



Sublethal concentrations of triclosan elicited oxidative stress, DNA damage, and histological alterations in the liver and brain of adult zebrafish

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Abstract

Triclosan (TCS), an antimicrobial agent, has been a pollutant of increasing concern owing to its potential health risk on humans and aquatic animals. The present study seeks to test the hypothesis that TCS could alter the oxidative stress-related parameters in the brain and liver, as well as eliciting DNA damage in hepatocytes of adult zebrafish. On the basis of the 96 h LC₅₀ (398.9 µg/L), adult zebrafish were separately exposed to 50, 100, and 150 µg/L TCS for 30 days. The brain and liver tissues from adult zebrafish were excised and assayed for a suite of antioxidant parameters and oxidative stress biomarkers including DNA damage in the liver. The induced effect by TCS on the activity of acetylcholinesterase (AChE) was also analyzed in the brain. Results showed a significant decrease in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the brain and liver of adult zebrafish. Also, the contents of the glutathione system (GSH and GSSH), as well as the activity of the glutathione reductase (GR), assayed in the liver, were reduced while the contents of malondialdehyde (MDA) were elevated in the liver. A comet assay revealed dose-dependent DNA damage in zebrafish hepatocytes. The 8-hydroxy-2'-deoxyguanosine (8-OHdG), MDA, and carbonyl protein contents in brain tissues significantly increased. Moreover, the AChE in the zebrafish brain was induced. Apparently, no obvious histological changes in brain tissues of zebrafish were observed compared with those of the control whereas atrophy and necrosis of hepatocytes and increased hepatic plate gap were observed in zebrafish hepatocytes after TCS exposure. The obtained results highlight that sublethal concentrations of TCS may be deleterious to the liver and brain of adult zebrafish upon subchronic exposure.

Keywords Oxidative stress · DNA damage · Liver cells · Triclosan · Zebrafish

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Introduction

Pharmaceuticals and personal care products (PPCPs) have been prolific environmental sources of emerging contaminants of great concern (Boxall et al. 2012). Triclosan (TCS), an antimicrobial agent in several products ranging from personal care products (PCPs) such as laundry detergents, soaps, toothpaste to cosmetics, and textiles (Dann and Hontela 2011; Olaniyan et al. 2016), has been a pollutant of increasing concern owing to its potential health risks on humans and wild animals (Yueh and Tukey 2016). Although TCS was banned by the US Food and Drug Administration from soap products in 2016 (Food and Drug Administration 2016) and hospital products by the end of 2018 (Food and Drug Administration 2017), it is permissible to have TCS in toothpaste, cosmetics, clothes or toys, and so on (Bever et al. 2018). The European

Union also limited its use in human hygiene biocidal products (EU 2016). Due to its widespread use, TCS is ubiquitous in various environmental matrices with relatively significant concentrations detected in the aquatic environments (Bedoux et al. 2012; Montaseri and Forbes 2016).

Since TCS is most commonly found in aquatic environment, aquatic organisms are at particular risk. The chronic and acute toxicity of TCS has been demonstrated in aquatic animals. For example, previous studies have reported the endocrine-disrupting potencies of TCS in vertebrates, including fish, and other aquatic species at $\mu\text{g/L}$ level (Dann and Hontela 2011; Wang and Tian 2015). Also, TCS can increase mortality, delay hatching, and heart edema; impair lipid metabolism; and induce hepatotoxicity in Japanese medaka (*Oryzias latipes*) (Ishibashi et al. 2004) and zebrafish (*Danio rerio*) (Haggard et al. 2016; Ho et al. 2016) at relatively high concentrations. However, at ng/L range, TCS also can disrupt development (Fort et al. 2011) and metabolism (Regnault et al. 2018) in *Xenopus tropicalis* for long-term exposure. In addition, an induced latent effect on mortality rate in adult fathead minnows was observed during sub-chronic exposure to 50 and 100 $\mu\text{g/L}$ TCS (Salierno et al. 2016). Therefore, it is promising to explore chemicals toxicity to aquatic animals upon low concentrations at long-term exposure conditions.

The coining of the term “oxidative stress” is when the dynamic equilibrium between the production and the elimination of reactive oxygen species (ROS) under the normal physiological condition is disrupted (Liu et al. 2014). These “double-edged sword” molecules, ROS, if not properly scavenged could be disturbing to cellular redox balance leading to oxidative damage to tissues including lipids and membranes, protein and nucleic acid (Wang et al. 2013). Coping with the probable damaging effect of ROS, the antioxidant defense system, both enzymatic and non-enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), reduced glutathione (GSH), oxidized glutathione (GSSG), and glutathione S-transferase (GST), offers a versatile mechanism to control intracellular ROS in maintaining redox homeostasis hence protecting the organism from oxidative damage (Ni et al. 2019). Therefore, evaluating the level of antioxidants can help environmental scientists to monitor the level of oxidative stress induced in the organisms exposed to certain chemicals.

