JCHR (2022) 12(3), 481-493

Journal of Chemical Health Risks

ORIGINAL ARTICLE

Evaluation of Blood Biochemical Parameters and Oxidative Stress Biomarkers in Common Carp (*Cyprinus carpio*) Exposed to Deltamethrin

Mehdi Banaei, Mohsen Forouzanfar*, Mojtaba Jafarinia

Department of Biology, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran

(Received: 6 December 2021 Accepted: 5 March 2022)

KEYWORDS

Delthamethrin;
Oxidative stress;
Biochemical parameters;
Common carp;
Pyrethroid pesticide

ABSTRACT: Deltamethrin has magnificent potential for agricultural pest control. The penetration of deltamethrin into aquatic ecosystems can endanger the life of aquatic organisms. In this study, common carp (*Cyprinus carpio*) was exposed to the sub-lethal concentrations of deltamethrin (0.0, 6, 12, and 18 µg L^{-1}) for 30 days. Then, the biochemical parameters of blood and the biomarkers of oxidative stress in fish were evaluated to assess the toxic effects of deltamethrin. Based on the results deltamethrin exposure altered antioxidant enzyme activities (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and glucose 6-phosphate dehydrogenase) and increased lipid peroxidation and protein carbonylation rate in hepatocytes. However, the course of these changes was dose-dependent to deltamethrin. There was a significant reduction in the total antioxidant and glycogen contents in the hepatocytes of fish challenged with deltamethrin. Conversely, exposure of *C. carpio* to deltamethrin increased aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, creatinine phosphokinase, alkaline phosphatase, and lactate dehydrogenase activities. Deltamethrin significantly inhibited butyrylcholinesterase activity and declined total protein and globulin levels. However, glucose, creatinine, cholesterol, and triglyceride levels significantly increased in the plasma of *C. carpio* exposed to deltamethrin. Therefore, these findings demonstrated the potential of deltamethrin to induce cytotoxicity in fishes by disrupting cellular homeostasis and producing reactive oxygen species-induced oxidative stress.

INTRODUCTION

Pyrethroid pesticides are most commonly used to control plant pests in the world [1]. Compared to organophosphate, organochlorine, and carbamate pesticides, pyrethroids have high bioavailability, low half-life, and lower toxicity. Deltamethrin is one of the most well-known pyrethroid esters, widely applied to control agricultural pests, preserve crops in silos, and kill insects and wild fish in aquatic ecosystems [2]. Although deltamethrin is moderately toxic to birds and mammals [3], it is highly toxic to fish and amphibians [4].

The lipophilic properties of deltamethrin and its metabolites cause this insecticide to rapidly penetrate into biological tissues [5]. Therefore, this pesticide may easily enter the the body of fish through the skin, gills, and digestive system and penetrate vital organs and nerve tissue through the circulatory system [4].

*Corresponding author: mforozanfar@yahoo.com (M. Forouzanfar)
DOI: 10.22034/jchr.2022.1945633.1463

Deltamethrin may be biodegraded in hepatocytes to facilitate its excretion from the body [6]. However, its metabolites and part of the parent substance may accumulate in the aquatic organisms [7]. Consequently, the bioaccumulation of this pesticide can endanger fish health due to the deficiency of the deltamethrin detoxification system in aquatic animals compared to mammals [8].

Deltamethrin is used in agricultural activities worldwide. Thus, deltamethrin may enter aquaculture ponds through the drainage of agricultural farms and surface runoff. Toxicology reports showed that deltamethrin residues in surface waters and sediments ranged from 0.73ng L^{-1} to 24µg L^{-1} and 8.27 to 473ng g^{-1}, respectively [9, 10]. Therefore, the overuse of deltamethrin in agriculture can severely threaten the aquaculture industry. In addition, the contamination of agricultural products with deltamethrin can affect consumers' health, including farmed fish [11].

It should be noted that deltamethrin is highly toxic to aquatic organisms; for example, the acute toxicity of deltamethrin was reported 0.449 µg L^{-1} in *Macrobrachium rosenbergii* [1], 31.51 µg L^{-1} in *Poecilia reticulata* [12], 1.94 µg L^{-1} in *Channa argus* [6], 14.6 µg L^{-1} in *Oreochromis niloticus* [13], and 7.33 µg L^{-1} in *C. punctatus* [14].

Fish blood in the gills is in direct contact with the aquatic environment, thus any change in biochemical parameters can reflect an alteration in the living conditions of the fish. Hence, the blood biochemical parameters of fish could be used as a biomarker to indicate the health status of fish and water quality. Some studies evaluated the effects of deltamethrin on blood biochemical parameters in *C. argus* [6], *Clarias gariepinus* [15], *Catla catla* [16], and *C. punctate* [17].

Literature reviews demonstrated that reactive oxygen species (ROS) production is the root of all pathological injuries in fish exposed to pesticides. The interaction of ROS with vital macromolecules, oxidative stress, disruption of cellular biochemical reactions and disturbance in cellular homeostasis, genetic mutations and gene damage, induction of apoptosis, changes in blood biochemical parameters and histopathological damages were reported in treated organisms with deltamethrin. Oxidative damage was also found in *C. argus* [18], *Macrobrachium nipponense* [19], and *Eriocheir sinensis* [20] exposed to deltamethrin. Hong et al. [20] investigated genotoxicity biomarkers in the Chinese mitten crab, *E. sinensis*, after exposure to deltamethrin. Changes in the expression of various genes were reported in common carp, *C. carpio* [21], and giant freshwater prawn, *M. rosenbergii* [1].

Therefore, it is hypothesized that exposure of fish to deltamethrin may lead to the disruption of cellular homeostasis and cytotoxicity. Hence, this study was focused on examining the effect of the cytotoxicity of deltamethrin on fish. More precisely, the current study aimed to evaluate the toxic effect of deltamethrin on biochemical parameters and oxidative stress in common carp (*C. carpio*) as a laboratory model.

MATERIALS AND METHODS

Insecticide

Deltamethrin (EC 2.5%) was purchased from Gyah-Corp Company, Iran. The deltamethrin stock was prepared by mixing commercial formulation with distilled water.

Fish

Juveniles common carps (*C. carpio*) were obtained from a local farm (Shiraz, Iran). The fishes were acclimated for two weeks in twelve tanks (100 L) equipped with aerators containing dechlorinated tap water (dissolved oxygen 6.5±0.5 mg L-1; temperature 24±2 °C, pH: 7.5±0.2, and photoperiod period: 16 light/8 dark). Fish were fed with commercial fish feed (Bayza Feed Mill, Iran). The proposal and all methods were reviewed and approved by the Ethics Committee on Animal Use of the Faculty of Basic Science, Marvdasht Branch, Islamic Azad University, Iran (162251822-IAU).

