Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Response of antioxidant system to formalin in the whole body of rainbow trout, *Oncorhynchus mykiss*

U. İspir[1], M. Kirici[2], M. E. Yonar[3*], S. Mişe Yonar[3]

[1] Inonu University, Fisheries Faculty, Battalgazi-Malatya, Turkey
[2] Bingol University, Agriculture Faculty, Department of Fisheries, Bingol, Turkey
[3] Firat University, Fisheries Faculty, Elazig, Turkey

Correspondence to: serpilmise@gmail.com
Received September 3, 2016; **Accepted** January 25, 2017; **Published** January 30, 2017
Doi: http://dx.doi.org/10.14715/cmb/2017.63.1.3

Abstract: Formalin bath treatments are widely used to control of parasitic infections in aquaculture. However, effects of formaldehyde on the lipid peroxidation and antioxidant system were not still need elucidation. Fish were exposed to formalin at doses of 50, 100 and 200 ppm for 1 h. Fish were then left to depurate for 24 h in formalin-free water. At the end of the test, whole bodies were isolated and homogenized to measured malondialdehyde (MDA) and reduced glutathione (GSH) levels and catalase (CAT) and glutathione peroxidase (GPx) activities. Results obtained showed that formalin significantly ($p < 0.05$) increased the MDA level. There was statistically significant decrease in the CAT activity of the experimental groups when compared to the control group. After recovery period, the CAT activity was still found to be lower than the control level. The GPx activity and GSH level decreased by formalin exposures and did not return to the control values during recovery periods. From the findings of our study, it can be interpreted that acute formalin inhalation may cause oxidative stress and thus, some secondary toxic effects in whole body.

Key words: Formalin; Rainbow trout; Oxidative stress; Antioxidant.

Introduction

Formalin is used predominantly as a chemical intermediate. It also has minor uses in agriculture, as an analytical reagent, in concrete and plaster additives, cosmetics, disinfectants, fumigants, photography, and wood preservation (1,2). Toxicological studies have suggested that Formalin is: causes hepatotoxicity, neurotoxicity; and induces the development of lung, nasopharyngeal and buccal cavity cancer in humans (3,4,5,6). Formalin is commonly used in the aquaculture industry against bacterial and parasitic infections of fish (7). This matter is an effective treatment against a number of fish pathogens including many external parasites like protozoans, and monogenetic trematodes.

Disinfectants can either increase or decrease lipid peroxidation and enzyme activities such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and protein content depending on the disinfectant type and concentration in the fish and the other animal species (8,9,10). In previous studies, oxidative stress caused by formalin was demonstrated in fish (11). But, no previous studies formalin related to oxidative stress mechanisms in the whole body of fry rainbow trout have been reported. In this study, therefore, was aimed at investigating the exposure to formalin in the fry rainbow trout (*Oncorhynchus mykiss*), as reflected in stress and health indicators; malondialdehyde (MDA) and reduced glutathione (GSH) levels and catalase (CAT) and glutatione peroxidase (GPx) activities.

Materials and Methods

Acute exposure to formalin were carried out with fry (0.38 ± 0.07 g). Fish were supplied from the Keban Fish Breeding Unit of IX. Region Directorate, the State Hydraulic Works in Turkey. Fish were kept for 2 week in 50 L glass tanks filled with continuous aeration, at water temperature (13-14 °C), before being used in experiments. The water quality characteristics were determined according to the APHA (12) guidelines. The mean quality parameters of water were as follows: dissolved oxygen 8.02 ± 0.5 mg/L , pH 7.3 ± 0.4, temperature 13 ± 2 °C . Fish were fed *ad libitum* with a commercial feed.

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. Fish exposed to three different concentrations of formalin (50 ppm; Group-A, 100 ppm; Group-B, and 200 ppm; Group-C) for 1 h. Fish were then left to depurate for 24 h (recovery) in formalin-free water. Control fish were sampled at the same intervals and were handled in the same way with treatment groups. After 1 h and 24 h , all of the fishes were sampled. The entire experiment was repeated two independent times; each replicate for each group contained 20 fish, for a total of 160 fish.

At the end of the experiments, 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 the MDA and GSH levels and the CAT and GPx activities.

The level of MDA as indices of oxidative stress was determined according to a modified method of Placer et al. (13) based on the reaction with thiobarbituric acid, and were expressed as nmol/mg protein. The CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (14), and was expressed as k/mg protein, where k is the first-order rate constant. The GPx activity was determined by the procedure described by Beutler (15). The procedure of analysis performed was based on the oxidation of GSH by GPx coupled to the disappearance of NADPH by GR measured at 37 °C and 340 nm and were expressed as U/mg protein. The GSH level was measured by a kinetic assay using a dithionitrobenzoic acid recycling method described by Ellman (16) and was expressed as µmol/mg protein. Protein concentrations were measured according to Lowry et al. (17).