Oxidative stress also may result in DNA damage in cells (Gonzalez-Hunt et al. 2018). By determining DNA damage at a cellular level, it can be helpful to easily evaluate DNA damage mechanisms induced by environmental pollutants and be further used for biomonitoring and genotoxicity testing. The hepatic and brain in fish are sensitive organs to environmental toxicants and their functions are associated with several physiological mechanisms including the redox homeostasis. Attenuations in antioxidant levels or oxidative damage biomarkers in these bodily function-dependent organs are

indicators of xenobiotic stressors/exposure. A few studies have reported the induced oxidative and genetic damage in fish exposure to TCS (Falisse et al. 2017; Ku et al. 2014; Oliveira et al. 2009; Parenti et al. 2019). However, the oxidative responses in target organs of adult fish exposure to TCS still remain vague and warrant scientific investigations.

In the present study, zebrafish was used as an animal model to investigate the oxidative stress responses of TCS in the liver and brain. Adding to the assessment of the induced effect of TCS on acetylcholinesterase (AChE) activity in the brain, the DNA damage to the zebrafish hepatocyte was examined using the comet assay. Hepatic and brain histological alterations were observed in zebrafish after subchronic exposure to low concentrations of TCS. The findings of the study will significantly illuminate the underlying mechanism of aquatic toxicology elicited by TCS in zebrafish.

Materials and methods

Chemicals and reagents

TCS, with purity > 97%, was purchased from Sigma-Aldrich (St. Louis, MO). TCS was dissolved in dimethyl sulfoxide (DMSO), which was used as the vehicle control in each assay at a final concentration of 0.005% v/v, to prepare a stock solution of 2 g/L and kept at 4 °C until needed for the experiment. All other chemicals and reagents were of analytical grade.

Zebrafish maintenance and experimental design

All experiments were performed on wild-type zebrafish (AB strain) obtained from the National Zebrafish Resources of China. The broodstock wild-type zebrafish were acclimated in inert glass tanks containing aerated dechlorinated tap water and maintained (in 26 ± 0.5 °C temp; 7.2–7.5 pH; 118–120 mg/L hardness; > 6.0 mg/L dissolved oxygen; 14 h light:10 h dark photocycle) for five generations. The cultured zebrafish were fed twice daily with freshly incubated brine shrimp nauplii (*Artemia salina*). To ensure the quality of the culture medium, the whole culture medium was renewed every day.

Five-month-old male zebrafish (0.27 ± 0.02 g weight and 3.0 ± 0.03 cm length) were utilized for all experiments. The zebrafish were randomly distributed into a 10-L glass tank containing different concentrations of TCS (0, 50, 100, and 150 $\mu\text{g/L}$), and the range of exposure concentrations was based on the information from the 96 h LC_{50} for male zebrafish in our preliminary test (Table S1, Supporting Information), and previously used for zebrafish (Oliveira et al. 2009), as well as the environmental freshwater levels of TCS were also considered (Bedoux et al. 2012). Fifteen

fish were exposed to each concentration in a semi-static system and the water was renewed every day. The solvent control and blank control groups were also designed. The solvent control and exposure groups received 0.01% DMSO (v/v), while the blank control received the dechlorinated tap water. Triplicates were performed for each treatment. Animals were challenged for 30 days and then sampled. Food was withheld 24 h prior to sampling.

Sampling and samples analysis

After 30-day exposure, all fish were euthanized in ice-water. Liver or brain tissues were dissected to remove the brain and liver tissues. Four fish of every replicate were rinsed with cold phosphate-buffered saline (PBS, pH 7.4), snap-frozen in liquid nitrogen, pooled as one sample ($n = 3$ pools per each treatment), and stored at $-80\text{ }^{\circ}\text{C}$ until needed for further biochemical analysis. Brain and liver tissues, except those for GSH and GSSH analysis, were thawed on ice and homogenized in 600 μL Tris-HCl buffer (100 mM, pH 7.4) phosphate buffer (50 mM, pH 7.4) using a sterile glass homogenizer. The supernatants, obtained from the homogenates centrifuged at 10,000g for 15 min at $4\text{ }^{\circ}\text{C}$, were collected for subsequent biochemical parameter analysis. Two individual fish per replicate from each treatment were immediately processed for the comet assay; three fish per replicate from each treatment were fixed in 4% paraformaldehyde for histological measurements.

Biochemical analysis

Supernatants were aliquoted and diluted separately for and assayed for the activities of enzymatic antioxidants (SOD, CAT, GPx, and GST), as well as the protein concentrations of tissue samples following standard protocols. Assaying kits were purchased from Nanjing Jiancheng Bioengineering Institute, China. Protein concentrations were measured following Bradford's method (1976) by using the bovine serum album standard. To assess the antioxidant response to TCS, activities of SOD and CAT were assayed in both brain and liver tissues according to the methods by Sinha (1972) and Marklund and Marklund (1974) respectively. Also, a modified method based on 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) as proposed by Hafeman et al. (1974) was used to measure the activity of GPx in tissue samples. The activity of GST was estimated for only liver samples using 1-Chloro-2,4-dinitrobenzene according to Habig et al. (1974). The cyclic reaction of GSH with DTNB was used to estimate the levels of GSH and GSSH (Baker et al. 1990; Tietze 1969) while the activity of glutathione reductase (GR) was assayed by monitoring the decrease in NADPH levels (Cribb et al. 1989) for only liver tissues.