Experimental design

After adaptation, common carps (*C. carpio*) were distributed in twelve tanks (In 4 experimental groups, each with three repetitions (4×3)) and exposed to different sub-

lethal concentrations of deltamethrin (0.0, 6, 12, and 18 µg L^{-1} which were equal to 0, 5, 10, and 15% of the 96 LC_{50} value: 126.11 (90.57-175.60 µg L^{-1})). Thus, each tank included 12 fish. The sub-lethal concentrations of deltamethrin were selected based on a previous study [21].

At the end of the experiment, twelve fish were randomly caught from each treatment and anesthetized with a decoction of clove powder (150 mg L^{-1}). Then the blood sample was collected from the caudal vein using a syringe containing anticoagulants and centrifuged at 6000 rpm for 15 min at 4°C. Next, the plasma was separated and kept at -26 °C until the biochemical analyses followed by autopsying the fish, and removing the liver tissue. The liver was then washed immediately with a 0.9 g L^{-1} saline solution, frozen in liquid nitrogen, and placed in the freeze at -80°C until the biochemical analysis.

Biochemical parameters assay

The aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) activities were estimated by the calorimetric technique of Moss and Henderson [22], describing the instructions for Pars Azmoun biochemical kits. Butyrylcholinesterase (BChE) activity was determined using butyrylcholine as a substrate following the method described in the Pars Azmoun biochemical kits [23]. Further, glucose [24], cholesterol, triglyceride [25], creatinine, total protein, and albumin [26] were measured by spectrophotometer methods as described in the instructions for Pars Azmoun biochemical kits. The globulin was estimated following the formula [27]: Total protein – albumin = globulin

Oxidative biomarkers

Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glucose 6-phosphate dehydrogenase (G6PDH) activities were measured by applying the technique in the biochemical kit instructions purchased from the Biorexfars Co., Iran. Moreover, catalase (CAT) activity was determined using a hydrogen peroxide solution as a substrate [28]. The total antioxidant contents were estimated following the ferric reducing ability of the plasma (FRAP) procedure using TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) as a substrate [29]. Additionally, malondialdehyde (MDA), a lipid peroxidation metabolite in the hepatocytes, was assayed by thiobarbituric acid as a substrate [30]. Similarly, carbonyl protein content in the hepatocytes was measured using the procedure in the biochemical kit guidance acquired from the KiaZist Co., Iran. Finally, glycogen content in the hepatocytes was determined by employing the method described in pervious research [31].

Statistical analysis

All data were subjected to statistical analysis using One-Way ANOVA to manifest the variation between different trial groups using Graph Pad Prism 8 software. The Shapiro-Wilk test was used to check the normality of the data. Statistical differences between different experimental groups were expressed at a confidence level of 5% ($P < 0.05$). Different letters of the alphabet explicitly showed a significant difference between the experimental groups ($P <0.05$).

RESULTS

The results of enzyme activities in plasma are illustrated in Figure 1. Based on the data, the activities of AST, GGT, and CPK in the plasma of *C. carpio* exposed to different concentrations of deltamethrin were higher than in control groups. After exposure to 12 and 18 µg L^{-1} deltamethrin, a significant increase in ALT, ALP, and LDH activities was detected in the plasma of *C. carpio* (Figure 1). Compared with the reference group, deltamethrin inhibited the BChE activity in the plasma of *C. carpio* (Figure 1).

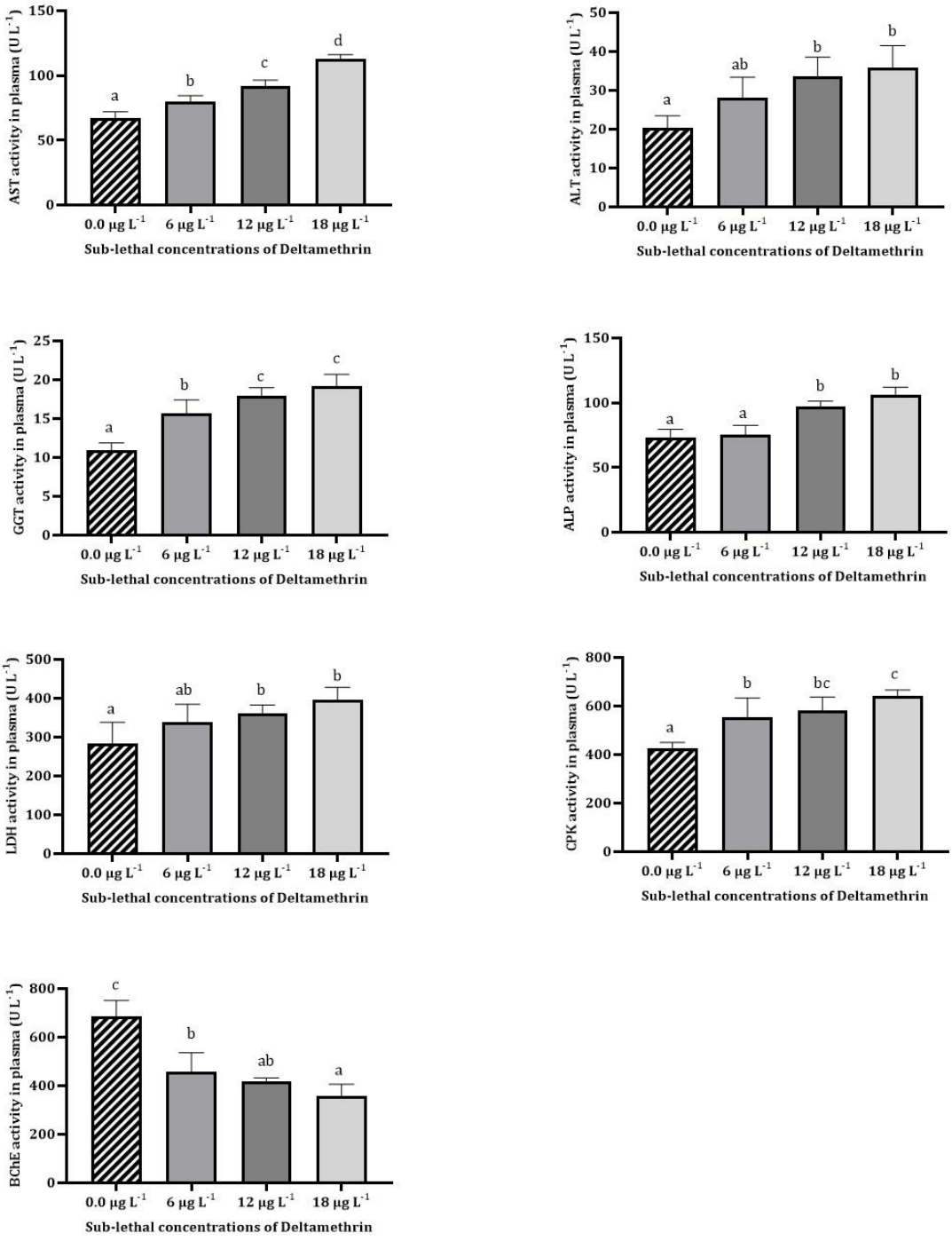

Figure 1. Effects of different concentrations of deltamethrin on enzyme activities in the plasma of *Cyprinus carpio*

A significant reduction in the total protein and globulin levels was recorded in the plasma of *C. carpio* exposed to deltamethrin compared to the control group. However, no significant changes were found in albumin levels (Figure 2). Based on the results, a significant increase in glucose and creatinine levels was observed in the plasma of *C. carpio* exposed to deltamethrin as compared to the control group (Figure 2). Cholesterol and triglyceride levels were observed to be significantly higher in the plasma of *C. carpio* exposed to 12 and 18 µg L^{-1} deltamethrin in comparison to the control group (Figure 2).