The means (± SE) of assayed parameters were calculated for each group of fish. One-way ANOVA tests were to compare values from individual experimental fish groups with those from controls. Significant differences were based on the $p < 0.05$ level.

Results

The clinical signs of the fish in the control and experiment groups were noted. Behavioural changes were observable within the first few hours. In the control and the 50 and 100ppm concentrations of formalin groups there were no behavioral changes throughout the experiment. In another concentration (200 ppm), the fish showed some abnormal swimming and tended to gather at the surface. They tried to avoid the toxic water with fast swimming; the fish were observed to have breathing difficulties and tried to breathe air from the surface water. However, no fish died during the experiment period of exposure to different formalin doses.

The effects of formalin exposures on the MDA and GSH levels and the CAT and GPx activities in the control and experimental groups are presented in Table 1.

The MDA levels in fish exposed to three different concentrations of formalin for 1h were higher than in the control group. When the fish were transferred into formalin-free water after treatment, significant decreases in the MDA level were observed. After recovery period, the MDA levels were still found to be higher than the control level ($p < 0.05$).

The CAT activities after 1 h of formalin treatment at all the concentrations was lower than that in the control fish. When the fish were transferred into formalin-free water after treatment, significant increases in the CAT activity were observed. However, the CAT activity was still lower than the control level after recovery period ($p < 0.05$).

Treatment with formalin caused a dose-dependent change in the GPx activity. The GPx activity decreased by formalin exposures and did not return to the control values during recovery periods ($p < 0.05$).

Statistically significant differences in the GSH levels were determined between the control and treatment groups in all exposure concentrations and periods. The GSH levels decreased in the groups that exposed to different concentrations of formalin. After recovery period, the GSH levels were still found to be lower than the control level ($p < 0.05$).

Discussion

Clinical signs of acute toxicity, such as grouping and loss of equilibrium were observed during the study in rainbow trout exposed to formalin. Similar data of clinical signs have also been described in brown trout, *Salmo trutta*, (18), *Oncorhynchus mykiss* (19) and *Esomus danricus* (20). Santos et al. (21) documented the Amazon ornamental fish, bluespotted coridora (*Corydoras melanistius*) exposed to formalin concentrations showed two patterns of behaviour during the first 24 hours. Fish exposed to higher concentrations presented agitation, followed by erratic swimming and positioning on the water surface immediately after the addition of the substance. While the fishes submitted to the lower concentrations remained standing, making small movements and resting at bottom of containers. Our results for behavioural manifestations showed similarity to the results in previous studies.

Table 1. Oxidant/antioxidant changes in the whole body of *O. mykiss* exposed to formalin (1 h) and its recovery response (24h).

Para-meters	Control		Group-A		Group-B		Group-C	
	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
MDA	$1.47 \pm 0.25^{x,a}$	$1.48 \pm 0.22^{x,A}$	$2.25 \pm 0.80^{x,b}$	$1.88 \pm 0.54^{y,B}$	$2.31 \pm 0.78^{x,b}$	$1.93 \pm 81^{y,B}$	$2.81 \pm 0.42^{x,c}$	$2.29 \pm 0.68^{y,C}$
CAT	$2.93 \pm 0.20^{x,a}$	$2.84 \pm 0.16^{x,A}$	$2.04 \pm 0.39^{x,b}$	$2.25 \pm 0.51^{y,B}$	$2.05 \pm 0.35^{x,b}$	$2.28 \pm 0.40^{y,B}$	$2.08 \pm 0.66^{x,b}$	$2.30 \pm 0.34^{y,B}$
GPx	$6.14 \pm 0.66^{x,a}$	$6.17 \pm 0.89^{x,A}$	$4.23 \pm 0.66^{x,b}$	$5.18 \pm 0.74^{y,B}$	$3.57 \pm 0.51^{x,c}$	$4.36 \pm 0.47^{y,C}$	$3.34 \pm 0.33^{x,d}$	$4.32 \pm 0.45^{y,C}$
GSH	$18.22 \pm 2.47^{x,a}$	$17.98 \pm 2.43^{x,A}$	$14.53 \pm 1.74^{x,b}$	$14.86 \pm 2.23^{x,B}$	$13.74 \pm 1.55^{x,c}$	$14.67 \pm 2.91^{y,B}$	$12.79 \pm 1.66^{x,d}$	$12.90 \pm 1.38^{x,C}$

Group-A: 50 ppm formalin, Group-B: 100 ppm formalin, Group-C: 200 ppm formalin

MDA: malondialdehyde level (nmol/mg protein), CAT: catalase activity (k/mg protein, where k is the first-order rate constant), GPx: glutathione peroxidase activity (U/mg protein), GSH: reduced glutathione level (µmol/mg protein).