Indices of oxidative damage, measurement of biomarkers, were conducted following the protocols of commercially

available kits (Nanjing Jiancheng Bioengineering Institute, China). For lipid peroxidation (LPO), malondialdehyde (MDA) concentrations were measured in both tissues (liver and brain) by the thiobarbituric method reported by Ohkawa et al. (1979) using commercial kits. The 8-hydroxy-2'-deoxyguanosine (8-OHdG), measured for only brain tissues, was done by biotinylated antibody-sandwich ELISA following the manufacturer's instruction as a biomarker for oxidative DNA damage in the brain. AChE activity in brain tissue was measured following the modified spectrophotometric protocol described by Ellman et al. (1961). Protein levels were estimated by Bradford's method (1976) using bovine serum albumin as a standard. Triplicate analysis was applied to each sample.

Single-cell gel electrophoresis assay (comet assay)

The cell suspension of the zebrafish liver was prepared according to the protocol in our laboratory. Briefly, after 30 days of exposure, livers from each fish were put into centrifuge tubes, rinsed with iced phosphate-buffered saline (PBS, pH 7.4) three times, and then digested with trypsin. After that, the cells were collected by centrifugation at 150g for 10 min at $4\text{ }^{\circ}\text{C}$ and re-suspended with 1 mL of PBS, and filtered through a 200-mesh sieve filter. Again, the cells were centrifuged at 150g for 10 min at $4\text{ }^{\circ}\text{C}$. At last, the primary liver cells were obtained and adjusted the density of cells with 200 mL of PBS for the comet assay. The comet assay was performed following the protocol according to Singh et al. (1988), with slight modifications. Briefly, hepatocytes were embedded in an agarose sandwich on frosted microscope slides, immersed in freshly prepared iced lysing solution (2.5 M NaCl, 100 mM EDTA-2Na, 10 mM Tris, 10% DMSO, 1% Triton X-100 and 1% Na sarcosinate; pH 10.0), and stored at $4\text{ }^{\circ}\text{C}$ in the dark for 4 h to remove cellular proteins. Further, the slides were placed on a horizontal gel electrophoresis unit filled with denaturation electrophoresis buffer (300 mM NaOH, 1 mM EDTA) and equilibrated for 20 min and followed by electrophoresis in the same buffer for 30 min at 0.75 V/cm and 200 mA. Then the slides were neutralized with 0.4 M Tris (pH 7.5) and stained with ethidium bromide (20 $\mu\text{g}/\text{mL}$) for microscopic examination. A total of 120 randomly selected cells, comprising of 40 cells from each of the three replicate slides, were analyzed per sample using the Cometscore (version 1.5). The DNA damage was quantified as % DNA in the tail, tail length, tail moment, and Olive tail moment.

Histology analysis

The excised tissues (liver and brain) fixed in 4% paraformaldehyde for 24 h were subsequently dehydrated in gradient ethanol, cleared in xylene, and then embedded in paraffin. A section of 4–5 μm on a rotary microtome (Paraffin machine, Leica RM, Germany), and mounted on glass microscope

slides. Sections of liver and brain tissues were stained with Delafied's hematoxylin and alcohol eosin and then air-dried. Histological images were captured using an optical microscope (Olympus, Japan).

Statistical analysis

Data were expressed as the mean \pm standard error of the mean (S.E.M.). Normality and homogeneity of variance of data, as well as assumptions of one-way analysis of variance (ANOVA), were checked using the Kolmogorov-Smirnov one-sample test and Levene's test respectively in SPSS 18. Statistical differences between groups were tested by ANOVA followed by Duncan's test. A significant cut-off was set at $p < 0.05$ or $p < 0.01$.

Results and discussion

Toxic effects of TCS on adult zebrafish

The 96 h LC₅₀ of 398.9 $\mu\text{g/L}$ for adult male zebrafish was calculated with a 95% confidence interval range of 389.2–415.3 $\mu\text{g/L}$. Previous studies have reported a 96 h LC₅₀ of 390 $\mu\text{g/L}$ and 399 $\mu\text{g/L}$ of TCS exposure to fish species, other than zebrafish, *Laber rohita* (Hemalatha et al. 2019) and Japanese medaka (Ishibashi et al. 2004), respectively. Also, Oliveira et al. (2009) reported a 96 h LC₅₀ of TCS exposure to adult zebrafish as 340 $\mu\text{g/L}$, which is slightly lower than our findings.