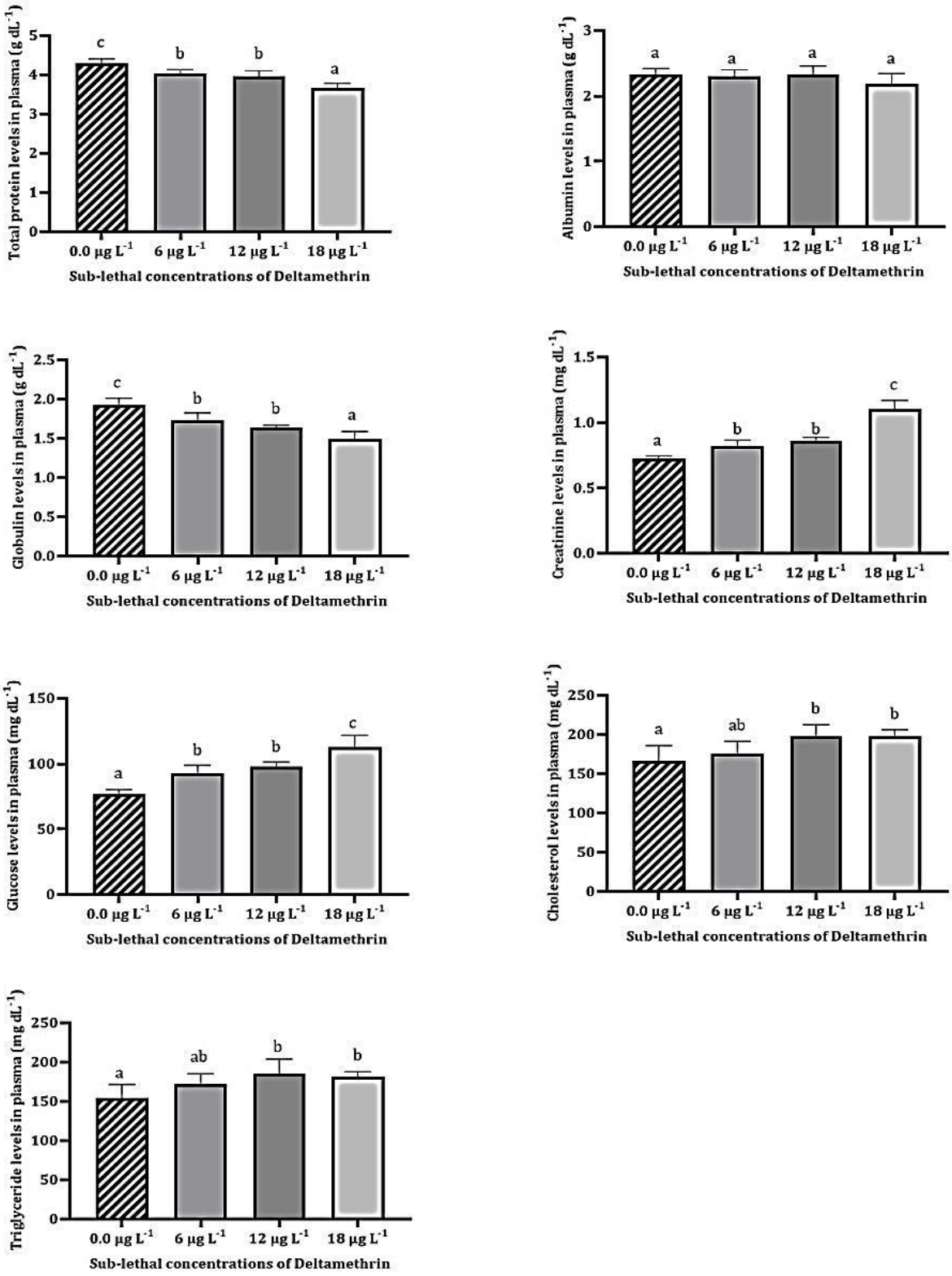

Figure 2. Effects of different concentrations of deltamethrin on blood biochemical parameters in *Cyprinus carpio*

A significant increase was observed in SOD activity in the hepatocytes of *C. carpio* exposed to deltamethrin (Figure 3). The results revealed that exposure to different concentrations of deltamethrin decreased GR, CAT, and G6PDH activities in the hepatocytes of *C. carpio* (Figure 3).

