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Research Article

Oxidative stress response in tilapia (GIFT, Oreochromis *niloticus*) liver after subacute exposure to subacute 1,3-dibromo-5,5-dimethylhydantoin (DBDMH)

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Abstract

1,3-dibromo-5,5-dimethylhydantoin (DBDMH) is a broad-spectrum and efficient disinfectant used in aquaculture. The present study aimed to know if the response of antioxidative defense system was reversible under different concentrations of DBDMH exposure. The fish were exposed to subacute concentrations of 0.06, 0.3, and 1.5 mg/L for 16 days and then transferred to DBDMH-free water for 10 days. Results showed that SOD/CAT (except 0.06), GPx (only 1.5), GR (only 0.3), T-GSH (only 1.5), and content GSH/GSSG ratio (0.06, 1.5) significantly increased at 1 day after exposure. SOD, CAT, GPx, GR, and T-GSH were decreased in all the groups at 2 days. The recovery results showed that SOD (0.3), GST (all groups), T-GSH contents (1.5), and GSH/GSSG ratios (1.5) were significantly increased, and it suggested the damage produced by 0.06~1.5 mg/L DBDMH were irreversible. The current study suggests such antioxidant responses could be used as the ecological biomarkers for monitoring residual DBDMH in aquatic environments of tilapia ponds, which also provides an early warning of DBDMH for wild fish because of the persistent existence in the contaminated water.

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Introduction

Rearing water disinfection is one of the major public health issues in the 21st century and the function is to reduce the risk of epidemics from water microbial pathogens (Meinelt *et al.*, 2015). The disinfectants in rearing water became some of the most frequently occurring water pollutants due to their wide use (Srivastav *et al.*, 2020), which may have toxicological effects on aquatic systems (Patange *et al.*, 2018). These disinfectants have the potential to introduce reactive oxygen species in fish and other aquatic animals.

The liver is apt to xenobiotic-induced injury due to its central role in xenobiotic metabolism, its portal location within the circulation, and its anatomic and physiologic structure (Burkina *et al.*, 2021). Intoxication with disinfectants may potentially modify the biochemical composition of fish liver, leading to oxidative stress in organisms (Palermo *et al.*, 2015). Oxidative stress can not only occur as damage to biological systems but also impair antioxidant defense systems (Cattaneo *et al.*, 2012). An antioxidant defense pathway in animals comprises antioxidant factors such as reduced glutathione (GSH), oxidized glutathione (GSSG) oxidation scavenger, glutathione-S-transferase (GST) phaseII detoxification enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx). Fish liver could potentially be used as an indicator for monitoring residual toxic present in the aquatic environment (Stara *et al.*, 2013).

1,3-dibromo-5,5-dimethylhydantoin (DBDMH) is a broad-spectrum and efficient disinfectant with low residual. It is classified as an acute toxic disinfectant for algae, bacteria, fish, shrimp, and crab (Lai *et al.*, 2014). DBDMH, a widely used disinfectant for *Oreochromis niloticus* (genetically improved farmed tilapia, GIFT) in Guangdong and Guangxi provinces of China, has been exposed in several biological studies for being easily fed under laboratory conditions. The chronic toxic effects of DBDMH on aquatic organisms, especially on fish, were scarcely investigated. The hepatic antioxidative defense system has been demonstrated in a reversible pattern (Meng *et al.*, 2014), and some parameters return to the normal level during the recovery period. The purpose of this study was to investigate the subacute toxic effects of DBDMH disinfectant on GIFT tilapia liver and to know if the response of the antioxidative defense system was reversible.

Materials and methods

Fish and chemicals

GIFT tilapia (126.4529.31 g; 19.57±2.08 cm) were supplied by the fish farm of the Freshwater Fisheries Research Center, CAFS. The fish were initially acclimated in aquaria containing 2000-L dechlorinated tap water with a 12L/12D photoperiod for 4 weeks. The experimental fish were all fed four percent body mass daily, with commercial fish feed (NingboTech-Bank Co., Ltd., Yuyao, China). Throughout the exposure period, the water conditions followed “the water quality standard for fisheries”. Feeding was suspended for 24 h before commencing the toxicity tests. DBDMH was obtained from Wuxi Zhongyi Biotechnology Co., Ltd (Wuxi, China).

