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EFFECTS OF FENBUTATIN OXIDE ON ANTIOXIDANT SYSTEM OF DIFFERENT TISSUES IN *Cyprinus carpio* (L., 1758)

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ABSTRACT

In this study, effects of sublethal concentrations of fenbutatin oxide on catalase (CAT) and superoxide dismutase (SOD) activities and lipid peroxidation in muscle, liver, kidney and brain tissues of *Cyprinus carpio* were investigated. The 96-hours LC_{50} value for fenbutatin oxide was determined as 1.544 mg/L for *C. carpio* in this study. 0.15 mg/L (1/10 of LC_{50}) and 0.30 mg/L (1/5 of LC_{50}) sublethal concentrations were applied for 96 h (4 days) in this experiment. The CAT activities in tissues were increased relation to doses applied. The SOD activities were decreased by 0.30 mg/ L. The tissue MDA levels were significantly increased in relation to dose applied.

KEYWORDS:
Cyprinus carpio, fenbutatin oxide, oxidative stress, lipid peroxidation.

INTRODUCTION

Fenbutatin oxide, (bis[tris(2-methyl-2-phenylpropyl)tin] oxide; FBTO) is an organotin acaricide with the molecular formula $C_{60}H_{78}OSn_2$. FBTO is very toxic to fish and accumulates in tissues, and it is also very toxic to aquatic organisms [1]. The accumulation and eco-toxicological effects of organotins along the food chain requires a whole classification of organotin levels tissue of organisms of lower and higher trophic stages [2].

Organotin compounds (OTCs) are one of the most widely used organometallic compounds. Over the last several decades, they have been utilized for a variety of industrial and agricultural applications including pesticides, fungicides and anti-fouling agents [3]. They are composed of an atom of tin that is covalently bonded to one or more organic chains and another functional group, such as chloride, oxide, or hydroxide, which are represented by methyl, butyl, octyl, and phenyltin groups [4]. OTCs are immunotoxic both in invertebrates and vertebrates. Mitochondria and membrane functions seem to be a preferred target of these lipophilic pollutants. The inhibition of key membrane-bound enzyme complexes such as Na, K-ATPases, accompanied by perturbation of hydromineral balance, membrane potential and bioenergetics, has been reported [5]. OTCs could be included in other biological routes occurring in cells, specifically in peroxide oxidation of lipids. The latter process is very important from the viewpoint of physiology, and it follows a radical chain mechanism. Acceleration of peroxide oxidation of lipids in cells leads to accumulation of hydroperoxides, degeneration of cell membranes, and various pathologies in organisms [6].

Reactive oxygen species (ROS), such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, are continuously formed in oxygen-consuming organisms. Exposure to xenobiotics or toxic chemical pollutants may produce an imbalance between these endogenous and exogenous ROS and can subsequently induce a decrease in antioxidant defenses or cause oxidative damage outright in organisms [7]. Defence systems that tend to inhibit ROS formation include the antioxidant enzymes such as catalase (CAT; EC 1.11.1.6), superoxide dismutase (SOD; EC 1.15.1.1) [8]. Lipid peroxidation (LPO) has also been used as a bioindicator of oxidative damage in aquatic organisms exposed in polluted environmental conditions. LPO can be increased in the tissues of fish, as indicated by an increased production of malondialdehyde (MDA). The most widely used test for oxidative stress is the measurement of MDA, a product of lipid peroxidation, by the thiobarbituric acid (TBA)-reacting substances assay [7].

Fish have been widely used as models to evaluate the health of aquatic ecosystems in toxicologic pathology [9]. In the present study, *Cyprinus carpio* was chosen as an experiment model, because of its wide availability and suitability for toxicity testing. We have studied the effects of sublethal concentrations of FBTO (0.15 and 0.30 mg/L) for 96 hours on oxidative stress biomarkers in muscle, liver, kidney and brain tissues of *C. carpio*.

MATERIALS AND METHODS

Chemicals. Fenbutatin oxide, (bis[tris(2-methyl-2-phenylpropyl)tin] oxide) was purchased as the Pestanal grade chemical (Sigma-Aldrich). The chemicals used for enzyme activity measurements were purchased from Sigma-Aldrich.

Toxicity test. For the toxicity tests, groups containing 10 fish were placed into glass aquarium. Fish were starved 2 days before the beginning of the experiments. Fish were divided into control and test groups. Different concentrations of FBTO were applied (0.05, 0.10, 0.50, 1.00, 1.50, 2.00 mg/L). During the 96-h experiment, the water was aerated continuously. Each test solution was renewed daily. The dead fish were removed and recorded. At the end of the experiment, median lethal concentrations (LC_{50}) were determined for 96-h. The LC_{50} values were calculated by probit analysis using SPSS Version 15.0 software. The 96-h LC_{50} value for *C. carpio* was determined as 1.544 mg/L for in this study.

