OXIDATIVE STRESS BIOMARKERS OF EXPOSURE TO DELTAMETHRIN IN RAINBOW TROUT FRY (*Oncorhynchus mykiss*)

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ABSTRACT

Deltamethrin (DM) is a widely used pesticide based on pyrethroids, and is reported to be extremely toxic to fish species. We investigated the effect of DM concentrations (0.010, 0.0125 and 0.0250 µg/L) on antioxidants in rainbow trout, *Oncorhynchus mykiss*, using standard laboratory conditions. 24 h exposure caused whole body induction of various antioxidant enzymes and nonenzymatic antioxidants. Results showed that DM significantly ($p< 0.05$) increased malondialdehyde (MDA) levels. Catalase (CAT) activity decreased in all experimental groups. Glutathione (GSH) was significantly ($p<0.05$) increased in the group with DM concentration of 0.0250 µg/L DM for 24 h. The activity of glutathione peroxidase (GSH-Px) was found to be statistical significantly ($p<0.05$) decreased in all other experimental groups except the 0.010 µg/L DM treated group. The parameters studied in this investigation can also be used as biomarkers of exposure to DM. It is suggested that appropriate ecotoxicological risk assessments should be made wherever DM is proposed for use in pest control activities.

KEYWORD: Deltamethrin, pesticide, rainbow trout, oxidative stress, antioxidant system

1. INTRODUCTION

The increasing use of synthetic pesticides is amplifying worldwide pollution risks. Pesticides are toxic and designed to kill unwanted organisms, but when applied to the land they can be washed into surface waters and kill or adversely influence the life of aquatic organisms [1,2]. Because of their beneficial qualities, synthetic pyrethroids, such as DM, are popular with farmers and health departments for use in pest control. However, these compounds are generally found to be highly toxic to fish [3-5].

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Oxidative stress and reactive oxygen species (ROS)-mediated toxicity have long been considered as the mechanisms responsible for DM-induced organ injury in mammals [6-8]. Effects of DM and other synthetic pesticides on lipid peroxidation (LPO) and antioxidant defense systems in fishes are reported [9-13].

The aim of this study is to comparatively investigate the effect of DM on the activities of CAT, GSH-Px and GSH and the content of MDA in the whole body tissue of rainbow trout. The relationship between antioxidant and LPO against oxidative stress in insecticides will also be investigated.

2. MATERIALS AND METHODS

Acute bioassays with DM were carried out with fry (0.27±0.06 g). Fish were kindly supplied by the Keban Fish Breeding Unit of IX. Region Directorate, the State Hydraulic Works in Turkey. Fish were kept for at least 2 weeks in 50 L glass tanks filled with continuous aeration, at a water temperature of 13-14 °C before experimental use. They were fed *ad libitum* with commercial food pellets. Experiments were conducted in static tests in 5 L glass aquaria. Before the test, fish were transferred to aquaria filled with 3 L of tap water. Experiments were done in duplicate: each trial containing 10 fish corresponds to 20 fish for each group. Treatments were a tap water control, and three DM concentrations (0.010, 0.0125 and 0.0250 µg/L). The test period was 24 h.

Water quality characteristics in the control units were determined according to APHA [14]. Dissolved oxygen and pH were determined by a digital oxygen meter and a pH meter. The mean quality parameters of water used for preparation of test solutions were as follows: dissolved oxygen 8.4 ± 0.4 mg L-1, pH 7.2 ± 0.2, alkalinity 137 ± 09 mg L^{-1} and total hardness 184 ± 13 mg L^{-1} as $CaCO_3$.

Fish were not fed during the test period. At the end of the test, fish were anaesthetized in icecold chilled water

and whole bodies were isolated for biomarker analysis. After rinsing with cold 0.09% NaCl solution and filtering to remove fluid, the fry samples were weighed meticulously. The homogenization was carried out in a Teflon-glass homogenizer with a buffer containing 1.15% KCl, to obtain 1:10 (w/v) whole homogenate. The homogenates were centrifuged at 18.000×*g* for 30 min at 4 °C to determine malondialdehyde (MDA) and reduced glutathione (GSH) concentrations, catalase (CAT) and glutathione peroxidase (GSH-Px) activities.