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DBDMH solution was prepared with ultrapure water. Kits for oxidative stress biomarker assays were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Experimental design and sampling

GIFT tilapia were randomly distributed into 500 L glass aquaria containing different concentrations of DBDMH (0, 0.06, 0.3, 1.5 mg/L), with 3 replicates per treatment. The range of exposure concentrations was based on the information from the previous study (96 h LC_{50}=3.8 mg/L) and the practical application concentration (0.3~0.4 mg/L). Thirty fish were introduced into each concentration in a semi-static system and half of the water was renewed daily. The experiment lasted for 26 days. After the 16 days exposure period, the left fish were transferred to DBDMH-free water for 10 days for carrying out for studying the recovery response.

All the protocols involving the use of animals were by approved guidelines of the Animal Care and Use Committee of the Nanjing Agricultural University (approval ID: 2011AA1004020012). The fish (n=9 per treatment, 3 tilapia per replicate) of the control and treatment groups were sampled at 1, 2, 4, 8, and 16 days after starting the experience and at 10 days after transferring to DBDMH-free water for the recovery test. To minimize the stress associated with handling, fish from each tank were sedated with an overdose of tricaine methanesulfonate (2%; MS-222; Suzhou Sciyoung BioMedicine Technology Co., Ltd., Suzhou, China) within 1 min after capture. The livers were quickly dissected from these fish and stored at −80°C until assays. A part of liver tissues (0.1 g) was homogenized (1:9, w/v) using plastic pestles (Beijing Haide Venture Biotechnology Co. Ltd., Beijing, China) with cold phosphate saline buffer (PBS, 0.02 M, pH 7.3) solution. The homogenates were centrifuged at 10,000×g for 10 min at 4°C, and the supernatants were collected for biochemical determination (Zheng *et al.*, 2016).

Biochemical analysis

Homogenate protein concentration and the detective antioxidant enzymes were determined using kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu; China) according to the manufacturer's instructions and followed by Zheng *et al.* (2016). The assay of SOD was based on a local method, while CAT activity was determined by measuring the rate of disappearance of H_2O_2 with slight modifications (using 1000 g for 10 min). GPx activity used GSH as a substrate to measure the conjugation of GSH and DTNB. GR activity monitored the consumption of NADPH during the regeneration of GSH from oxidized glutathione (GSSG) at 340 nm. GST activity was detected by evaluating the conjugation of GSH. T-GSH and GSSG contents were determined at 412 nm, using the calculation formulae GSH=T-GSH-2GSSG. Protein contents were determined by the Coomassie blue method.

Statistical analysis

Statistical analysis was undertaken using SPSS25.0 (SPSS Inc., Chicago, Illinois, USA). All values were expressed as mean±SD (standard deviation). Data were

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subjected to a one-way analysis of variance (ANOVA) followed by Duncan's test. Differences were measured against the control values and considered statistically different at $p<0.05$.

Results

SOD and CAT activity

Neither mortality nor visible disease signals were observed in the tilapia exposed to sublethal concentrations of DBDMH during the experimental study. As can be seen from Figure1a, SOD activities in 0.3 and 1.5 mg/L groups were significantly increased compared with those in the control group ($p<0.05$) after 1 day of DBDMH exposure, subsequently significantly decreased at 2 d, 8 d (only 0.06 mg/L) and 16 d ($p<0.05$). SOD activities in all the groups reached a minimum at 16 days and were 40.32, 67.78, and 71.15 U/mg protein, respectively. There was no significant difference in all the groups on day 4 ($p>0.05$). CAT activities in 0.3 and 1.5 mg/L groups increased significantly ($p<0.05$) compared with the control group after 1 day of DBDMH exposure (Fig.1b). From the 2nd day, the CAT activities in all groups had a reducing tendency. CAT activities in the 0.06 and 1.5 mg/L DBDMH groups showed a significant decrease at 4 d ($p<0.05$), while those in 1.5 mg/L DBDMH groups revealed a significant increase at 8 d ($p<0.05$). CAT activities in the 0.06 and 0.3 mg/L DBDMH groups showed a significant increase at 16 d, while those in 1.5 mg/L DBDMH groups revealed a significant decrease at 16 d ($p<0.05$).