Test animals and treatment. *Cyprinus carpio* (mean weight: 19.00±0.90 g; mean length 9.90±0.30 cm) were obtained from Mersin University, Fisheries Faculty, Aquaculture Department and transferred to laboratory to where the temperature was kept at 23±2°C (12:12 L:D). Throughout the experiments, dechlorinated tap water with pH value of 7.85, an alkalinity of 326 mg/L $CaCO_3$, and oxygen concentration of 6.70 mg/L was used. The fish were allowed to acclimatize to these conditions for 2 weeks. The fish were fed at a rate of 2% body weight/day with a commercial pellet diet (Camli-Yem, Izmir-Turkey) during the acclimation period. Experiments were conducted in glass aquaria containing 100 L test solution. Fish were exposed to 0.15 (1/10 of LC_{50}) and 0.30 (1/5 of LC_{50}) mg/L sublethal concentrations of fenbutatin oxide for 96 h (4 days). Stock solution was prepared by fenbutatin and diluting it in acetone to give the dosing concentrations. The water was refreshed every 2 days to compensate for the pesticide lost in the exposure medium.

Twenty-four fish were divided into three experimental groups (n: 8 in each group), as follows: Control (Cont) group; Treatment1 (T1) group (0.15 mg/L FBTO) and Treatment2 (T2) group (0.30 mg/L FBTO). The control group was exposed to acetone at the highest concentration of stock solution used in the fenbutatin oxide-exposed groups (the absence of insecticide). At the end of exposure period, eight fish were removed from each tank, and killed by transaction of the spinal cord. The muscle, liver, kidney and brain tissues of both control and treated fish were dissected. Tissues were homogenized to 1/5 (w/v) ratio in physiological saline solution (0.8 % NaCl) with homogenizer and then centrifuged at 13.500 rpm for 10 min in a Sigma 2-16K centrifuge at +4 ºC, and supernatant was used for biochemical analyses.

Enzyme Assays. The CAT activities of liver tissues were determined according to the method of Aebi [10]. The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. The enzyme activities are given in U/mg protein.

The SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2 generated by the xanthine/xanthine oxidase system [11]. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate. The reduction in NBT by superoxide anion to blue formazan was measured at 560 nm. The enzyme activities are given in U/mg protein.

Measurement of MDA levels. The levels of MDA homogenized tissue, as an index of LPO, were determined by TBA reaction using the method of Yagi [12]. Malondialdehyde and other aldehydes when boiled with thiobarbituric acid at acid pH give a pink-colored product that can be assayed spectrophotometrically. Briefly, a 50 µL of tissue homogenate was mixed with 750 µL of TBARS reagent. The mixture was incubated for 30 min in a boiling water bath. After cooling, the mixture was centrifuged at 3,500 rpm for 15 min. Absorption was measured at 532 nm, and the values are expressed as nanomoles of MDA/mg protein.

Protein determination. The tissue protein contents were measured only to determine the specific activity of antioxidant enzymes according to the method developed by Lowry et al. [13] using bovine serum albumin as standard. Absorbance of samples were measured at 750 nm wavelength by spectrophotometer.

Statistical analysis. The LC_{50} values were calculated by probit analysis using SPSS Version 24.0 software (SPSS Inc., USA). Data were presented as mean±standard error of the mean (SEM) and analyzed by one-way analysis of variance (ANOVA). The significant means were compared by Duncan's multiple range tests at $p<0.05$.

RESULTS

Toxicity Assay. In the present study, different concentrations of FBTO were administrated to *C. carpio*. The mortality rates of fish were calculated as a percentage after 96 hours of insecticide treatment. The mortality of *C. carpio* increased depending on the dose of fenbutatin oxide. The data were obtained from the toxicity test was evaluated using the Probit Analysis Method. The LC_{50} 96 h value for *C. carpio*

was found to be 1.544. 95% confidence limits were between 1.331-1.806 mg/L (Table 1).

Biochemical Assay. The CAT activities in tissues of fish were given in Fig. 1. FBTO did not make any significant changes in muscle tissue enzyme activity compared to control. The CAT activity of liver and brain were significantly increased (P<0.05) by 0.15 and 0.30 mg/L concentrations of FBTO, but, in kidney tissue were significantly increased (P<0.05) by 0.30 mg/L. 0.30 mg/L concentration caused a significant elevation by 46%, 34% and 44% (P<0.05), respectively, in liver, kidney and brain tissues compared to control.