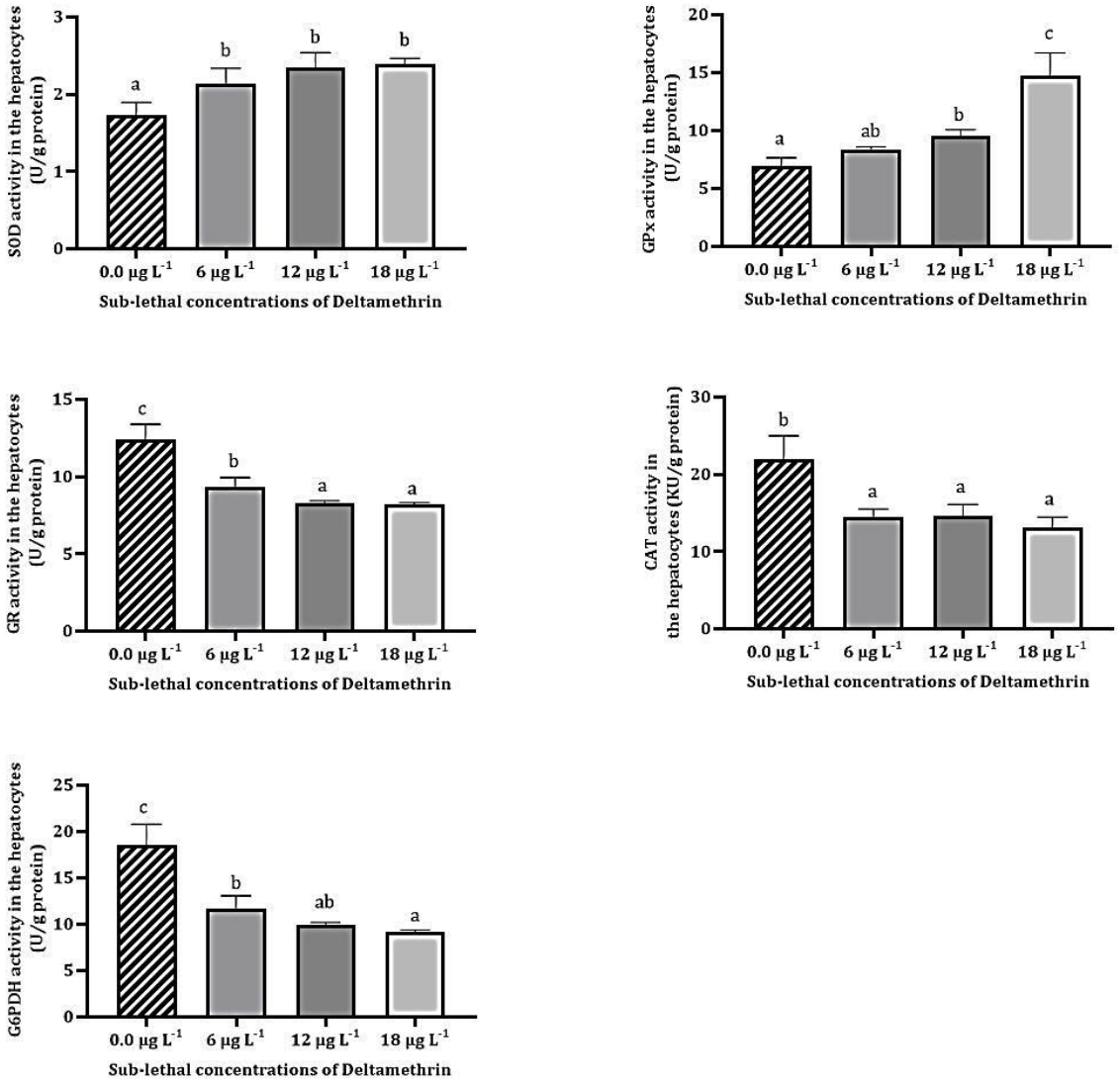

Figure 3. Effects of different concentrations of deltamethrin on antioxidant enzyme activities in the hepatocytes of *Cyprinus carpio*

A significant increase was observed in the MDA and CA levels in the hepatocytes of deltamethrin exposed *C. carpio* compared to the control group (Figure 4). After exposure to different concentrations of deltamethrin, TAO levels in the hepatocytes of *C. carpio* significantly decreased as compared to the control group (Figure 4). Based on the result, glycogen levels in the hepatocytes of *C. carpio* were significantly reduced in all the deltamethrin-treated fish in comparison to the control group (Figure 4).

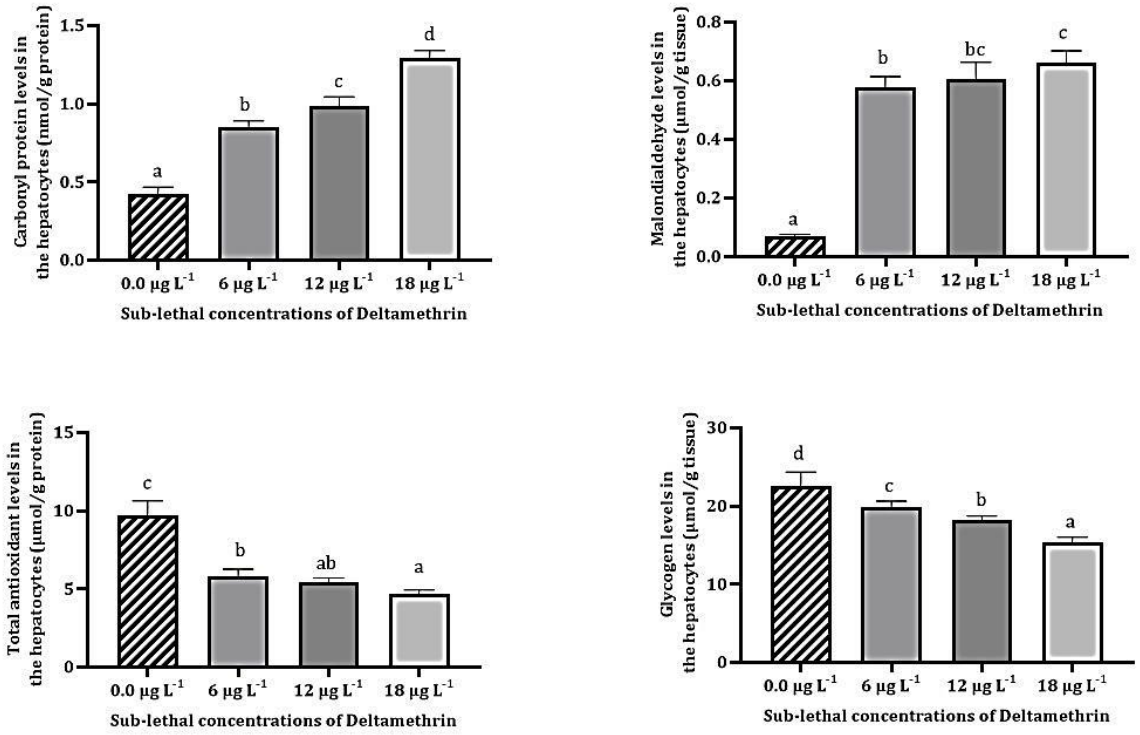

Figure 4. Effects of different concentrations of deltamethrin on CA, MDA, TAN, and glycogen levels in the hepatocytes of *Cyprinus carpio*

DISCUSSION

The present study examined the toxicity effects of deltamethrin on the common carp, *C. carpio*, on biochemical parameters and oxidative markers. No mortality was observed in all experimental groups and the control group. Deltamethrin was originally designed to disrupt the function of ion conduction channels and transmit nerve signals to the insect nervous system [32]; moreover, it can have toxic effects on aquatic animals, including fish [9]. The extreme sensitivity of aquatic animals to deltamethrin may be due to its lipophilic nature [12]. Deltamethrin can be simply absorbed through the gills, skin, and gastrointestinal tract, thus gaining direct access to the blood supply and the sites of toxic action [10]. Deltamethrin, even at low concentrations in the blood, readily crosses the capillaries of the central nervous system and disrupts it [32]. Exposure to deltamethrin can induce liver mixed-function oxidases and detoxification mechanisms. Furthermore, some studies reported alterations in cellular biochemical homeostasis [14, 6]. In the present study, a significant elevation in AST, GGT, and CPK activities was observed in the plasma of *C. carpio* exposed to different concentrations of deltamethrin (Figure 1). There was a significant increase in ALT, ALP, and LDH activities in the plasma of *C. carpio* exposed to 12 and 18 µg L^{-1} deltamethrin (Figure 1). Exposure to deltamethrin led to the disturbance and rupture of the cellular membrane. Hence, cytoplasmic enzymes leaked into the plasma. The increased ROS generation during deltamethrin detoxification facilitated lipid peroxidation and cell membrane disintegration. These results are consistent with similar changes reported in *Labeo rohita* [33], *Oreochromis niloticus* [34], *C. carpio* [35, 36], and *Carassius auratus* [37], after exposure to glyphosate, nano-cadmium, chlorpyrifos, dimethoate, and methylene blue, respectively.