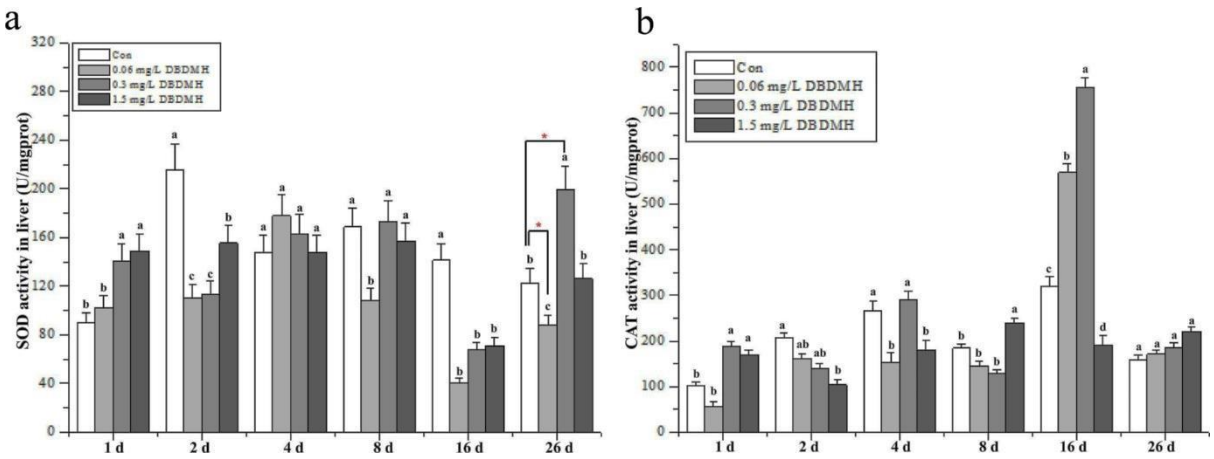

Figure 1: Liver SOD (a) and CAT (b) activities in GIFT tilapia, *Oreochromis niloticus* exposed to sublethal concentrations of DBDMH. Different lower-case letters indicate significant difference among concentrations at the same exposure period, with $p<0.05$ being considered significant.

GPx, GR, and GST activity

GPx activities in 1.5 mg/L DBDMH groups showed a significant increase at 1 d ($p<0.05$, Fig. 2a), while revealing a significant decrease in 0.06 and 0.3 mg/L DBDMH groups at 2 d ($p<0.05$). GPx activities in 0.06 and 1.5 mg/L DBDMH groups showed significant decreases at 16 d ($p<0.05$). There was no significant difference in all the groups on day 4 and day 8 ($p>0.05$).

GR activities were observed as a significant increase only in 0.3 mg/L DBDMH group as compared with the control group at 1 d

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(p<0.05, Fig. 2b), which significantly decreased in all groups at 2 d (p<0.05). GR activities revealed a significant increase in 1.5 mg/L DBDMH groups (4 d and 8 d), while showed a significant decrease in 0.3 mg/L DBDMH groups (4 d) and 1.5 mg/L DBDMH groups (16 d) respectively (p<0.05). GST activities demonstrated significant decreases both in 0.06 and 1.5 mg/L DBDMH groups at 1 d (p<0.05, Fig. 2c). It showed an increasing, decreasing trend ranging from 0.06 to 1.5 mg/L DBDMH groups at 2, 4, and 8 days, respectively (p<0.05). GST activities in 0.06 and 1.5 mg/L DBDMH groups revealed a significant decrease at 16 d (p<0.05).