The SOD activities in tissues of fish were shown in Fig. 2. FBTO did not make any significant changes in muscle tissue SOD activities compared to control. 0.30 mg/L concentration of FBO caused a significant inhibition (P<0.05) by 28% and 16% respectively, in liver and brain tissues compared to control. The SOD activity in kidney was increased (26%) by 0.30 mg/L.

TABLE 1
96 hours toxicity results of the fenbutatin oxide bioassay on *C. carpio*
(LC: Lethal concentration; SE: Standart Error)

Points of Lethal Concentration	Concentration (mg/L)	95% Confidences Limits (mg/L)	Intercept±SE
LC 1.00	0.579	0.325-0.768	1.030± 0.249
LC 5.00	0.771	0.505-0.959	
LC 10.00	0.899	0.637-1.083	
LC 30.00	1.238	1.010-1.428	
LC 50.00	**1.544**	**1.331-1.806**	
LC.80.00	2.203	1.873-2.915	
LC.90.00	2.653	2.181-3.843	
LC 99.00	4.123	3.006-7.565	

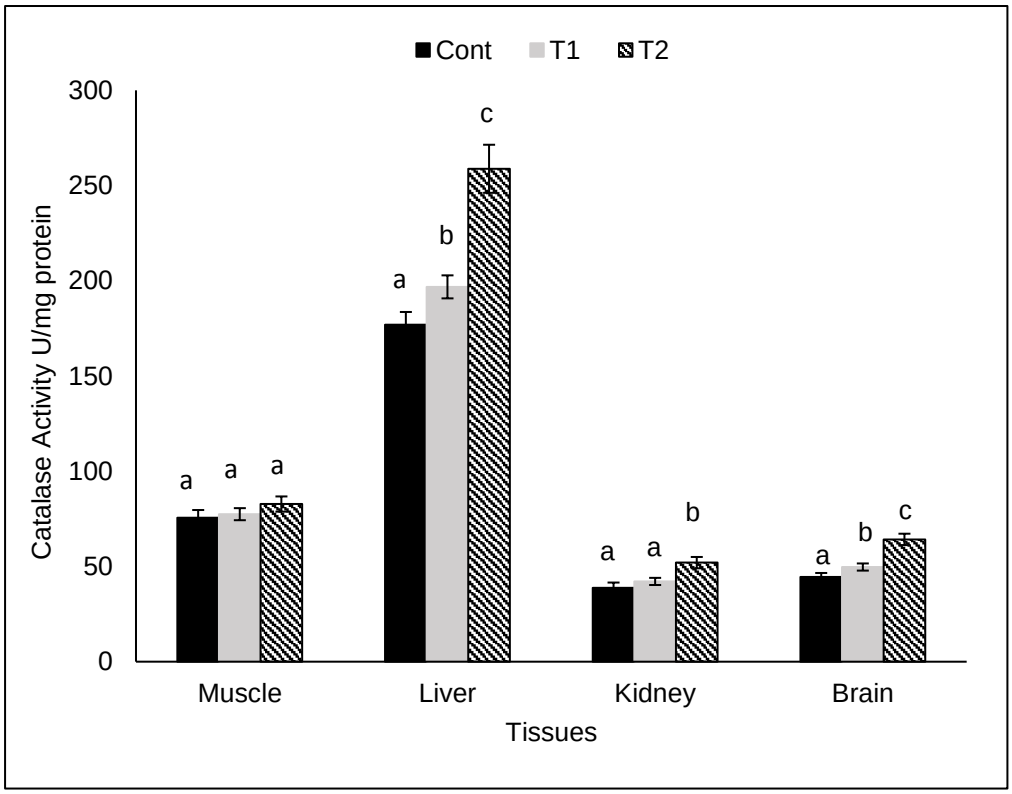

FIGURE 1
CAT activities in tissues of *C. carpio* exposed to sublethal concentrations of FBTO; 0.15 mg/L (T1) and 0.30 mg/L (T2). Each value is the mean ± standard error (n = 8). Multiple comparisons were made separately for each tissue and means with different superscript in tissues are significantly different (P< 0.05).