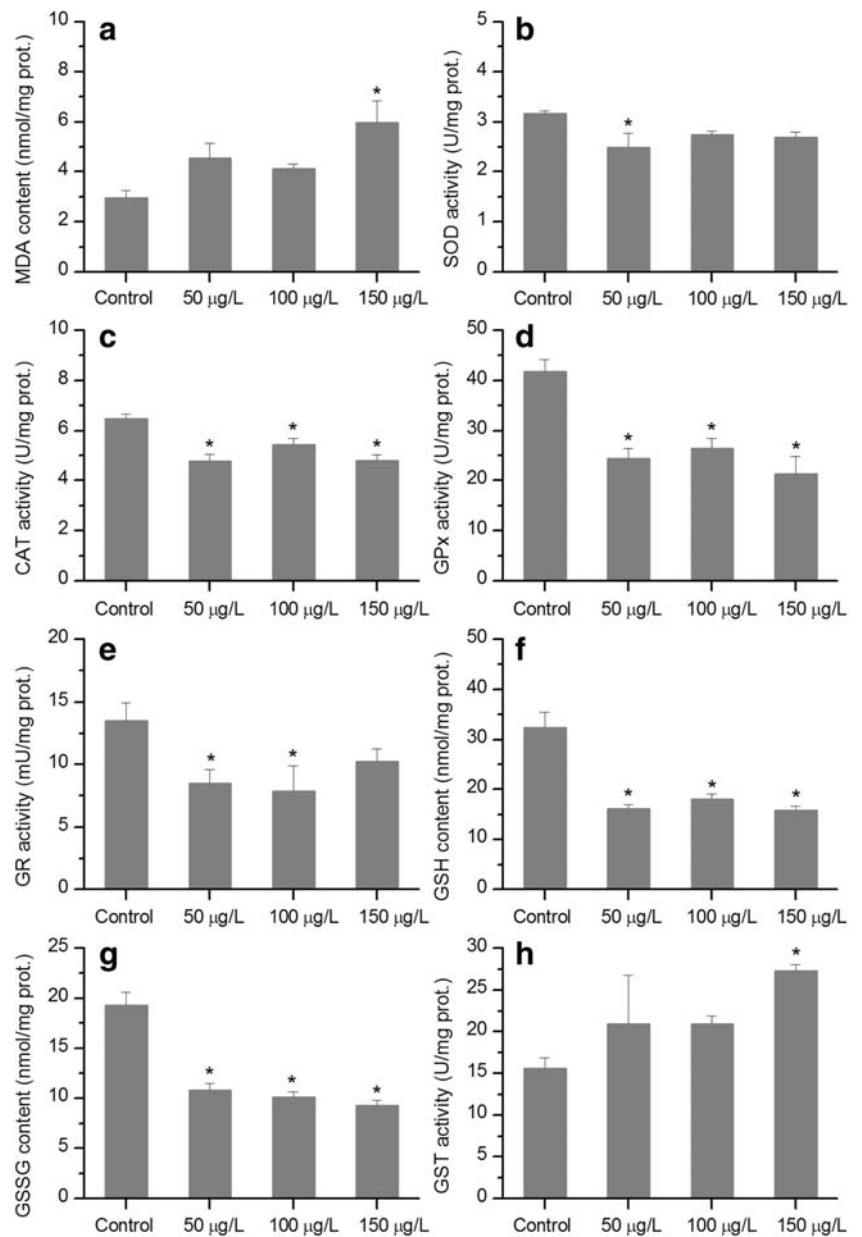
Changes of biochemical parameters in the liver

The activities of SOD, CAT, and GPx enzymes in the liver of adult zebrafish exposed to varying concentrations of TCS are presented in Fig. 1. Excessive production of ROS is a plausible cause of enzyme inactivation when the antioxidant defense does not thwart these ROS. The removal of ROS is initiated by the conversion of oxygen-free radicals to hydrogen peroxide molecules by SOD which is in turn degraded by CAT and GPx into H₂O (Ni et al. 2019). Hence, a decrease in the activities of these significant first-line defenses could result in the build-up of H₂O₂ as well as its degradable products (Wu et al. 2011). The here-obtained results showed inhibition in SOD activities in all treatment groups compared with the control. However, the induced inhibition in SOD compared with that in the control was significant at the lowest concentration of 50 $\mu\text{g/L}$ ($p < 0.05$). The relatively reduced levels of SOD activity in liver tissue among the treatment groups could in part be due to ROS generated by triclosan exposure. The CAT and GPx are crucial in metabolizing/converting H₂O₂ to innocuous water and oxygen molecules which are vital in maintaining intracellular redox balance (Ni et al. 2019). The trend in

the activities of CAT and GPx was similar to that of SOD activity in the liver. A significant reduction in CAT activities was observed between treatment and control groups ($p < 0.05$). Differences between treatment groups regarding the inhibition of CAT activities in the liver were significant at $p < 0.05$ with the effect of inhibition being much pronounced at the lowest concentration (50 $\mu\text{g/L}$). The observed reduction in CAT activity is an indicative measure that the generated H₂O₂ might not be rapidly degraded by CAT reflecting an imbalance in cellular redox. Companied with the trend of inhibition in the CAT activity, GPx activity was also decreased (Fig. 1) in the liver. Inadequate modulation of antioxidant enzymes activities could result in a higher level of ROS hence decreasing the competency of the antioxidant system. This hypothesis is enlightened by the theory that “the decrease of SOD has been oxidatively modified by excessive ROS, leading to the loss of enzyme activity and function” (Butterfield et al. 1998). The findings of our study albeit preliminary have revealed that subchronic exposure to low concentrations of TCS could cause a dysfunctioning of the vital first-line antioxidant defense system in the liver of adult zebrafish.