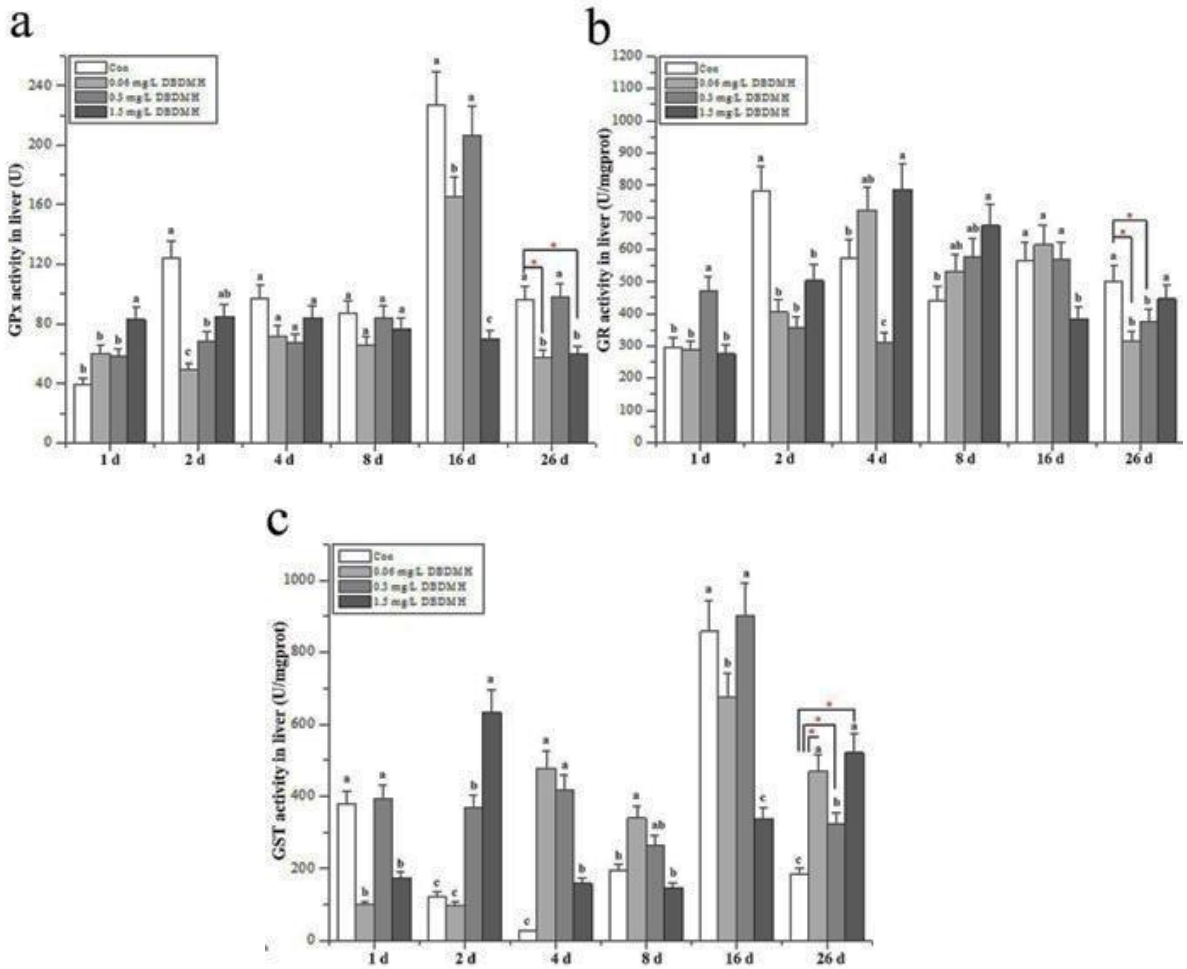

Figure 2: Liver GPx (a), GR (b), and GST (c) activities in GIFT tilapia, *Oreochromis niloticus* exposed to sublethal concentrations of DBDMH. Different lower-case letters indicate significant differences among concentrations at the same exposure period, with p＜0.05 being considered significant.

T-GSH content and GSH/GSSG ratio

T-GSH contents in 1.5 mg/L DBDMH group significantly increased at 1 d (p<0.05, Fig. 3a), which revealed a significant decrease at 2 d for all the groups (p<0.05). It showed a significant decrease in 1.5 mg/L DBDMH groups at 4 d and 16 d respectively, which also showed a

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significant decrease in both 0.06 and 0.3 mg/L DBDMH groups at 8 d (p<0.05). From Figure 3b, GSH/GSSG ratios showed a significant increase in both 0.06 and 1.5 mg/L DBDMH groups at 1 d and 2 d, while revealing a significant decrease in all the groups at 8 d (p<0.05). It demonstrated a significant decrease in 0.3 and 1.5 mg/L DBDMH groups at 16 d (p<0.05). There was no significant difference in all the groups on day 4 (p>0.05).