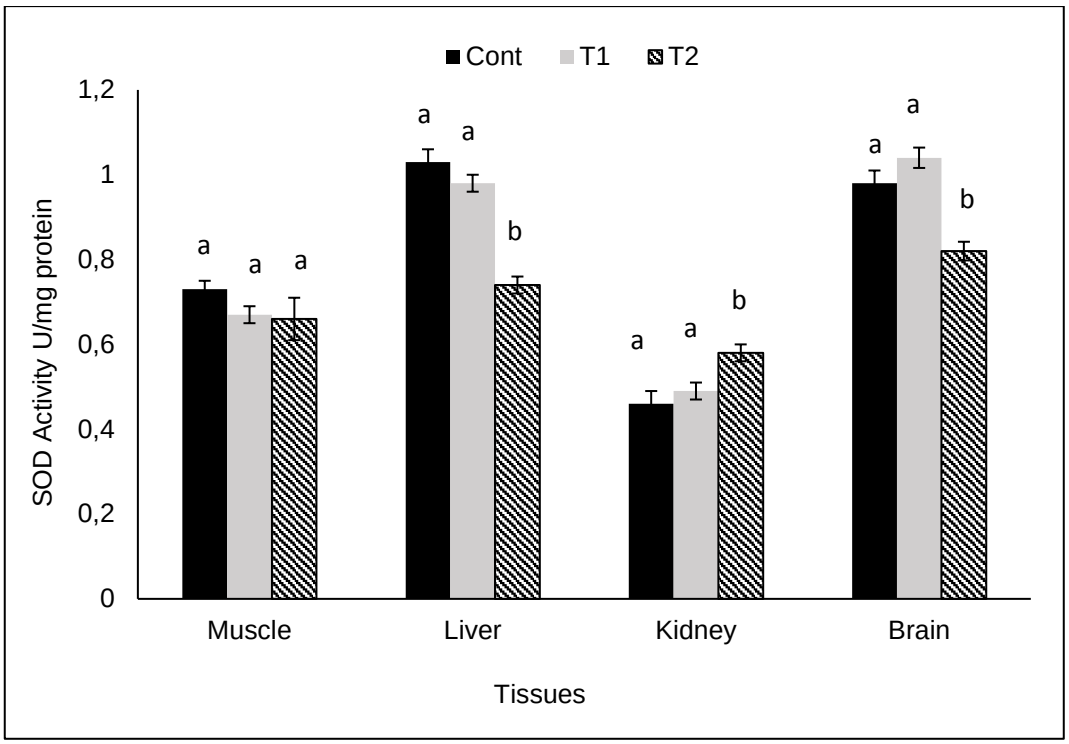

FIGURE 2
SOD activities in tissues of *C. carpio* exposed to sublethal concentrations of FBTO; 0.15 mg/L and 0.30 mg/L. Each value is the mean ± standard error (n = 8). Multiple comparisons were made separately for each tissue and means with different superscript in tissues are significantly different (P< 0.05).