Exposure to deltamethrin significantly inhibited BChE activity in the plasma of *C. carpio* compared to the control group (Figure 1). BChE is a neurotransmitter that plays an essential role in neurotransmission [38]. Therefore, a decrease in BChE may interfere with the transmission of

neural signals. The interaction of deltamethrin and its metabolites with the structure of BChE may inhibit its activity. BChE is mainly synthesized in the liver and found in blood plasma, liver, heart, kidneys, and intestines [23]. Decreased BChE may be related to impaired biosynthesis in hepatocytes. Likewise, BChE is involved in the detoxification of xenobiotics and hydrolysis of acetylcholine [23]. In addition, BChE in aquatic organisms, especially fish, can prevent toxicity by binding to toxins before reaching the target molecules [39]. The conjugation of deltamethrin and its metabolites to BchE may reduce its levels.

The total protein and globulin levels in the plasma of *C. carpio* exposed to deltamethrin were significantly lower than the control group. However, no significant changes were detected in albumin levels (Figure 2). The results showed that exposure to deltamethrin led to decline in protein biosynthesis in the hepatocytes. Furthermore, decreased total protein may be due to reduced protein storage in the liver, degradation, and possible use of amino acids in the energy supply cycle to counteract cytotoxic effects. A significant decrease in globulin levels was related to reduction in protein biosynthesis in the liver of fish exposed to deltamethrin. Decreased total protein and globulin were reported in *Pontastacus leptodactylus* [40], *Coturnix japonica* [27], *Emys orbicularis* [41], and *Cirrhinus cirrhosus* [42] exposed to chlorpyrifos and glyphosate, deltamethrin microplastics, and crude oil, respectively.

The increased glucose levels may indicate metabolic stress. Increased cellular energy demand may be a physiological mechanism for counteracting the cytotoxicity of deltamethrin [43]. Further, a decreased glomerular filtration rate in the kidneys of fish exposed to deltamethrin may elevate creatinine levels. When nephrons are hurt, creatinine levels will be increased in the plasma [44]. Studies on Nile tilapia (*Oreochromis niloticus*) showed that deltamethrin could increase serum creatinine [43, 45]. A similar change was observed in glucose and creatinine levels in the blood of *C. carpio* [46] exposed to paraquat.

The increased cholesterol and triglyceride levels may be due to disturbances in the balance between the intestinal absorption rate, hepatic synthesis, excretion, and storage of lipids in the adipose tissue. Farag et al. [47] found that Bifenthrin increased cholesterol and triglyceride in the serum of *O. niloticus* [47]. A similar increase was observed in cholesterol and triglyceride in the serum of *C. carpio* exposed to profenofos [48].

Elevated SOD activity was a physiological response to increasing superoxide anions in the hepatocytes. A significant increase in SOD activity could facilitate the conversion of superoxide anions to hydrogen peroxide. Additionally, a significant increase in SOD activity was reported in the hepatopancreas of *E. sinensis* exposed to 0.073 μg L^{-1} deltamethrin [49]. However, our results contradict the findings of Elia et al. [50], indicating that deltamethrin exposure resulted in a significant reduction in SOD in *O. mykiss* [50]. The change in SOD activity may be due to the flexibility of the antioxidant defense system of fish in neutralizing ROS. Furthermore, Hong et al. [20] revealed that changes in SOD activity in aquatic organisms in response to deltamethrin exposure could be dose-dependent [49].

The activity of GPx in fish exposed to 12 and 18 μg L^{-1} deltamethrin was significantly higher than in the control group (Figure 3). GPx plays a role in the detoxification of hydrogen peroxide (H_2O_2). Therefore, the increase in GPx activity may be due to the increased accumulation of H_2O_2 in hepatocytes. Consistent with our observations, a significant increase in GPx activity was reported in the hepatocytes of *C. punctatus* exposed to deltamethrin [17].

GR plays a role in reducing glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH). A significant decrease in GR activity could reduce the capacity of cellular GSH. Ceyhun et al. [54] found that exposure to 0.25 μg L^{-1} deltamethrin reduced GR activity in the hepatocytes of *O. mykiss.*

According to evidence [51], G6PDH contributes the biosynthesis of Nicotinamide adenine dinucleotide phosphate (NADPH), which is essential for the reduction of cellular GSH. Therefore, a significant decline in G6PDH activity could negatively affect the function of GR and the

regeneration of GSH. Thus, the ability of cellular radical scavengers may also decrease due to reduced cellular GSH. The effect of some drugs and xenobiotics on inhibiting the activity of G6PDH in fish is consistent with our findings [52, 36, 53]. Similarly, Ceyhun et al. [54] reported a significant decrease in G6PDH activity in rainbow trout in response to exposure to deltamethrin [54].

Morover, CAT exerts a role in the decomposition of H_2O_2 into water and oxygen. A significant decrease in CAT activity can inhibit the neutralization of H_2O_2. As a result of the inhibition of CAT activity, the lipid peroxidation rate may represent an increase. A significant decrease in CAT activity may be related to the downregulation of *CAT* gene expression in fish exposed to deltamethrin [45]. A reduction in CAT activity was observed in the hepatocytes of *Channa punctatus* after a challenge with deltamethrin [55]. In the previous study, deltamethrin declined CAT activity in the hepatocytes of *C. punctatus* [17], which is in line with our findings.

MDA and CP levels in the hepatocytes of deltamethrin exposed *C. carpio* showed a significant increase in the MDA and CA generation in deltamethrin treated groups compared to the control group (Figure 4). MDA is a final product of lipid peroxidation. Therefore, a significant increase in MDA levels indicated an imbalance between per-oxidants and the cellular antioxidant defence system. CP as a biomarker of protein peroxidation is often formed due to the interaction of ROS with proteins [32]. The denaturation of some functional proteins and enzymes may disrupt many biochemical processes and cell physiology. A significant increment in lipid peroxidation and protein carbonylation was reported in the hepatocytes of *C. carpio* [55], *Channa punctate* [17], and *Bombina variegata* [56] exposed to deltamethrin. In their study, Dorts et al. [57] found that deltamethrin exposure increased lipid peroxidation and protein carbonylation rate in the hepatopancreas of black tiger shrimp, *Penaeus monodon* [57], which corroborates with our results.

Decreased cellular total antioxidants are important biomarkers demonstrating the collapse of the cellular antioxidant defence system and the oxidative stress in fish. Reduced TAO contents were reported in *C. carpio* exposed to chlorprifos [35], dimethoate [36], and paraquat [58]. Similar results were detected in Zebra cichlid, *Cichlasoma nigrofasciatum* [53], and freshwater snail, *Galba truncatula* [59] exposed to malathion and dimethoate, respectively.