The non-enzymatic antioxidant defense system (the glutathione system) GSH and GSSG, as well as the GSH-related enzymes (GR and GST), are considered as the second-line system of defense against oxidative damage (Wu et al. 2011). The rejuvenation of GSH as a defense system for oxidative stress depends greatly on the activity of GR. During the metabolic action of GSH, it gets converted into an oxidized form (GSSG); hence, the recycling of GSH from GSSG by GR is important in maintaining cellular antioxidant defense system (Elia et al. 2003). Moreover, it is worth noting that the detoxification of ROS and H₂O₂ via the oxidation of GSH to GSSG is mediated by GPx hence the inhibition in GPx activity could affect the contents of glutathione and its conjugate. In our study, GSH levels in the liver of adult zebrafish treated with TCS were significantly reduced relative to the control ($p < 0.05$) as presented in Fig. 1. Similarly, GSSH levels in TCS exposed groups were significantly lower than the control ($p < 0.05$). However, the decrease in GSSG levels was not significant in treatment groups, except for the 100 $\mu\text{g/L}$ TCS exposure group. The lowered GSH/GSSG contents indicate lowered glutathione contents following exposure to TCS. The activities of GR in treatment groups were found to be lower than the control. However, the lowered GR activities were not significant between groups ($p < 0.05$) except, for 50 $\mu\text{g/L}$ and 100 $\mu\text{g/L}$ groups compared to the control group. A lowered GPx and GR activities observed in the present study could account for the lowered contents of GSH/GSSG resulting from the fact that the GSH could not be regenerated to restore

Fig. 1 Contents of MDA (A); activities of SOD (B), CAT (C), GPx (D), GR (E), contents of GSH (F) and GSSG (G); activities of GST (H) in the liver of adult zebrafish exposed to 50, 100, and 150 µg/L TCS, compared to control (mean ± standard error). * represents a significant difference between treatment and control group at $p < 0.05$



its normal level in the liver upon exposure to TCS concentrations. These findings revealed the failure of the antioxidant responses in the liver of adult zebrafish after subchronic exposure to TCS. Contrary to the activities of GPx and GR, elevated GST activity in the liver of treatment groups compared with that in the control group was observed in the study. The GST is a biotransformation enzyme that is crucial during the conjugation of glutathione with several pollutants (Ni et al. 2019). During phase II detoxification, GST helps to protect cells from oxidative damage via the catalysis of the tripeptide GSH with electrophilic substrates (Regoli and Principato 1995). Differences in the increased activities of GST between groups were not significant ($p < 0.05$) except, for

that between 150 µg/L group and the control at $p < 0.05$. The findings of the study are consistent with the increased GST activity observed in zebrafish larvae exposed after TCS (250–350 µg/L) (Oliveira et al. 2009).

The removal of excess ROS by antioxidant and detoxification systems might alleviate LPO and subsequently result in tissue damage (Ni et al. 2019). Malondialdehyde (MDA) has been widely used as a biomarker to evaluate the extent of LPO and indicator of oxidative damage/stress (Chen et al. 2017). The MDA contents in the liver of adult zebrafish in treatment groups increased significantly compared with those in the control ($p < 0.05$). However, the differences within treatment groups were not significant except, for that between 150 µg/L TCS exposure group and the control. The observed increased

levels of MDA in the liver could be attributed to the failure in the liver's antioxidant system to sufficiently eliminate ROS induced by TCS upon subchronic exposure. The here-obtained results indicated that the liver is prone to oxidative damage resulting from an untimely scavenged ROS after exposure to TCS.

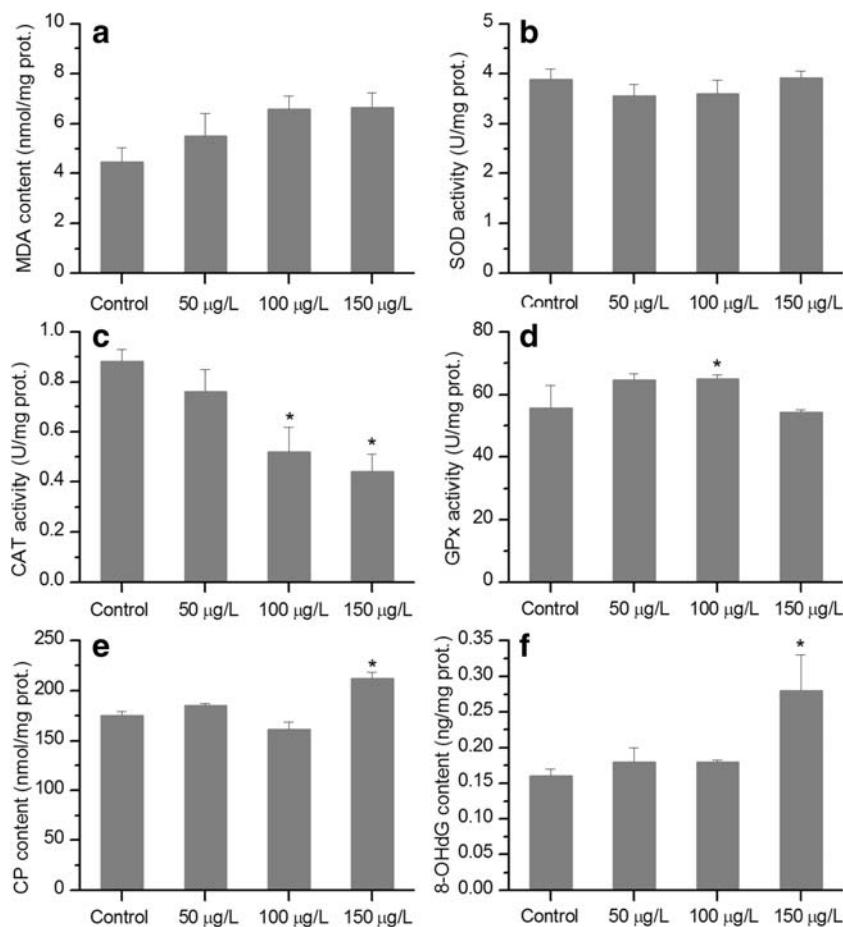
Changes of biochemical parameters in the brain