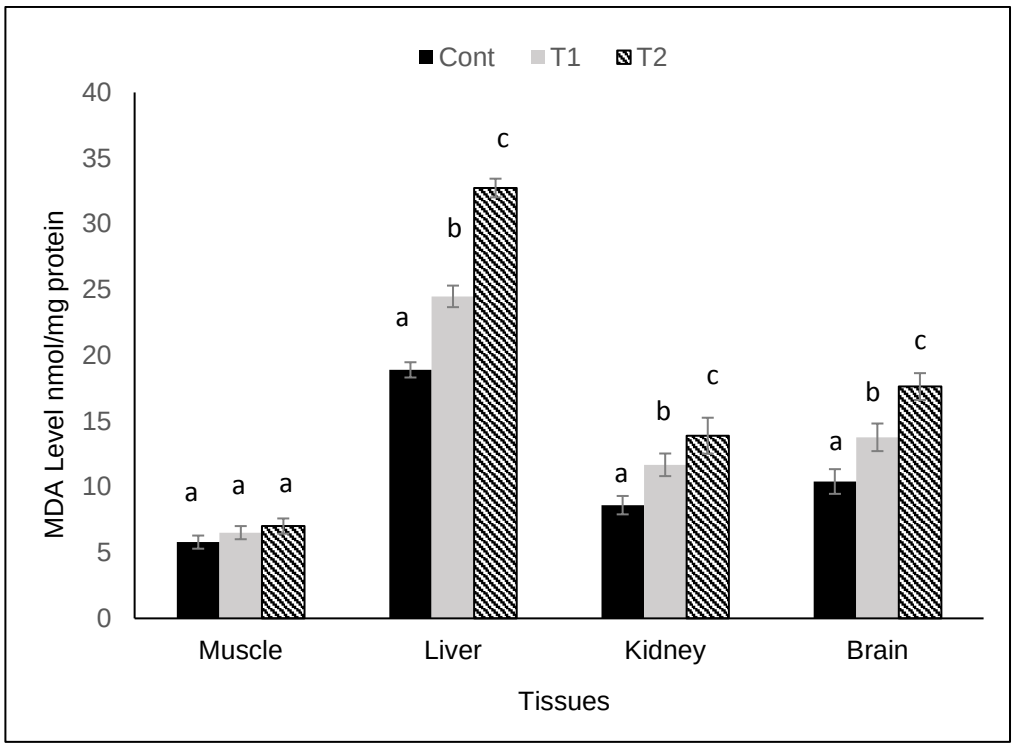

FIGURE 3
MDA levels in tissues of *C. carpio* exposed to sublethal concentrations of FBTO; 0.15 mg/L (T1) and 0.30 mg/L (T2). Each value is the mean ± standard error (n = 8). Multiple comparisons were made separately for each tissue and means with different superscript in tissues are significantly different (P< 0.05).

The levels of MDA in tissues were given in Fig. 3. The MDA levels of tissues (except muscle) were significantly increased ($P<0.05$) by both of concentrations of insecticide. 0.30 mg/L concentration of FBTO caused a significant elevation by 73%, 61% and 70% respectively, in liver, kidney and brain tissues compared to control.

DISCUSSION

Fenbutatin oxide is one of the toxic insecticide for fish. The 48-h LC_{50} values of FBTO for fish have been determined to be 1.70 µg/L for rainbow trout, 2.8 µg/L for mysid shrimp [1]. There is no data about LC_{50} values of this pesticide for *C. carpio* in previous studies. The 96-h LC_{50} value for FBTO was determined as 1.544 mg/L for *C. carpio* in this study. The different sensitivity of fish may be due to differences of sensitivity of enzymes activation [14] and species, strain, age, and sex of the animal, the dose and route of exposure, and the effect of various environmental, nutritional and physiological factors [15]. As a result of the ecological risk assessment for the 1994 Reregistration Eligibility Decision (RED), the agricultural use of FBTO was classified as restricted use due to high acute toxicity to aquatic organisms [16].

The antioxidant system responses constitute important sensors in connecting the pollutant effects and the metabolic alteration in aquatic organisms [17]. In general, the measurement of enzyme activities can provide an indication of the antioxidant status of fish and can also serve as biomarkers of oxidative stress [8]. In the present study, we observed an increase in CAT activities of liver, kidney and brain tissues following 96 h of toxicity by FBTO concentrations. SOD activities were decreased in liver and brain tissues but increased in kidney. There is no data on the effects of FBTO on antioxidant defence systems in fish. Also, there are limited data on the toxicity of OTCs in fish. Zhang et al. [18] showed that with an increase in tributyltin (TBT: one of the organotin) dose, the activities of total SOD, CAT and glutathione peroxidase (GSH-Px) in liver of *Danio rerio* were significantly decreased compared to the control. Conversely, Wang et al. [19] reported that hepatic SOD and CAT activities were significantly induced when *Sebastiscus marmoratus* were exposed to TBT for 4 days. Wang et al. [20] showed that GST activity was induced by the lower doses of TBT, while it tended to be inhibited by the higher doses of TBT. Al-Ghais and Ali [21] showed that organotin compounds (tributyltin :TBT, triphenyltin; TPT, and dibutyltin; DBT) inhibited Glutathion-S-Transferase (GST) activities of liver and kidney in *Siganus canaliculatus* and *Sparus sarba*. SOD activities in brain of *C. carpio* were slightly increased in lower concentration group and significantly decreased in the highest concentration of the TBT [22]. Compared with the control, a significant lower CAT activity was observed in fish exposed to higher concentrations [22]. Researchers have explained that the same chemical having opposite effects may be related to the differences of fish species, exposure condition and concentrations of TBT [18]. The toxicity level of OTCs may be related to its concentration, the timing of exposure, bioavailability, and sensitivity of the biota, as well as to the persistence of various compounds in the environment [4].