Increased cellular energy demand can lead to the breakdown of stored glycogen in the liver and muscles into glucose [60]. As a result, the necessary energy for the physiological activities of the cells, including detoxification, will be provided after the increase in blood glucose. Thus, the biodegradation of glycogen could help supply energy to relieve the cytotoxic effect of deltamethrin. Similar to our finding, a reduction in glycogen contents was observed in the liver of *G. truncatula* [59], *Heteropneustes fossilis* [61], and cichlid, *Australoheros facetus* [62], exposed to dimethoate, chlorpyrifos, and azoxystrobin, respectively

CONCLUSIONS

Overall, our findings revealed that although sub-lethal concentrations of deltamethrin could not lead to short-term mortality of fish, long-term exposure of fish could cause physiological and biochemical disorders. Increased plasma AST, ALT, GGT, LDH, ALP, and CPK activities indicated severe cell membrane damage. Furthermore, a decrease in the total protein and globulin and an increase in glucose, creatinine, cholesterol, and triglyceride levels showed the disturbance of homeostasis in the cells. Changes in oxidative stress biomarkers also displayed oxidative stress in fish hepatocytes exposed to deltamethrin. Therefore, exposure of fish to the sub-lethal concentrations of deltamethrin can endanger fish life in the long run.

ACKNOWLEDGEMENTS

The authors appreciate the Vice Chancellor for Research of the Islamic Azad University, Marvdasht Branch, for the spiritual support of this dissertation.

Conflict of interests

The authors declare that they have no conflict of interest.

REFERENCES

1. Jiang Q., Jiang Z., Ao S., Gao X., Zhu X., Zhang Z., Zhang X., 2021. Multi-biomarker assessment in the giant freshwater prawn *Macrobrachium rosenbergii* after deltamethrin exposure. Ecotoxicology and Environmental Safety. 214, 112067. doi: 10.1016/j.ecoenv.2021.112067.

2. Wu X., Zhang C., An H., Li M., Pan X., Dong F., Zheng Y., 2021. Biological removal of deltamethrin in contaminated water, soils and vegetables by *Stenotrophomonas maltophilia* XQ08. Chemosphere. 279, 130622. doi: 10.1016/j.chemosphere.2021.130622.

3. Ullah S., Li Z., Ul Arifeen M. Z., Khan S.U., Fahad S., 2019. Multiple biomarkers based appraisal of deltamethrin induced toxicity in silver carp (*Hypophthalmichthys molitrix*). Chemosphere. 214, 519-533. doi:10.1016/j.chemosphere.2018.09.145.

4. Bamber S., Rundberget J.T., Kringstad A., Bechmann R.K. 2021. Effects of simulated environmental discharges of the salmon lice pesticides deltamethrin and azamethiphos on the swimming behaviour and survival of adult Northern shrimp (*Pandalus borealis*) Aquatic Toxicology. 240, 105966. doi: 10.1016/j.aquatox.2021.105966.

5. Bothe S.N., Lampert A., 2021. The insecticide deltamethrin enhances sodium channel slow inactivation of human Nav1.9, Nav1.8 and Nav1.7. Toxicology and Applied Pharmacology. 428, 115676. doi: 10.1016/j.taap.2021.115676.

6. Kong Y., Li M., Shan X., Wang G., Han G., 2021. Effects of deltamethrin subacute exposure in snakehead fish, *Channa argus*: Biochemicals, antioxidants and immune responses. Ecotoxicology and Environmental Safety. 209, 111821. doi: 10.1016/j.ecoenv.2020.111821.

7. Li M., Liu X., Feng X., 2019. Cardiovascular toxicity and anxiety-like behavior induced by deltamethrin in zebrafish (*Danio rerio*) larvae. Chemosphere. 219, 155-164. doi:10.1016/j.chemosphere.2018.12.011.

8. Zhang L., Hong X., Zhao X., Yan S., Ma X., Zha J., 2020. Exposure to environmentally relevant concentrations of deltamethrin renders the Chinese rare minnow (*Gobiocypris rarus*) vulnerable to Pseudomonas fluorescens infection. Science of The Total Environment. 715, 136943. doi: 10.1016/j.scitotenv.2020.136943.

9. Zhou S., Dong J., Liu Y., Yang Q., Xu N., Yang Y., Ai X., 2021. Effects of acute deltamethrin exposure on kidney transcriptome and intestinal microbiota in goldfish (*Carassius auratus*). Ecotoxicology and Environmental Safety. 225, 112716. doi: 10.1016/j.ecoenv.2021.112716.

10. Jijie R., Solcan G., Nicoara M., Micu D., Strungaru S.A., 2020. Antagonistic effects in zebrafish (*Danio rerio*) behavior and oxidative stress induced by toxic metals and deltamethrin acute exposure. Science of The Total Environment. 698, 134299. doi: 10.1016/j.scitotenv.2019.134299.

11. Wu Y., Li W., Yuan M., Liu X., 2020. The synthetic pyrethroid deltamethrin impairs zebrafish (*Danio rerio*) swim bladder development. Science of the Total Environment. 701, doi:10.1016/j.scitotenv.2019.134870.

12. Salako A.F., Amaeze N.H., Shobajo H.M., Osuala F.I. 2020. Comparative acute toxicity of three pyrethroids (Deltamethrin, cypermethrin and lambda-cyhalothrin) on guppy fish (*Poecilia reticulata peters*, 1859). Scientific African. 9, e00504. doi: 10.1016/j.sciaf.2020.e00504.

13. El-Sayed Y.S., Saad T.T., 2008. Subacute intoxication of a deltamethrin-based preparation (butox® 5% EC) in monosex nile tilapia, *Oreochromis niloticus* L. Basic and Clinical Pharmacology and Toxicology. 102(3), 293-299. doi:10.1111/j.1742-7843.2007.

14. Singh S., Tiwari R.K., Pandey R.S., 2018. Evaluation of acute toxicity of triazophos and deltamethrin and their inhibitory effect on AChE activity in *Channa punctatus*. Toxicology Reports. 5, 85-89. doi: 10.1016/j.toxrep.2017.12.006.

15. Eni G., Ibor O.R., Andem A.B., Oku E.E., Chukwuka A. V., Adeogun A. O., Arukwe A. 2019. Biochemical and endocrine-disrupting effects in *Clarias gariepinus* exposed to the synthetic pyrethroids, cypermethrin and deltamethrin. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 225, 108584. doi: 10.1016/j.cbpc.2019.108584.

16. Vani T., Saharan N., Mukherjee S. C., Ranjan R., Kumar R., Brahmchari P. K. 2011. Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of vitamin C. Pesticide Biochemistry and Physiology. 101(1), 16-20. doi: 10.1016/j.pestbp.2011.05.007.

17. Kaur M., Atif F., Ansari R.A., Ahmad F., Raisuddin S., 2011. The interactive effect of elevated temperature on deltamethrin-induced biochemical stress responses in *Channa punctata* Bloch. Chemico-Biological Interactions. 193(3), 216-224. doi: 10.1016/j.cbi.2011.06.011.