The enzymatic activities of SOD, CAT, and GPx measured in the brain of adult zebrafish upon subchronic treatment with TCS are presented in Fig. 2. The SOD activity slightly decreased for (50 µg/L and 100 µg/L) treatments compared to the control groups, while a slight increase in SOD activity was observed in the brain treated by 150 µg/L TCS. However, variations in SOD activity between treatment and control groups were not statistically significant ($p < 0.05$). The slight loss of enzyme activity of SOD may be due to oxidation at the active-site of SOD caused by excess ROS generated by TCS. Similar to SOD activity in the brain, the activity of CAT decreased among the treatment groups relative to the control. The lower CAT activity was statistically significant ($p < 0.05$) for that which existed between the control and higher treatments (100 µg/L and 150 µg/L) of TCS. The

inhibition in SOD and CAT activities indicated that TCS could alter the antioxidant system of the brain of zebrafish. However, the mean values of SOD activity were relatively higher in the brain than in the liver for all treatments of TCS, also the mean values of CAT activity were appreciably lower in the brain than that of the liver for all treatments. Variations in numerical values of antioxidant enzymatic activities might be due to the different physiological functions of the organs. Contrary to the activities of SOD and CAT, GPx in the brain of zebrafish treated by 50 µg/L and 100 µg/L TCS increased in activities, while a decrease in GPx activity in 150 µg/L treatment was observed compared to the control group. However, variations in GPx activities were not statistically significant ($p < 0.05$). The observed increase in GPx activities indicated an augment in the conversion of hydrogen peroxide to water hence the possibility of maintaining intracellular balance in the brain.

Generally, ROS has the tendency to damage, either directly or indirectly, all biological molecules (Fedorova et al. 2014). To explore the oxidative damage induced by TCS in the brain of adult zebrafish, protein carbonylation, lipid peroxidation, and the 8-OHdG contents were measured as oxidative responses to evaluate the extent of damage of biomolecules (proteins, lipids, and DNA respectively) in the brain. Protein

Fig. 2 Contents of MDA (A), activities of SOD (B), CAT (C) and GPx (D), contents of CP (E) and 8-OHdG (F) in the brain of adult zebrafish exposed to 50, 100, and 150 µg/L TCS, compared to control (mean ± standard error). * represents a significant difference between treatment and control group at $p < 0.05$



carbonylation/protein carbonyl (an irreversible post-translational modification) has been widely used as a marker for protein oxidation induced by ROS (Weimann et al. 2012) and have been linked to several human disorders, such as Alzheimer's disease, rheumatoid arthritis, diabetes, and lung disease (Fedorova et al. 2014). The present study revealed that exposure to 50 and 150 $\mu\text{g/L}$ TCS increased levels of carbonyl protein in the zebrafish brain compare with those of the control, while a slight decrease was observed in the 100 $\mu\text{g/L}$ exposure group. Differences between the levels of carbonyl protein in the brain were statistically significant at $p < 0.05$. Elevated carbonyl protein level has been postulated by Levine and Stadtman (2001) to be dependent on age supporting the ROS philosophy of aging on the cellular or organism level. The results of our study could, therefore, indicate that TCS could cause protein oxidation in the brain of zebrafish. Interestingly, the antioxidant enzymes (CAT and SOD) in the brain showed an opposite trend, signifying an excess ROS induced by TCS with the probability of resulting in oxidative damage. The contents of MDA in the exposure groups were relatively higher than the control. However, the differences between groups were not statistically significant ($p < 0.05$). The increased levels of MDA in the brain indicated an insufficient removal of ROS induced by the antioxidant system. The results indicated that TCS enhanced lipid peroxides in the brain of zebrafish and could subsequently result in oxidative damage. The observed levels of MDA in all treatments were relatively higher in the brain than those observed in the liver for which the variations are primarily attributed to the different physiological functions of the two organs. For DNA damage induced by TCS in the zebrafish's brain, the contents of 8-OHdG were assayed and presented in Fig. 2. The results of the study presented a statistically significant ($p < 0.05$) concentration-dependent increased levels of 8-OHdG in the brain of zebrafish treated with TCS compared with the control. The induced genotoxicity, measured as 8-OHdG, by excess ROS resulting from exposure to environmental stressors has been postulated and hypothesized. In concordance with this conception, our study observed suppression of CAT and SOD activities, as well as an elevation in 8-OHdG levels in zebrafish's brain upon subchronic exposure to low concentrations of TCS. The relatively high levels of the DNA damage biomarker, 8-OHdG, observed in the study could be accounted for by the enhanced ROS resulting in reduced antioxidant capacity in the brain upon exposure to TCS.