Similar results have also been reported in other fish species exposed to different pesticides. Zhang et al. [23] observed that CAT activity in the liver of *C. auratus* were increased although SOD activity was inhibited gradually with 2,4-dichlorophenol concentration increasing. Thomaz et al. [24] reported that CAT activity was increased and SOD activity was decreased in liver of *O. niloticus* exposed to the insecticide trichlorfon for 96 h. Lushchak et al. [25] found that the activity of CAT in liver and kidney of *C. auratus* was elevated by exposure to glyphosate. Exposure to methyl parathion resulted in a significant induction of CAT activity in *Brycon cephalus* liver [26]. Özkan et al. [27] reported that an increase in CAT activity following 96 h of toxicity by chlorpyrifos concentrations but a decrease SOD activity of liver tissue in *O. niloticus*. CAT and SOD enzymes have related functions. SOD catalyzes the dismutation of the superoxide anion radical to H_2O and H_2O_2, which is detoxified by both CAT and GSH-Px activities. Due to the inhibitory effects on ROS formation, the SOD–CAT system provides the first defense line against oxygen toxicity and usually used as an indirect biomarker indicating ROS production [8, 28]. An increase in CAT enzyme activity is probably a response toward increased ROS generation in pesticide toxicity [26]. Usually, an induction of tissue SOD activities was observed when exposed to organic pollutant [29]; however, the excess production of superoxide radicals or after their transformation to H_2O_2 causes an oxidation of the cysteine in the enzyme and deactivates SOD [20]. The decreased antioxidant enzymes result in increased oxidative stress, an indication of impaired antioxidant defense mechanism due to excessive generation of free radicals generated by pesticide [31]. The toxicity of FBTO in present study may be caused by the unbalance between free radicals and antioxidants, which might have resulted in inhibition of SOD activity.

The activity of the antioxidant enzymes could be increased or inhibited by xenobiotic exposure depending on the intensity and the duration of the stress applied, as well as the susceptibility of the exposed species. It is not a general rule that an increase in xenobiotic concentrations induces antioxidant activity [32]. Antioxidant enzymes also show tissue-specific differences in activities that reflect the functions of the tissues and the oxidative stress load that they ex-

perience [25]. In the present work, the studied enzymes responded in a different level in liver, kidney and brain tissues. The response of antioxidant system to oxidative stress in various tissues shows differences from one species to another due to the differences in free radical generation and different antioxidant potential of these tissues.

In this study, lipid peroxidation end product MDA were significantly elevated in liver, kidney and brain tissues of *C. carpio* exposed to FBTO compared with control group. Previous investigations have reported on the induction of LPO in the tissues by different OTCs. Li et al. [22] reported that a significant evaluation in MDA level in brain of *C. carpio* exposed to TBT when compared with the control. Wang et al. [20] found *Sebastiscus marmoratus* exposed to TBT for up to 7 days showed a rise in liver MDA level. MDA content was significantly increased with TBT exposure in liver of *D. rerio* [18]. Earlier investigations have reported on the induction of LPO in the tissues by different pesticides Ozkan-Yilmaz et al. [33] found that MDA levels were increased in the liver of methidathion-treated in *O. niloticus*. Hai et al. [34] reported that level of LPO was elevated in liver and brain of catfish (*Ictalurus nebulosus*) exposed to dichlorvos. Lipid peroxidation is the reaction of oxidative deterioration of membrane polyunsaturated fatty acids (PUFA). Fish tissues are characterized by high concentration of PUFA and may therefore be susceptible to lipid peroxidation [35]. Pesticide exposure could produce free radicals, which resulted in the increase in lipid peroxidation. The increase in lipid peroxidation may be attributed to alterations in the antioxidant defense system [36]. An increase in the MDA levels of tissues might be induced by the possible involvement of ROS in FBTO induced toxicity. In the present work, elevation of the MDA contents may be due to the fact that when the amount of ROS and other radicals exceeds the capacity of antioxidant enzymes. Excess production of radicals may increase in ROS level, while a rise in ROS may increase lipid peroxidation, as measured by MDA intensity [18]. Our results showed that the capacity of FBTO to produce lipid peroxidation.

In the present work, antioxidant enzymes responded in a different level in muscle, liver, kidney and brain tissues. The response of antioxidant system to oxidative stress in various tissues shows differences from one species to another due to the differences in free radical generation and different antioxidant potential of these tissues [37]. In this study, liver tissue was found to be more sensitive than other tissues. In the previous studies, it was found that liver was a more sensitive target organ to OTCs oxidative damage than kidney and muscle [38]. Also, it has been reported that organotin compounds accumulation at higher levels in liver than in most other organs [2, 39].

CONCLUSION

Fenbutatin oxide exposure can induce significant changes in activities CAT, SOD and MDA levels in the tissues of the *C. carpio*. The increasing doses are essential factors when evaluating oxidative stress responses caused by sublethal concentrations of FBTO in liver, kidney and brain tissues of fish. Compared to liver, muscle, kidney and brain was more sensitive to the oxidative damage.