18. Kong Y., Li M., Guo G., Yu L., Sun L., Yin Z., Li R., Chen X., Wang G., 2021. Effects of dietary curcumin inhibit deltamethrin-induced oxidative stress, inflammation and cell apoptosis in *Channa argus* via Nrf2 and NF-κB signaling pathways. Aquaculture. 540, 736744. doi: 10.1016/j.aquaculture.2021.736744.

19. Jiang Q., Ao S., Ji P., Zhou Y., Tang H., Zhou L., Zhang X., 2021. Assessment of deltamethrin toxicity in Macrobrachium nipponense based on histopathology, oxidative stress and immunity damage. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 246, 109040. doi: 10.1016/j.cbpc.2021.109040.

20. Hong Y., Yang X., Huang Y., Yan G., Cheng Y., 2018. Oxidative stress and genotoxic effect of deltamethrin exposure on the Chinese mitten crab, *Eriocheir sinensis*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 212, 25-33. doi: 10.1016/j.cbpc.2018.06.004.

21. Arslan H., Altun S., Özdemir S., 2017. Acute toxication of deltamethrin results in activation of iNOS, 8-OHdG and up-regulation of caspase 3, iNOS gene expression in common carp (*Cyprinus carpio* L.). Aquatic Toxicology. 187, 90-99. doi: 10.1016/j.aquatox.2017.03.014.

22. Moss D.V., Henderson A.R., 1999. Clinical enzymology," in Tietz Textbook of Clinical Chemistry. 3rd ed., C. A. Burtis and E. R. Ashwood, Eds., Philadelphia, W.B. Saunders Company. 617-721.

23. Johnson G., Moore S.W. 2012. Why has butyrylcholinesterase been retained? structural and functional diversification in a duplicated gene. Neurochemistry International. 61(5), 783-797. doi:10.1016/j.neuint.2012.06.016.

24. Sacks D.B., 1999. Carbohydrates, in *Tietz Textbook of Clinical Chemistry. 3rd ed*, C. A. Burtis and E. R. Ashwood, Eds., Philadelphia, W.B. Saunders Company. 766-785.

25. Rifai N., Bachorik P.S., Albers J.J., 1999. Lipids, lipoproteins and apolipoproteins, in Tietz Textbook of Clinical Chemistry (3rd Edition), Philadelphia, W.B. Saunders Company. 809-861.

26. Johnson A.M., Rohlfs E.M., Silverman L.M., 1999. Proteins, in Tietz Textbook of Clinical Chemistry. 3rd Ed, Philadelphia, W.B. Saunders Company,.77-540.

27. Hamidipoor F., Pourkhabbaz H.R., Banaee M., Javanmardi S., 2015. Sub-lethal toxic effects of deltamethrin on blood biochemical parameters of japanese quail, *Coturnix japonica*. Toxicological and Environmental Chemistry. 97(9), 1217-1225.

28. Góth L.A., 1991. Simple method for determination of serum catalase and revision of reference range. Clinica Chimica Acta, 196,143-152.

29. Benzie I., Strain J., 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power: The FRAP Assay. Analytical Biochemistry. 239, 70-76.

30. Banihashemi E.A., Soltanian S., Gholamhosseini A., Banaee M., 2022. Effect of microplastics on yersinia ruckeri infection in rainbow trout (*Oncorhynchus mykiss*). Environmental Science and Pollution Research. 29, 11939–11950

31 Ibrahim A.T.A., Banaee M., Sureda A., 2019. Selenium protection against mercury toxicity on the male reproductive system of *Clarias gariepinus*. Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology. 225, 108583.

32. Lu Q., Sun Y., Ares I., Anadón A., Martínez M., Martínez-Larrañaga M.R., Yuan Z., Wang X., Martínez M.A. 2019. Deltamethrin toxicity: A review of oxidative stress and metabolism. Environmental Research. 170, 260-281, doi: 10.1016/j.envres.2018.12.045.

33. Geetha N., 2021. Mitigatory role of butyrylcholinesterase in freshwater fish *Labeo rohita*

exposed to glyphosate based herbicide Roundup®. Materials Today: Proceedings. 47(9), 2030-2035. doi: 10.1016/j.matpr.2021.04.281, 2021.

34. Ibrahim A.T.A., Banaee M., Sureda A., 2021. Genotoxicity, oxidative stress, and biochemical biomarkers of exposure to green synthesized cadmium nanoparticles in *Oreochromis niloticus* (L.). Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology. 242, 108942. doi:10.1016/j.cbpc.2020.108.

35. Hatami M., Banaee M., Nematdoost Haghi B., 2019. Sub-lethal toxicity of chlorpyrifos alone and in combination with polyethylene glycol to common carp (*Cyprinus carpio*). Chemosphere. 219: 981-988. doi: 10.1016/j.chemosphere.2018.12.077.

36. Rezaei Shadegan M., Banaee M., 2018. Effects of dimethoate alone and in combination with Bacilar fertilizer on oxidative stress in common carp, *Cyprinus carpio*. Chemosphere. 208, 101-107.

37. Soltanian S., Gholamhosseini A., Banaee M., 2021. Effects of exposure to a therapeutic level of methylene blue on antioxidant capacity, haemato-immunological responses and resistance of goldfish, *Carassius auratus* to *Aeromonas hydrophila*. Aquaculture Research. 52(6), 2640-2650.

38. Hayat N.M., Sabullah M.K., Shukor M.Y., Syed M.A., Daha-Lan F.A., Khalil K.A., Ahmad S.A., 2014. Effect of pesticides on cholinesterase activity by using fish as a biomarker. Nanobiology Bionanotechnology. 1, 17-25.

39. Salles J.B., Cunha Bastos V.L.F., Silva Filho M.V., Machado O.L.T., Salles C.M.C., Giovanni De Simone S., Cunha Bastos J. 2006. A novel butyrylcholinesterase from serum of *Leporinus macrocephalus*, a neotropical fish. Biochimie. 88(1), 59-68. doi:10.1016/j.biochi.2005.06.017.

40. Banaee M., Akhlaghi M., Soltanian S., Sureda A., Gholamhosseini A., Rakhshaninejad M., 2020. Combined effects of exposure to sub-lethal concentration of the insecticide chlorpyrifos and the herbicide glyphosate on the biochemical changes in the freshwater crayfish *Pontastacus leptodactylus*. Ecotoxicology. 29(9), 1500-1515.

41. Banaee M., Gholamhosseini A., Sureda A., Soltanian S., Fereidouni M.S., Ibrahim A.T.A., 2021. Effects of microplastic exposure on the blood biochemical parameters in the pond turtle (*Emys orbicularis*). Environmental Science and Pollution Research. 28(8), 9221 - 9234.