Following the evidence on the plausible effects of TCS in the antioxidant competence and the probable oxidative damage therein the zebrafish's brain, alterations in the activity of acetylcholinesterase (AChE) was examined (Fig. 3) as a biomarker for neurotoxicity. The AChE has a significant catalytic role in neurotransmission by hydrolyzing acetylcholine, hence, the activity of AChE has been widely used as an indicator for xenobiotic exposure

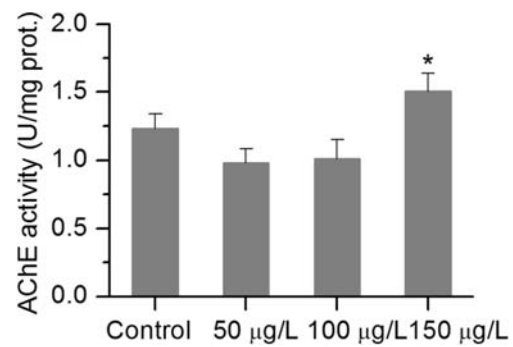


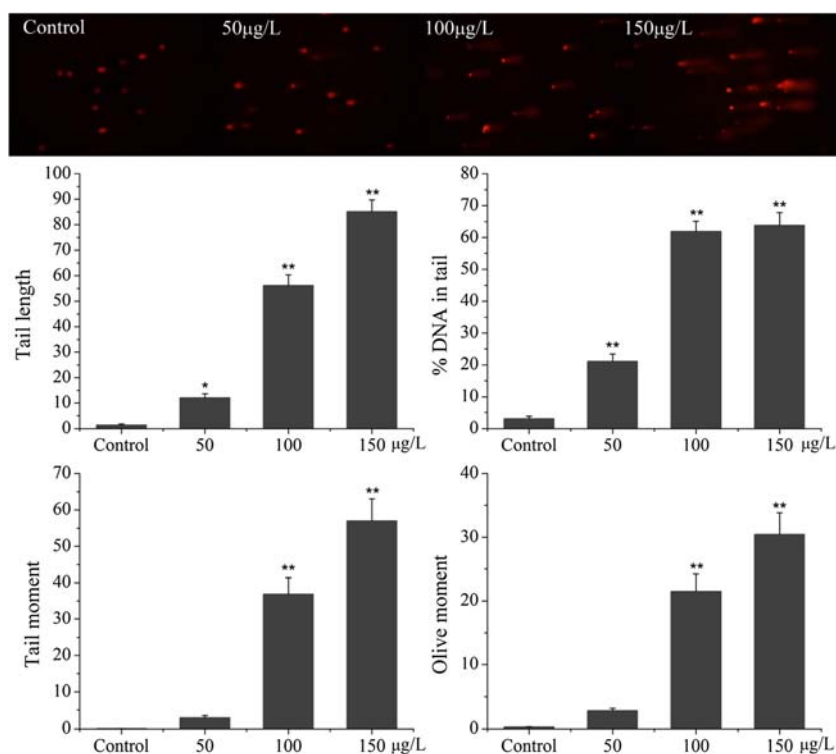
Fig. 3 The activity of AChE in the brain of adult zebrafish exposed to 50, 100, and 150 $\mu\text{g/L}$ TCS, compared to control (mean \pm standard error). * represents a significant difference between treatment and control group at $p < 0.05$

(Manzo et al. 1995). The results of the study reported an inhibition in AChE activity in zebrafish treated with 50 $\mu\text{g/L}$ and 100 $\mu\text{g/L}$, while a slightly elevated activity was observed in the 150 $\mu\text{g/L}$ treatment relative to the control. The trend in AChE was similar to that observed for SOD activities in the brain observed in the study. Differences in the inhibited activity of AChE between groups were not significant at $p < 0.05$ except, for the elevated activity of AChE observed in 150 $\mu\text{g/L}$ group. Inhibition of AChE activity in zebrafish's brain has been observed for several xenobiotics, including pyrethroid, organophosphorus pesticides, and phthalates (DEP and DBP) exposures (Almroth et al. 2019; Xu et al. 2013). Findings of the present study suggested that lowered activity of SOD resulting from excess ROS induced by TCS could subsequently lead to inhibition in AChE activity in the brain, hence, the brain AChE might be a potential target for TCS toxicity (Fig. 4).