The aquatic environment is contaminated by a number of foreign chemical. Fish antioxidant responses are very sensitive to environmental contamination. These are used as a biological indicator of aquatic environmental health. There is limited available data on OTCs toxicity in animals. For this reason, more detailed research is needed to explain the mechanism.

REFERENCES

[1] Environmental Protection Agency (EPA) (1994) Reregistration eligibility decision for fenbutatin oxide. September, United States EPA-738-F-94-021.

[2] Antizar-Ladislao, B. (2008) Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment: a review. Environ. Int., 34: 292-308.

[3] Buck-Koehntop, B., Porcelli, F., Lewin, J.L., Cramer, C.J., Veglia, G. (2006) Biological chemistry of organotin compounds: interactions and dealkylation by thiols. Journal of Organometallic Chemistry, 691: 1748-1755.

[4] Graceli, J.B., Sena, G.C., Lopes, P.F.I., Zamprogno, G.C., da Costa, M.B., Godoi, A.F.L., Dos Santos, D.M., de Marchi, M.R.R., Dos Santos Fernandez, M.A. (2013) Organotins: a review of their reproductive toxicity, biochemistry, and environmental fate. Reprod. Toxicol., 36: 40-52.

[5] Pagliarani, A., Nesci, S., Vittoria Ventrella, V. (2013) Toxicity of organotin compounds: Shared and unshared biochemical targets and mechanisms in animal cells. Toxicology in Vitro, 27: 978-990.

[6] Pellerito, C., Nagy, L., Pellerito, L., Attila Szorcsik, A. (2006) Biological activity studies on organotin(IV)n+ complexes and parent compounds Journal of Organometallic Chemistry. 691: 1733-1747.

[7] Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M. (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotox. Environ. Safe. 64: 178-189.

[8] Van der Oost, R., Beyer, J., Vermeulen, N.P. (2003) Fish bioaccumulation and biomarkers in

environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13: 57-149.
[9] Peixoto, F., Alves-Fernandes, D., Santos, D., Fontainhas-Fernandes, A. (2006) Toxicological effects of oxyfluorfen on oxidative stress enzymes in tilapia *Oreochromis niloticus*. Pestic. Biochem. Phys., 85: 91-96.
[10] Aebi, H. (1974) Catalase. In: Bergmayer, H.U. (ed.) Methods of enzymatic analysis, Academic Press, New York, pp 671-684.
[11] Sun, Y., Oberley, L.W., Ying, L. (1988) A simple method for clinical assay of superoxide dismutase. Clin. Chem., 34: 497-500.
[12] Yagi, K. (1998) Simple procedure for specific enzyme of lipid hydroperoxides in serum or plasma. Methods in Mol. Biol., 108: 107-110.
[13] Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.
[14] Gungordu, A. (2013) Comparative toxicity of methidathion and glyphosate on early life stages of three amphibian species: *Pelophylax ridibundus*, *Pseudepidalea viridis*, and *Xenopus laevis*. Aquat. Toxicol., 140-141: 220-228.
[15] Buhler, D.R., Williams, D.E. (1988) The role of biotransformation in the toxicity of chemicals. Aquat. Toxicol., 11: 19-28.
[16] Environmental Protection Agency (EPA) (2002) Fenbutatin oxide analysis of risks to endangered and threatened salmon and steelhead, Environmental field Branch Office of Pesticide Programs, November 29, 112 p.
[17] Lushchak, V.I. (2011) Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicology, 101: 13-30.
[18] Zhang, C.N., Zhang, J.L., Ren, H.T., Zhou, B.H., Wu, Q.J., Sun, P. (2017) Effect of tributyltin on antioxidant ability and immune responses of zebrafish (*Danio rerio*). Ecotoxicology and Environmental Safety, 138: 1-8.
[19] Wang, C.G., Chen, Y.X., Li, Y., Wei, W., Yu, Q. (2005) Effects of low dose tributyltin on activities of hepatic antioxidant and phase II enzymes in *Sebastiscus marmoratus*. Bull. Environ. Contam. Toxicol., 74 (1): 114-119.
[20] Wang, C., Zhao, Y., Zheng, R., Ding, X., Wei, W., Zuo, Z., Chen, Y. (2006) Effects of tributyltin, benzo[a]pyrene, and their mixture on antioxidant defense systems in *Sebastiscus marmoratus*. Ecotoxicol. Environ. Saf., 65: 381–387.
[21] Al-Ghais, S.M., Ali, B. (1999) Inhibition of glutathione *S*-transferase catalyzed xenobiotic detoxication by organotin compounds in tropical marine fish tissues. Bull. Environ. Contam. Toxicol., 62, 207-213.
[22] Li, Z.H., Li, P., Shi Z.C. (2015) Chronic Exposure to Tributyltin Induces Brain Functional Damage in Juvenile Common Carp (*Cyprinus carpio*). PLoS ONE, 10(4): 13pp.
[23] Zhang, J., Shen, H., Wang, X., Wu, J., Xue, Y. (2004) Effects of chronic exposure of 2,4- dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. Chemosphere, 55: 167-174.
[24] Thomaz, J.M., Martins, N.D., Monteiro, D.A., Rantin, F.T., Kalinin, A.L. (2009) Cardio-respiratory function and oxidative stress biomarkers in Nile tilapia exposed to the organophosphate insecticide trichlorfon (NEGUVONs). Ecotox. Environ. Safe. 72: 1413-1424.
[25] Lushchak, O.V., Olha, I., Kubrak, O.I., Storey, J.M., Storey, K.B., Lushchak, V.I. (2009) Low toxic herbicide roundup induces mild oxidative stress in goldfish tissues. Chemosphere, 76: 932-937.
[26] Monteiro, D.A., Almeida, J.A., Rantin, F.T., Kalinin, A.L. (2006) Oxidative stress biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Com Biochem Phys C 143:141-149.
[27] Ozkan, F., Gunduz, S.G., Berkoz, M., Ozluer-Hunt, A., Yalin, S. (2012) The protective role of ascorbic acid (vitamin C) against chlorpyrifos-induced oxidative stress in *Oreochromis niloticus*. Fish Physiol. Biochem. 38(3): 635-643.
[28] Pandey, S., Parvez, S., Sayeed, I., Haquer, R., Bin-Hafeez, B., Raisuddin, S. (2003). Biomarkers of oxidative stress: A comparative study of river Yamuna fish *Wallago attu* (Bl. Schn). Sci. Total Environ., 309: 105-115.
[29] Palace, V.P., Dick, T.A., Brown, S.B., Baron, C.L., Klaverkamp, J.F. (1996) Oxidative stress in Lake Sturgeon (*Acipenser fulvescens*) orally exposed to 2,3,7,8-tetrachlorodibenzofuran. Aquat. Toxicol. 35: 79-92.
[30] Dimitrova, M.S.T., Tsinova, V., Velcheva, V. (1994) Combined effect of zinc and lead on the hepatic superoxide dismutase–catalase system in carp (*Cyprinus carpio*). Comp. Biochem. Physiol. 108: 43-46
[31] Kavitha, P., Rao, V. (2008) Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, *Gambusia affinis*. Environ. Toxicol. Phar. 26: 192-198.
[32] Oruc¸ E.O., Usta, D. (2007) Evaluation of oxidative stress responses and neurotoxicity potential of diazinonin different tissues of Cyprinus carpio. Environ Toxicol Phar 23:48–55
[33] Ozkan-Yilmaz, F., Ozluer-Hunt, A., Gunduz, S.G., Berkoz, M., Yalin, S., Sahin, N.O. (2015) Effects of methidathion on antioxidant system and expression of heat shock protein 70

(HSP70) gene in the liver of *Oreochromis niloticus* L. 1758. Fresen. Environ. Bull., 24(8a): 2650-2658.
[34] Hai, D.Q., Varga, S.I., Matkovics, B. (1997) Organophosphate effects on antioxidant system of carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*). Comp. Biochem. Phys. C, 117: 83-88.
[35] Stephan, G., Guillaumeb, J., Lamoura, F. (1995) Lipid peroxidation in turbot (*Scophthahus maximus*) tissue: effect of dietary vitamin E and dietary n-6 or n-3 polyunsaturated fatty acids. Aquaculture, 130:251–268
[36] Ademuyiwa, O., Agarwal, R., Chandra, R., Behari, J.R. (2009) Lead induced phospholipidosis and cholesterogenesis in rat tissue. Chem. Biol. Interact., 179(2-3): 314-320.
[37] Adonaylo, V.N., Oteiza. P.I. (1999) Lead intoxication: antioxidant defenses and oxidative damage in rat brain. Toxicology, 135: 77–85.
[38] Tsuda, T., Nakanishi, H., Aoki, S., Takebayashi, J. (1986) Bioconcentration of butyltin compounds by round crucian carp. Toxicol. Environ. Chem., 12(1-2): 137-138.
[39] Strand, J., Jacobsen, J.A. (2005) Accumulation and trophic transfer of organotins in a marine food web from the Danish coastal waters. Sci. Total. Environ., 350: 72–85.

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