The concentrations of MDA as indices of oxidative stress were determined according to a modified method of Placer et al. [15] based on the reaction with thiobarbituric acid, and were expressed as nmol/g protein. CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi [16], and was expressed as k/g protein, where k is the first-order rate constant. GSH concentration was measured by a kinetic assay using a dithionitrobenzoic acid recycling method described by Ellman [17] and was expressed as µmol/g protein. GSH-Px activity was determined by the procedure described by Beutler [18]. The procedure of analysis performed was based on the oxidation of GSH by GSH-Px coupled to the disappearance of NADPH by glutathione reductase measured at 37 °C and 340 nm and were expressed as U/g protein. Protein concentrations were measured according to Lowry et al. [19].

The parametric test for ANOVA was used to compare the groups and applied considering $p < 0.05$. Each value was expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

DM exposure elicited hyperactivity characterized by opercula movement, erratic swimming, loss of equilibrium, hanging in the water vertically and gasping for air. However, no fish died after the 24h period of exposure to different DM doses.

The effects of the acute DM exposure on MDA in rainbow trout are presented in Table 1, where it can be seen that MDA levels were significantly ($p < 0.05$) increased. The increases in the mean values of MDA in experimental groups were 42.64%, 32.50%, and 47.48%, respectively, over control values.

Changes in CAT activity in rainbow trout are shown in Table. 1. The enzyme activities at all the concentrations were moderately decreased from those in the control group. In all the DM treated groups, CAT activity was not significantly ($p > 0.05$) induced, with an approximate 19.36% decrease in the 0.0250 µg/L DM group compared with the control group.

Changes in GSH activity are shown in Table 1. In all experimental groups, the GSH level was higher than that in control fish. With DM concentration increasing, GSH activity increased significantly ($p < 0.05$), reaching a maximum of approximately 28.10% at 0.0250 µg/L DM treatment compared to the control group.

Treatment of rainbow trout with DM caused a dose-dependent change in GSH-Px activity. These data are shown in Table 1. GSH-Px activities were continuously induced at 0.0125 and 0.0250 µg/L of DM concentrations showing a decrease ($p < 0.05$).

In our study, DM exposure elicited hyperactivity characterized by opercula movement, erratic swimming, loss of equilibrium, hanging in the water vertically and gasping for air. Similar findings in rainbow trout and other fish species given DM treated have been seen [20-22]. Stress responses are identified via various changes, including behavioral responses, clinical toxic symptoms and, primarily, the biochemical indicators. The abnormal behavioral responses were predominantly respiratory and nervous manifestations, ascribed to the neurotoxic effect of the DM [23]. Respiratory distress is one of the early symptoms of pyrethroid poisoning. The clinically observed toxic signs in fish may be attributed to the irritant and inflammatory effects of DM [24, 25]. Comparable clinical signs have been observed in fish species exposed to cypermethrin, a synthetic pyrethroid [26-28].

LPO may be due to oxidation of molecular oxygen to produce superoxide radicals. This reaction is also the source of H_2O_2, which causes the production of MDA by initiating the peroxidation of unsaturated fatty acids in the membrane. Both H_2O_2 and O^{-2} produce highly reactive hydroxyl radicals in the Haber-Weiss reaction. The hydroxyl radical can initiate lipid peroxidation, which is a free radical chain leading to loss of membrane structure and function [29,30].

LPO has been extensively used as a marker for oxidative damage induced in fish tissues by water pollutants [31]. The delay of significant LPO induction in both the liver and intestine of fish presumably results from the compensatory response of the antioxidant defense systems following acute DM exposure. In this study, the relevant

TABLE 1 - Biochemical parameters of control and experimental groups of fish exposed to 0.010, 0.0125 and 0.0250 µg/L deltamethrin for 24 h.

Parameters	Control	0.010 µg/L	0.0125 µg/L	0.0250 µg/L
MDA (nmol/g protein)	156.90 ± 32.8	223.40 ± 45.9[a]	207.90 ± 34.6[a]	231.40 ± 47.2[a]
CAT (k/g protein)	3.08 ± 0.67	2.99 ± 0.27	2.79 ± 0.45	2.49 ± 0.55
GSH (µmol/g protein)	18.46 ± 1.68	22.70 ± 5.75	20.62 ± 3.86	23.65 ± 1.76[a]
GSH-Px (U/g protein)	6.87 ± 0.63	6.10 ± 0.50	5.49 ± 0.55[a]	4.16 ± 0.16[a, b, c]

a: control; b: 0.010 µg/L DM; c: 0.0125 µg/L DM; d: 0.0250 µg/L DM groups, $p < 0.05$