DNA damage

Xenobiotics induce oxidative damage to DNA during the induction of ROS and the attenuation of the antioxidant defenses. Excess ROS may directly react with biological molecules such as DNA or indirectly act as signal molecules to mediate genotoxicity (Alak et al. 2019). The close relationship between oxidative stress and DNA damage resulting from environmental insults have been well established (Oliveira et al. 2009). Oxidative stress induced by TCS resulted in concentration-dependent DNA damage in earthworms (Lin et al. 2012), also TCS elicited genotoxicity in hemocytes of zebra mussel (Binelli et al. 2009; Hemalatha et al. 2019). The present study employed the comet assay in measuring the induced oxidative damage of DNA in the hepatocytes of zebrafish after treatment with TCS. Scaling oxidative DNA damage on the comet assay parameters, our study revealed that exposure to TCS induced significant dose-dependent

Fig. 4 Effects of TCS on DNA damage by comet assay. Tail length, tail DNA%, tail moment and olive moment of zebrafish liver cells induced by TCS. An asterisk indicates significant differences at $p < 0.05$ (*) or $p < 0.01$ (**)



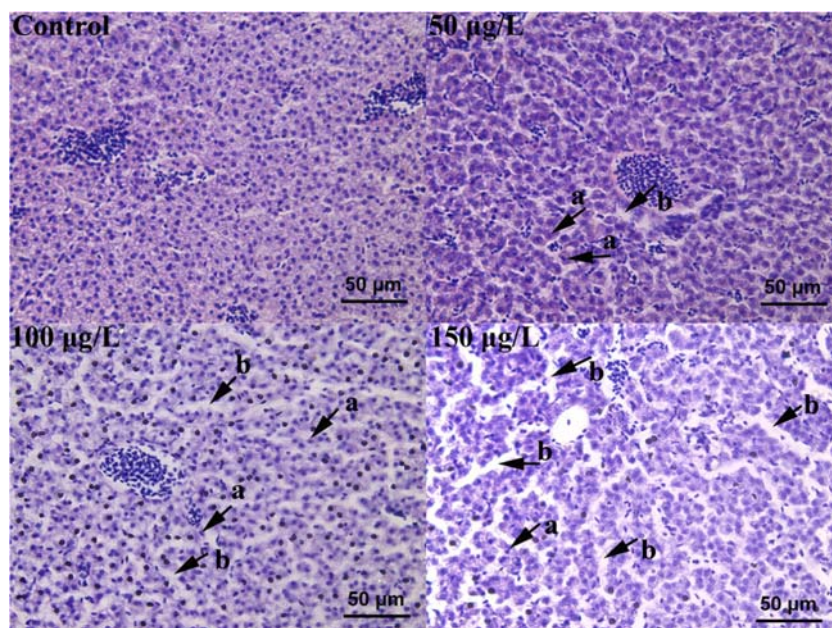
DNA damage in the zebrafish liver hence indicates the susceptibility of the liver to TCS exposure.

Liver and brain histopathological analysis

To further investigate the effects of TCS on liver and brain injury, a histopathological analysis was conducted. Exposure to TCS elicited much hepatocellular atrophy and necrosis, and increased hepatic plate gap in the liver in a concentration-

dependent manner compared with the control groups (Fig. 5). Particularly, a chronic exposure (6 hpf to 90 dpf) to TCS also showed an obvious histopathological alteration in the zebrafish liver (Liu et al. 2019) at concentrations similar to our work. Therefore, the damaging effects of TCS on the liver warrant scientific attention since the liver is a pivotal organ in the metabolism and detoxification of xenobiotics. On the contrary, TCS exposure did not produce obvious histopathological alteration in brain tissues of zebrafish compared to the

Fig. 5 Histological changes in the liver of zebrafish exposed to different concentrations of TCS; “a arrow” shows atrophy and necrosis of hepatocytes; “b arrow” shows the hepatic plate; scale bars, 50 µm



control (Fig. S2). This observation could in part be due to the low bioaccumulation of TCS in the brain (Escarrone et al. 2016; Weatherly and Gosse 2017) and relative short-term exposure to TCS. The changes in biochemical parameters also show that TCS has less effect on the brain than the liver in the present study. Interestingly, at concentrations similar to our works, a recent study indicates longer-term exposure (60 days) to TCS could induce brain injury in zebrafish via abnormal expression of miR-125 regulated by PKC α /Nrf2/p53 signaling pathways (Wang et al. 2020), however, it does not show the histopathological analysis. Therefore, further works are needed to study the TCS induced brain injury.

Conclusion

In summary, an in vivo model based on adult zebrafish revealed that exposure to sub-lethal dose 50, 100, and 150 $\mu\text{g/L}$ respectively representing 1/8, 1/4, and 1/2.7 of 96 h LC₅₀ (398.9 $\mu\text{g/L}$) induced oxidative stress in liver and brain, significantly attenuated the first-line antioxidant defense system (SOD and CAT) activities as well as the MDA contents. Also, the contents of GSH and GSSH were reduced in the liver of zebrafish. Moreover, the zebrafish brain is vulnerable to induction on the activity of AChE while contents of carbonyl protein and 8-OHdG examined in the brain showed a significant increase. Although the zebrafish's antioxidant might be activated for the removal of generated ROS, the comet assay revealed dose-dependent DNA damage in zebrafish liver indicating the susceptibility of the liver to TCS exposure. Concentrations of TCS had no obvious histological changes in brain tissues of zebrafish compared with those of the control. However, hepatocellular atrophy and necrosis, and increased hepatic plate gap were observed in zebrafish hepatocytes after TCS exposure. The overall results of our study highlighted the concern regarding the waterborne of TCS at sublethal concentrations. Studies focusing on elucidating the mechanism of hepatotoxicity and neurotoxicity of TCS on other aquatic wildlife, as well as investigating other toxicity endpoints including transcription levels of stress-related genes and proteomics are also recommended.

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