos was still observed in the GSH co-exposure group when compared with the control group (**Fig. 8b**).

3 Discussion

The present study was conducted to ascertain whether T-2 toxin was developmentally toxic at early life stages, using a useful model of zebrafish embryos, and to elucidate its potential mechanism. From the data presented, the developmental toxicity and teratogenicity of T-2 toxin have been demonstrated in zebrafish embryos. The results indicated the responsiveness of this organism to the toxin through production of types of developmental abnormalities, such as tail deformities, cardiovascular defects and behavioral changes that had not been previously reported (**Figs. 2** and **3**). Furthermore, we found that T-2 toxin exposure resulted in hyper-generation of ROS in zebrafish

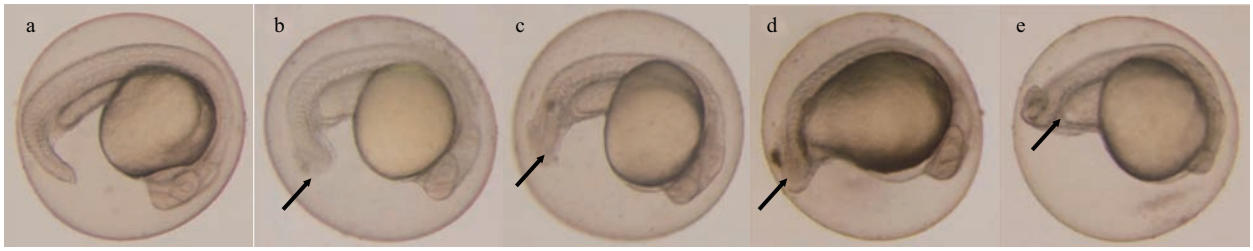

Fig. 7 Representative photographs of tail deformities induced by 0.30 μmol/L T-2 toxin alone and after co-exposure with 200 μmol/L GSH at 24 hpf. Tail abnormalities are indicated by arrows. (a) control; (b, c) slight tail deformities; (d, e) severe tail deformities.

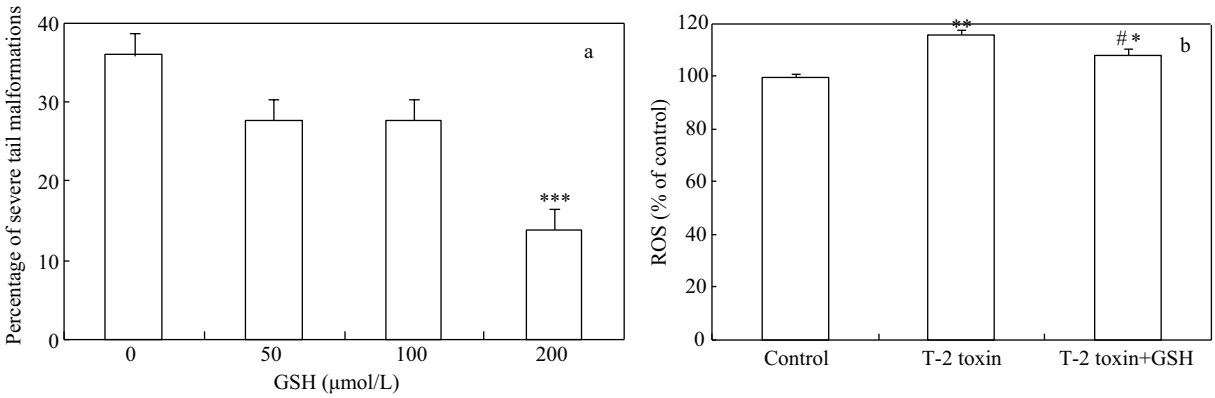

Fig. 8 (a) Percentage of zebrafish embryos exhibiting severe tail deformities in the presence of 0.30 μmol/L T-2 toxin and increasing concentrations of GSH co-exposure at 24 hpf. (b) Effect of 200 μmol/L GSH co-exposure with 0.30 μmol/L T-2 toxin on the level of ROS in the embryos at 24 hpf. Values that were significantly different from the control are indicated by asterisks, values in the group co-exposed with 200 μmol/L GSH significantly different from that exposed to 0.30 μmol/L T-2 toxin alone are indicated by pound signs (one-way ANOVA, followed by a *post hoc* test, LSD: $^{*,\#}p < 0.05$; $^{**}p < 0.01$, $^{***}p < 0.001$).

embryos and induced cell apoptosis in the tail at 24 hpf. In addition, by using an antioxidant GSH as scavenger, we further demonstrated the role of ROS in T-2 toxin-induced developmental toxicity (**Figs. 5–8**).