induction of LPO observed after 24 h in the whole body of rainbow trout fry suggests an oxidative stress condition due to over-accumulation of ROS. A similar response was found in fish species exposed subchronically to industrial pollutants [32]. The oxidative stress causes injury to biomolecules such as nucleic acids, proteins, structural carbohydrates and lipids [33]. Among these targets, the peroxidation of lipids is basically damaging because the formation of LPO products generates a cascade of free radical reactions [34]. As a result, LPO can greatly alter the physicochemical properties of membrane lipid bilayers, resulting in severe cellular dysfunction. In addition, LPO products might react with some amino-acid side-chains of proteins or with reducing sugars or their oxidation products [35]. The development of oxidative stress conditions in different tissues following DM exposure has therefore been suggested as a main cause of toxicity in freshwater fish *Channa punctatus* [10]. MDA has been shown to increase in plasma and some tissues in DM and in other synthetic pyrethroid toxicities [9-13]. Our study found similar results to the latter study on increased MDA levels in the whole body of rainbow trout.

We found that CAT levels in all experimental groups were lower than those in the control group, but there was no significant difference between these groups. This decrease in catalase activity could be due to the flux of superoxide radicals, which have been reported to inhibit CAT activity [36]. Sayeed et al. [10] have also reported the LPO inducing effect of DM, a synthetic pyrethroid, in all tissues in a freshwater fish, *Channa punctatus*, together with decreased CAT activity. In our present study, we also found decreases in whole body CAT activity in DM-treated rainbow trout.

GSH activities increased in all experimental groups with the exception of the control group. The GSH activities in rainbow trout fry receiving 0.0125 and 0.0250 µg/L DM were higher than in the 0.010 µg/L DM group, but there was no significant difference between these three groups. However, there was a statistically significant difference between the 0.0250 µg/L DM group and the control group. Increased GSH has been described as one of the protective mechanisms that fish adopt in the initial phases of exposure to aquatic pollutants [37,38]. We believe these findings indicate a moderately increased response to oxidative stress in the 0.0250 µg/L DM treated group.

Our study indicates that GSH-Px activity decreased in all the experimental groups compared to the control. This is likely to be the result of oxidative stress. GSH-Px is involved in protecting cytosol and plasma membrane from LPO [39]. In fact, this enzyme transforms lipid hydroperoxides produced at the membrane level into less reactive species. GSH-Px activity was reduced after 24 h DM exposure. Our data are in accordance with reports on *Channa punctatus* in which reduced GSH-Px activity was observed in the gills of DM exposed fish [10]. The lower enzymatic activity of GSH-Px in our study could indicate facilitation of increased MDA due to the lack of the protective effect of this antioxidant enzyme. The decreased activity of GSH-Px may be the result of O^{-}_{2} production [40] or a direct action of pesticides on the synthesis of the enzyme [41]. Oxidative stress causes an elevation in GSH-Px activity, which probably reflects an adaptation to the oxidative conditions to which the fish has been exposed [42]. Sayeed et al. [10] reported increased GSH-Px levels in the liver and kidney of *Channa punctatus* exposed to DM. However in this case, GSH-Px levels were depleted in the gills. Consequently, in this study, whole body MDA levels were high in rainbow trout fry after 24 h exposure. GSH is responsible for cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes, including the GSH peroxidases [43].

This study showed antioxidant system alterations in the whole body of rainbow trout exposed to DM. The activities of CAT and GSH-PX, the most sensitive enzymes to ROS production in aquatic organisms, decreased after pesticide administration. The parameters studied in this investigation could be used as biomarkers of exposure to DM. However, the induction pattern of antioxidant enzymes under long-term and high dose exposure conditions needs to be investigated.

REFERENCES

[1] Hayes, W.J. and Laws, E.R. (1991). Handbook of Pesticide Toxicology. Academic Press, San Diego, 1576 p.

[2] Rand, G.M. (1995). Fundamentals of Aquatic Toxicology. Taylor and Francis Publishing, Washington, DC.

[3] Coats, J.R. and O'Donnell-Jeffrey, N.L. (1979). Toxicity of four synthetic pyrethroid insecticides to rainbow trout. Bulletin of Environmental Contamination and Toxicology, 23: 250–255.

[4] Bradbury, S.P. and Coats, J.R. (1989). Toxicological and toxicodynamics of pyrethroid insecticide in fish. Environmental Toxicology and Chemistry, 8: 373–386.