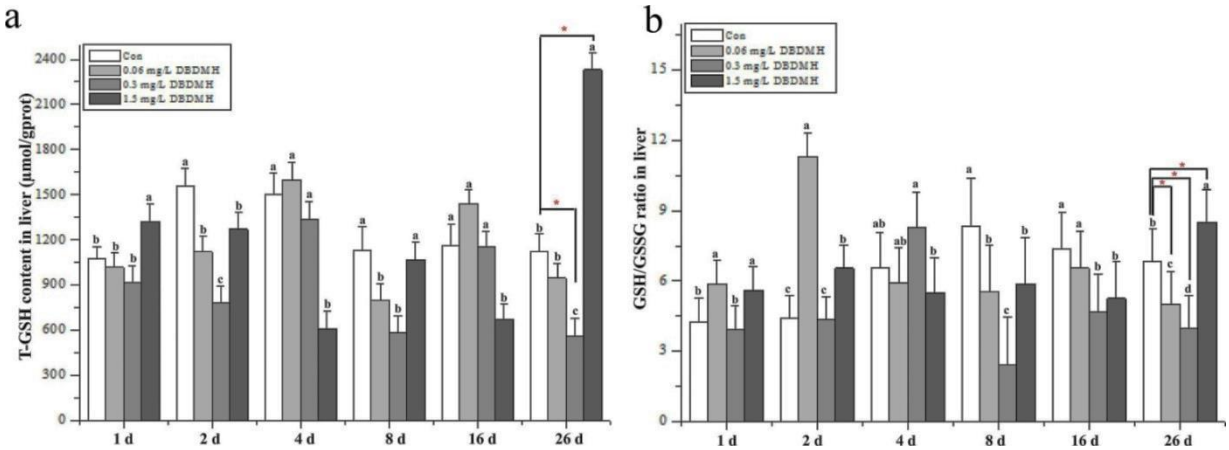

Figure 3: Liver T-GSH contents (a) and GSH/GSSG ratios (b) in GIFT tilapia, *Oreochromis niloticus* exposed to sublethal concentrations of DBDMH. Different lower-case letters indicate significant differences among concentrations at the same exposure period, with P＜0.05 being considered significant.

Parameters in the recovery test

When fish were transferred into DBDMH-free water, SOD (0.3 mg/L), GST (all groups), T-GSH contents (1.5 mg/L), and GSH/GSSG ratios (1.5 mg/L) were significantly increased in comparison to control (p<0.05). GPx (0.06 and 1.5 mg/L) were significantly decreased (p<0.05). Nevertheless, compared with the control, no significant differences in CAT activities in 0.06, 0.3, and 1.5 mg/L groups were observed (p>0.05).

Discussion

SOD, CAT, and GPx are the major enzymes in eliminating reactive oxygen species (ROS) in the liver tissues, and their induction provides a first line of defense against ROS (Meng *et al.*, 2014). After 1 day of exposure, DBDMH-induced SOD (except 0.06), CAT (except 0.06), and GPx (only 1.5) activities in the liver of GIFT tilapia in almost all the groups, however, after the second day SOD, CAT, and GPx activities were restrained until 16 days exposure. Stara *et al.* (2012) found that SOD, CAT, and GPx activities in common carp liver increased between day 14 and day 28 after exposure, but decreased after 60 days of exposure when fish has been exposed to simazine at 2 and 4 mg/L. The increment in SOD activity may be due to the increased production of ROS, and the reduction in SOD activity may result in amounts of hydrogen peroxide (Puerto *et al.*, 2010). CAT and GPx activities in organ tissues can be a response to H_2O_2 produced by SOD, since CAT and GPx are responsible for the detoxification of H_2O_2 to water (Xu *et al.*, 2013). It can be seen that

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the inducement peak appeared in the medium concentration groups on the first day of the present study, which hinted that the adaptive response occurred when tilapia were exposed to DBDMH at 1 d. CAT activity decreased until 16 days of exposure, and it showed a dose-effect relationship at day 2/16 in the current study. It is clear that the increment of CAT activity proved to protect the cells from oxidative damage (Jayaseelan *et al.*, 2014). Another report found CAT activity has been affected by dose and exposure time (Hou *et al.* 2014). The possible reason for the deduced CAT activity is that CAT depletion occurred (revealed as inhibition), and the liver cells of fish may be damaged by the high concentration of DBDMH. While immense increase (Morachis-Valdez *et al.*, 2015) and decrease (Saïdi *et al.*, 2015) in GPx activity has been observed in some studies. The results indicate disruption of the normal oxidation process, suggesting a failure of antioxidant defense systems represented by SOD, CAT, and GPx (Morachis-Valdez *et al.*, 2015).