Malformation has been reported in zebrafish embryos exposed to environmental toxicants, such as BDE 47 (Lema et al., 2007), TCDD (Antkiewicz et al., 2005; Yamauchi et al., 2006) and copper (Johnson et al., 2007). Other results also suggest that malformation may be a general end-response of fish embryos exposed to toxicants (Zhang et al., 2008; Hu et al., 2009). To determine the sensitivity of zebrafish to T-2 toxin during gastrulation, somitogenesis, and organogenesis, embryos were observed for developmental toxic effects when exposed to a range of T-2 toxin concentrations between 0.05 and 0.80 μmol/L. Exposure to T-2 toxin also caused malformations. It should be noted that the malformations mainly occurred in the tail areas at 24 hpf in our study, which suggests that the developing tail may be an important potential target for T-2 toxin in zebrafish. Since bone malformations such as incomplete ossification, absence of bones, wavy bones and fused bones are one of the most frequently observed fetotoxicities of T-2 toxin (Hood et al., 1978), we investigated the mechanism of tail deformity in T-2 toxin-exposed zebrafish embryos. In addition, cell apoptosis is reported to be chemically induced, and induces teratogenic effects (Zakeri and Ahuja, 1997). Using AO staining in the embryos, we determined whether T-2 toxin induced apoptotic cell death at 24 hpf. AO staining showed that the major apoptosis occurred in the tail area. This effect was possibly due to the high percentage of apoptotic cells in the tail and may partly explain the observed tail deformities.

It has been reported that six teratogenic mechanisms are associated with chemical exposure: folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption and specific receptor- or enzyme-mediated teratogenesis (van Gelder et al., 2010). Environmental pollutants are well-known inducers of ROS, and ROS can further cause the depletion of antioxidant defenses and mediate other oxidation-reduction reactions through different metabolic pathways (Livingstone, 2001). Embryonic development may be especially sensitive to ROS and the resulting oxidative stress, as even a 15%–20% increase in ROS can tip progenitor cells into premature cell cycle arrest and differentiation (Shi and Zhou, 2010); and oxidative stress-induced apoptosis is thought to contribute to abnormal development during embryogenesis (Yamashita, 2003). Our results demonstrated that T-2 toxin induced ROS overproduction in zebrafish embryos at 24 hpf. However, when the increase of ROS

reached the highest level, it may result in necrosis in cells followed by a fall of ROS levels. This may explain why ROS concentration in the embryos treated with 0.20 and 0.30 μmol/L of T-2 toxin was lower than for treatment with 0.10 and 0.15 μmol/L (**Fig. 6**). Therefore, the developmental toxicity caused by T-2 toxin exposure could be explained, at least in part, by induction of ROS generation and subsequent cellular apoptosis in zebrafish embryos.

Oxidative stress may occur either due to the overproduction of ROS or to decrease of cellular antioxidant levels. Pronounced increases in thiobarbituric acid reactive substances (the product of lipid peroxidation) were reported in liver homogenate of T-2 toxin-treated rats and in differentiated murine embryonic stem cell exposed to T-2 toxin, due to oxidative damage (Rizzo et al., 1994; Fang et al., 2012). Exposure to T-2 toxin resulted in significant ROS generation, which may result in the depletion of antioxidant defenses. GSH was initially tested to determine the sub-lethal concentrations, and it was shown that GSH did not induce tail deformities on its own at any concentration tested. GSH was then tested at several sub-lethal concentrations in combination with 0.30 μmol/L T-2 toxin to determine its potential to modify T-2 toxin-induced tail deformities. Our results showed that exogenous GSH could mitigate T-2 toxin-induced developmental toxicity in zebrafish embryos, and the percent of the embryos exhibiting severe tail deformities was noticeably diminished by GSH co-exposure. GSH and T-2 co-exposure induced a significant decrease of ROS production in the embryos as compared to T-2 toxin exposure alone. Taken together, the results suggest that oxidative stress is partly involved in T-2 toxin-induced tail deformities in the zebrafish embryos.

In summary, the results of the present study demonstrate the occurrence of developmental toxicity in zebrafish embryos exposed to T-2 toxin, as indicated by increased malformation and reduced survival in the embryos and larvae. The mechanism of this developmental toxicity appears to be the generation of ROS and the consequent triggering of apoptosis. However, a future study of expressions of oxidative stress- and apoptosis-related genes would likely provide more information in terms of the mechanism of developmental toxicity in zebrafish embryos. Therefore, further studies on the relationship between certain gene functions and T-2 toxin-induced developmental toxicity are underway in our laboratory.

Acknowledgments

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