[5] Szegletes, T., Polyhos, C.S., Balint, T., Rady, A.A., Lang, G., Kufcsak, O. and Nemcsok, J. (1995). In vivo effects of deltamethrin on some biochemical parameters of carp (*Cyprinus carpio* L.). Environmental Monitoring and Assessment, 78: 1–15.

[6] Rehman, H., Ali, M., Atif, F., Kaur, M., Bhatia, K. and Raisuddin, S. (2006). The modulatory effect of deltamethrin on antioxidants in mice. Clinica Chemica Acta, 369: 61–65.

[7] Yousef, M.I., Awad, T.I. and Mohamed, E.H. (2006). Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. Toxicology, 227: 240–247.

[8] Tuzmen, N., Candan, N., Kaya, E. and Demiryas, N. (2008). Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver. Cell Biochemistry and Function, 26: 119–124.

[9] Uner, N., Oruc, O., Canli, M. and Sevgiler, Y.M. (2001). Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* (L.). Bulletin of Environmental Contamination and Toxicology, 67: 657–664.

[10] Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R. and Raisuddin, S. (2003). Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicology and Environmental Safety, 56: 295–301.

[11] Parvez, S. and Raisuddin, S. (2006). Copper modulates non-enzymatic antioxidants in the freshwater fish *Channa punctata* (Bloch) exposed to deltamethrin. Chemosphere, 62: 1324–1332.

[12] Harper, H.E., Pennington, P.L., Hoguet, J. and Fulton, M.H. (2008). Lethal and sublethal effects of the pyrethroid, bifenthrin, on grass shrimp (*Palaemonetes pugio*) and sheepshead minnow (*Cyprinodon variegatus*). Journal of Environmental Science and Health Part B, 43: 476–483.

[13] Sepici-Dinçel, A., Karasu Benli, A.C., Selvi, M., Sarıkaya, R., Şahin, D., Özkul, I.A. and Erkoç, F. (2009). Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: Biochemical, hematological, histopathological alterations. Ecotoxicology and Environmental Safety, 72: 1433–1439.

[14] APHA (American Public Health Association). (1985). Standard methods for the examination of water and wastewater, 16th edn. American Public Health Association, Washington, DC.

[15] Placer, Z.A., Cushman, L. and Johnson, B.C. (1966). Estimation of products of lipid peroxidation (Malonyl dialdehyde) in biological fluids. Analytical Biochemistry, 16: 359–364.

[16] Aebi, H. (1983). Catalase. In: Bergmeyer HU (Ed), Methods in Enzymatic Analysis. Academic Press, New York, 276–286 pp.

[17] Ellman, G.L. (1959). Tissue sulphydryl groups. Archives of Biochemistry and Biophysics, 82: 70–77.

[18] Beutler, E. (1975). Red cell metabolism. In: A Manual of Biochemical Methods, Grune Strottan, New York, 67–69 pp.

[19] Lowry, O.H., Rosenberough, N.J., Farr, A.L. and Randal, R.J. (1951). Protein measurement with folinphenol reagent. Journal of Biochemistry, 193: 265–275.

[20] Viran, R., Ünlü Erkoç, F., Polat, H. and Koçak, Ö. (2003). Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). Ecotoxicology and Environmental Safety, 55 (1): 82–85.

[21] Ural, M.Ş. and Sağlam, N. (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). Pesticide Biochemistry and Physiology, 83: 124–131.

[22] Yildirim, M.Z., Karasu Benli, A.C., Selvi, M., Ozkul, A., Erkoc, F. and Kocak, O. (2006). Acute toxicity, behavioral changes, and histopathological effects of deltamethrin on tissues (gills, liver, brain, spleen, kidney, muscle, skin) of Nile tilapia (*Oreochromis niloticus* L.) fingerlings. Environmental Toxicology, 21(6): 614–620.

[23] Golow, A.A. and Godzi, T.A. (1994). Acute toxicity of deltamethrin and dieldrin to *Oreochromis niloticus* (LIN). Bulletin of Environmental Contamination and Toxicology, 52: 351–354.

[24] El-Sayed, Y.S., Saad, T.T. and El-Bahr, S.M. (2007). Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and haematological effects. Environmental Toxicology and Pharmacology, 24: 212–217.