When fish are exposed to pollutants, they can purge the foreign contaminants by conjugation with GSH directly or by means of GSH-related antioxidant system (GPx, GR, GST, GSH, and GSSG comprise the GSH-related antioxidant system), which leads to a depletion of GSH levels (Meng *et al.*, 2014). The change in GSH-related antioxidant enzymes is considered a potential indicator of environmental stress. The current study demonstrated that long-term exposure to DBDMH in GIFT tilapia induced an immense decrease in T-GSH contents and GSH/GSSG ratios, suggesting the induction of ROS production. In addition, GPx inductions caused high production of GSSG in tilapia which also supported the present results (Atli and Canli, 2010). Eroglu *et al.* (2015) explained a continuous decrease in GSH/GSSG ratio due to the destroyed adaptive mechanism of GSH related to the huge amount of GSH consumption in the liver of freshwater fish GIFT at day 7, it was similar to our present GSH/GSSG ratio results from day 8. With the extension of DBDMH exposure time, GSH consumption causes oxidative stress. Based on the results, the increase in GR activity could be interpreted as an adaptive response to oxidative stress. Just as Gyimah *et al.* (2020) found elevated GR activity in the liver exposed to triclosan in zebrafish at 30 days. GST plays an important role in homeostasis as well as in the detoxification and clearance of many xenobiotic compounds, thereby protecting tissues from more serious oxidative damage (Rehman *et al.*, 2020). The results of the current study showed that GST activity decreased on the first day and increased from day 2. Severe oxidative stress may suppress GST activity due to the exhaustion of GSH and the disruption of its synthesis. GST activity can be induced due to an adaptive mechanism to counteract marginal oxidative stress. We found a similar relationship between GST and GSH in the present study, which confirmed the catalytic action of GST in the conjugation of DBDMH with GSH.

The recovery test is mainly used to study whether the target factors in organisms return to normality after eliminating pollutant factors or not. When the tilapia exposed to 0.06, 0.3, and 1.5 mg/L DBDMH were transferred to DBDMH-free water for 10 days, most antioxidant

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activities and contents in the fish liver could not return to the control values, except CAT activity values. Danion *et al.* (2014) observed a similar result, after 15 days in clean water, SOD activity was restored, but GSH content and GPx activity were still disturbed in rainbow trout (*Oncorhynchus mykiss*) exposed to pendimethalin. Meng *et al.* (2014) found when GIFT was transferred to methomyl-free water for 18 days, GPx, GR, and GST activities as well as GSH, and GSSG contents in the fish liver could not return to the control values. Our study results indicated that 0.06~1.5mg/L DBDMH could cause irreversible damage to GIFT tilapia.

Conclusions

Slight oxidative stress occurred in tilapia under 0.06~1.5mg/L DBDMH exposure due to compensatory response. The present results showed there was a very rapid response in the activity and the content of antioxidants after the fish contract with DBDMH. The activities of SOD, CAT, GPx, GST, GR, GSH/GSSG ratios and the contents of T-GSH in the liver of GIFT tilapia exposed to 0.06, 0.3, and 1.5 mg/L were significantly affected during the 16-day exposure period. These changes indicated the antioxidant defense system of GIFT tilapia could cause significant oxidative damage if exposed to the concentrations of DBDMH in 0.06 mg/L and more. The results of the recovery data showed that the toxicity produced by DBDMH concentrations between 0.06 and 1.5 mg/L was irreversible within 10 days after stimulus withdrawal. This study reveals a relationship between biochemical changes in the liver and DBDMH duration exposure and concentration. It also serves as a warning of potentially negative impacts of DBDMH for wild fish, especially in case of persistent contact with contaminated water.

Acknowledgments

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Conflicts of interest

The authors declare that they have no conflict of interest.

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