42. Hamidi S., Banaee M., Pourkhabbaz H.R., Sureda A., Khodadoust S., Pourkhabbaz A.R., 2022. Effect of petroleum wastewater treated with gravity separation and magnetite nanoparticles adsorption methods on the blood biochemical response of mrigal fish (*Cirrhinus cirrhosus*). Environmental Science and Pollution Research. 29, 3718-3732. doi:10.1007/s11356-021-15106-8.

43. Dawood M.A.O., Abdo S.E., Gewaily M.S., Moustafa E.M., SaadAllah M.S., AbdEl-kader M.F., Hamouda A.H., Omar A.A., Alwakeel R.A., 2020. The influence of dietary β-glucan on immune, transcriptomic, inflammatory and histopathology disorders caused by deltamethrin toxicity in Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology. 98, 301-311. doi: 10.1016/j.fsi.2020.01.035.

44. Sayed A.E.D.H., AbdAllah E.A., Hamed M., Soliman H.A.M., 2020. Hepato-nephrotoxicity in late juvenile of *Oreochromis niloticus* exposed to gibberellic acid: Ameliorative effect of *Spirulina platensis*. Pesticide Biochemistry and Physiology. 167, 104600.

45. Dawood M.A.O., Moustafa E.M., Gewaily M.S., Abdo S.E., AbdEl-kader M.F., SaadAllah M.S., Hamouda A.H., 2020. Ameliorative effects of *Lactobacillus plantarum* L-137 on Nile tilapia (*Oreochromis niloticus*) exposed to deltamethrin toxicity in rearing water. Aquatic Toxicology. 219, 105377. doi: 10.1016/j.aquatox.2019.105377.

46. Banaee M., Tahery S., Nematdoost Haghi B., Shahafve S., Vaziriyan M., 2019. Blood biochemical changes in common carp (*Cyprinus carpio*) upon co-exposure to titanium dioxide nanoparticles and paraquat. Iranian Journal of Fisheries Sciences. 18(2), 242-255. doi:10.22092/ijfs.2019.118174.

47. Farag M.R., Alagawany M., Khalil S.R., Abd El-Aziz R.M., Zaglool A.W., Moselhy A.A.A., Abou-Zeid S.M., 2022. Effect of parsley essential oil on digestive enzymes, intestinal morphometry, blood chemistry and stress-related genes in liver of Nile tilapia fish exposed to Bifenthrin. Aquaculture. 546, 737322.

48. Rahman A.N.A., Mohamed A.A.R., Mohammed H.H., Elseddawy N.M., Salem G.A., El-Ghareeb W.R., 2020. The ameliorative role of geranium (*Pelargonium graveolens*)

essential oil against hepato-renal toxicity, immunosuppression, and oxidative stress of profenofos in common carp, *Cyrinus carpio* (L.). Aquaculture. 517, 734777. doi: 10.1016/j.aquaculture.2019.734777.

49. Hong Y., Huang Y., Yan G., Huang Z., 2019. Effects of deltamethrin on the antioxidant defense and heat shock protein expression in Chinese mitten crab, *Eriocheir sinensis*. Environmental Toxicology and Pharmacology. 66,1-6.

50. Elia A.C., Giorda F., Pacini N., Dorr A.J.M., Scanzio T., Prearo M., 2017. Subacute toxicity effects of deltamethrin on oxidative stress markers in rainbow trout. Journal of Aquatic Animal Health. 29(3), 165-172.

51. Capoluongo E., Giardina B., Minucci A., 2013. Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency," in *Brenner's Encyclopedia of Genetics (Second Edition)*, K. H. Stanley Maloy, Ed., Academic Press. 2013, 340-342. doi: 10.1016/B978-0-12-374984-0.00569-6.

52. Ciftci M., Turkoglu V., Coban T.A., 2007. Effects of some drugs on hepatic glucose 6-phosphate dehydrogenase activity in Lake Van fish (*Chalcalburnus tarischii Pallas*, 1811). Journal of Hazardous Materials. 142(1-2), 415-418. doi: 10.1016/j.jhazmat.2006.09.053.

53. Banaee M., Sureda A., Shahaf S., Fazilat N., 2015. Protective Effects of Silymarin Extract on Malthion-Induced Zebra Cichlid (*Cichlasoma nigrofasciatum*) Hepatotoxicity. Iranian Journal of Toxicology. 9(28), 1239-1246, 2015.

54. Ceyhun S.B., Şentürk M., Ekinci D., Erdoğan O., Çiltaş A., Kocaman E.M., 2010. Deltamethrin attenuates antioxidant defense system and induces the expression of heat shock protein 70 in rainbow trout. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 152(2), 215-223.

55. Sayeed I., Parvez S., Pandey S., Bin-Hafeez B., Haque R., Raisuddin S., 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicology and Environmental Safety. 56(2), 295-301. doi: 10.1016/S0147-6513(03)00009-5.

56. Radovanović T.B., Gavrilović B.R., Petrović T.G., Despotović S.G, Gavrić J.P., Kijanović A., Mirč M., Kolarov N.T., Faggio C., Prokić M.D., 2021. Impact of desiccation pre-exposure on deltamethrin-induced oxidative stress in *Bombina variegata* juveniles. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 250, 109191.

57. Dorts J., Silvestre F., Tu H. T., Tyberghein A. E., Phuong N. T., Kestemont P. 2009. Oxidative stress, protein carbonylation and heat shock proteins in the black tiger shrimp, *Penaeus monodon*, following exposure to endosulfan and deltamethrin. Environmental Toxicology and Pharmacology, 28(2), 302-310.

58. Sharifinasab Z., Banaee M., Mohiseni M., Noori A., 2016. Vitamin C and Chitosan Alleviate Toxic Effects of Paraquat on Some Biochemical Parameters in Hepatocytes of Common Carp. Iranian Journal of Toxicology. 10(1), 31-40.

59. Banaee M., Sureda A., Taheri S., Hedayatzadeh F., 2019. Sub-lethal effects of dimethoate alone and in combination with cadmium on biochemical parameters in freshwater snail, *Galba truncatula*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 220, 62-70.

60. Paul T., Shukla S.P., Kumar K., Poojary N., Kumar S., 2019. Effect of temperature on triclosan toxicity in Pangasianodon hypophthalmus (Sauvage, 1878): Hematology, biochemistry and genotoxicity evaluation. Science of The Total Environment, 668, 104-114. doi: 10.1016/j.scitotenv.2019.02.443.

61. Tripathi G., Shasmal J., 2011. Concentration related responses of chlorpyriphos in antioxidant, anaerobic and protein synthesizing machinery of the freshwater fish, *Heteropneustes fossilis*. Pesticide Biochemistry and Physiology. 99(3), 215-220.

62. Crupkin A.C., Fulvi A.B., Iturburu F.G., Medici S., Mendieta J., Panzeri A.M., Menone M.L., 2021. Evaluation of hematological parameters, oxidative stress and DNA damage in the cichlid *Australoheros facetus* exposed to the fungicide azoxystrobin. Ecotoxicology and Environmental Safety. 207, 111286. doi: 10.1016/j.ecoenv.2020.111286.