[25] WHO (World Health Organization). (1999). Report of the Third WHOPES Working Group Meeting. Review of Deltamethrin 1% SC and 25% WT Etofeprox 10% EC and 10% EW, 23–24 September, Geneva, Switzerland.

[26] Polat, H., Erkoc, F.U., Viran, R. and Kocak, O. (2002). Investigation of acute toxicity of beta-cypermethrin on guppies *Poecilia reticulata*. Chemosphere, 49: 39–44.

[27] Velisek, J., Wlasow, T., Gomulka, P., Svobodova, Z., Dobsikova, R., Novotny, L. and Dudzik, M. (2006). Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). Veterinarni Medicina, 51(10): 469–476.

[28] Sarıkaya, R. (2009). Investigation of acute toxicity of alpha-cypermethrin on adult Nile Tilapia (*Oreochromis niloticus* L.). Turkish Journal of Fisheries and Aquatic Sciences, 9: 85–89.

[29] Ray, D.E. (1991). Pesticides derived from plants and other organism. In: Hayes, W.Y. Jr. and Lows, E.R. Jr. (eds), Handbook of Pesticides Toxicology. Academic Press, San Diego CA, 585–636 pp.

[30] Kale, M., Rathore, N., John, S. and Bhatnagar, D. (1999). Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicology Letters, 105: 197–205.

[31] Fatima, M., Ahmed, I., Sayeed, I., Achar, M. and Raisuddin, S. (2000). Polluant induced overactivation of phagocytes is concomitantly associated with peroxidation of phagocytes in fish tissue. Aquatic Toxicology, 49: 243–250.

[32] Lima, P.L., Benassi, J.C., Pedrosa, R.C., Dal Magro, J., Oliviera, T.B. and Wilhem-Filho, D. (2006). Time course variations of DNA damage and biomarkers of oxidative stress in tilapia (*Oreochromis niloticus*) exposed to effluents of a swine industry. Archives of Environmental Contamination and Toxicology, 50: 23–30.

[33] Sies, H. and Cadenas, E. (1985). Oxidative stress: damage to intact cells and organs. Philosophical Transactions of the ***Royal*** Society ***B***: ***Biological*** Sciences, 311: 617–631.

[34] Catalá, A. (2006). An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. The International Journal of Biochemistry & Cell Biology, 38: 1482–1495.

[35] Stadtman, E.R. and Levine, R.L. (2000). Protein oxidation. Annals of The New York Academy of Sciences, 899: 191–208.

[36] Kono,Y. and Fridovich, I. (1982). Superoxide radical inhibits catalase. Journal of Biological Chemistry, 257: 5751–5754.

[37] Hasspielar, B.M., Behar, J.V. and Di Giulio, R.T. (1994). Glutathione dependent defense in channel catfish (*Ictalurus punctatus*) and brown bullhead (*Ameiurus nebulosus*). Ecotoxicology and Environmental Safety, 28: 82–90.

[38] Stephensen, E., Sturve, J. and Forlin, L. (2002). Effects of redox cycling compounds on glutathione content and activity of glutathione related enzymes in rainbow trout liver. Comparative Biochemistry and Physiology Part C, 133: 435–442.

[39] Halliwell, B. and Gutteridge, J.M.C. (1989). Free Radical in Biology and Medicine. Clarendon Press, Oxford, 96–98 pp.

[40] Bagnasco, M., Camoirano, A., De Flora, S., Melodia, F. and Arillo, A. (1991). Enhanced liver metabolism of mutagens and carcinogens in fish living in polluted seawater. Mutation Research Letters, 262: 129–137.

[41] Bainy, A.C.D., Arisi, A.C.M., Azzalis, L.A., Simizu, K., Barios, S.B.M., Videla, L.A. and Jungueira, V.B.C. (1993). Differential effects of short-term lindane administration on parameters related to oxidative stress in rat liver and erythrocytes. Journal of Biochemical and Moleculer Toxicology, 8 (4): 187–194.

[42] Lenartova, V., Holovska, K., Pedrajas, J.R., Martinez-Lara, E., Peinado, J., Lopez-Barea, J., Rosival, I. and Kosuth, P. (1997). Antioxidant and detoxifying fish enzymes as biomarkers of river pollution. Biomarkers, 2(4): 247–252.

[43] Cathcart, R.F. (1985). Vitamin C: The nontoxic, nonrate-limited, antioxidant free radical scavenger. Medical Hypotheses, 18: 61–77.

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