Each value is the mean ± standard error (n = 20).

^{x,y} in the same row indicate significant differences in the same group ($p < 0.05$).

^{a,b,c,d} in the same row indicate significant differences between groups for 1 h ($p < 0.05$).

^{A,B,C} in the same row indicate significant differences between groups for 24 h ($p < 0.05$).

Lipid peroxidation has been extensively used as a biomarkers of oxidative stress (22,23,24). MDA are produced by lipid peroxidation and considered as indicators of oxidative stres, which results from the free radicals damage to membrane complements of cells (25). The findings of this study showed that lipid peroxidation in whole body increased in the groups that exposed to different formalin concentrations when compared with the control group. Some investigators have reported an association between MDA and disinfectants-induced toxicity in fish and rat (26,27,28). In addition, Tkachenko et al. (9) demonstrated that MDA increased in fish heart tissue following formalin exposure. On the other hand, in a study carried out in rainbow trout, Yonar and Mişe Yonar (29) reported that malachite green exposure increased MDA levels in fish blood, liver, kidney, spleen and gills. Sreejai and Jaya (30) investigated the effect of bath administrator of hydrogen sulfide on the malondialdehyde levels and antioxidant status in Oreochromis mossambicus. They documented that significant increase in MDA levels were found in the liver, gill, kidney and brain of fish exposed to hydrogen sulfide. Our findings show that MDA levels in the experimental groups were higher than in the control group. A possible explanation for the enhancement of MDA level may be excessive production of reactive oxygen species (ROS) in fish expoed to formalin.

CAT is an enzyme located in peroxisomes and facilitates the removal of H_2O_2, which is metabolized to molecular oxygen and water (31,32). Trivedi et al. (33) studied the effect of copper sulphate pentahydrate influence on antioxidant enzymes of gold fish (*Carassius auratus)*. They reported that copper sulphate pentahydrate significantly decreased the CAT activity when compared to the controls. Yonar and Mişe Yonar (29) documented that malachite green caused oxidative stress by decreasing of the CAT activity in the blood, liver, kidney, spleen and gill muscle of rainbow trout. In this study, significant declines in the CAT activity were detected in the whole body of rainbow trout exposed to formalin in different concentrations. The decrease in CAT activity may be due to its restriction by the surplus production of ROS, as demonstrated by increased in the malondialdehyde levels in the present study. But, Tkachenko et al., (9) reported that rainbow trout were bath with different disinfectants such as chloramine-T, chlorine dioxide, formalin, peracetic acid and hydrogen peroxide had affected antioxidant enzymes. They observed that there appeared to be a trend of increasing in the CAT activity of fish exposure to disinfactants including formalin. In our study, however, the CAT activity in the whole body was increased by formalin bath. Differences in fish size may account for the differences between the results of study of Tkachenko et al. (9).

GPx catalyses the reduction of hydrogen peroxide and lipid peroxides and is considered an efficient protective enzyme against lipid peroxidation at the expense of GSH (34,35,36). Elia et al. (8) investigated hepatic antioxidant enzymes and total glutathione of *Cyprinus carpio* exposed to three disinfectants, chlorine dioxide, sodium hypochlorite and peracetic acid. Differences in biochemical parameters were observed in specimens following exposure to these disinfectants, and mainly chlorine compounds induced marked biochemical variations of carp liver compared to those induced by peracetic acid treatment. Monteiro et al. (28) studied the effect of inorganic mercury, a disinfectan, in a tropical freshwater fish, *Brycon amazonicus*. Significant alterations in the expression of the antioxidant enzymes such as SOD, CAT, GST, GPx, and GR were observed. These results indicate that affected of antioxidant mechanisms in *B. amazonicus* exposed to inorganic mercury. The present results showed the GPx activity was reduced in the groups that exposed to formalin in different concentrations. The reduction could be due to its exhaustion or restriction as a result of the increased production of free radicals. This result signify that the increase in the MDA levels of the fish exposure to different concentrations of formalin may be related to the diminish in the CAT and GPx activities.

Glutathione redox cycle is very important in intracellular antioxidant system and is essential for the tissues to protect themselves against the ROS damage. Glutathione is one of the necessary compounds for providing cell stability because of its reducing properties (24, 37,38,39). In this study, a decrease in the GSH level was observed. The reduction in the GSH level in this study may be due to direct conjugation of GSH with electrophiles species which are produced increasingly by formalin exposure or due to inhibition of enzymes such as GR and GPx, which are involved in GSH synthesis and regeneration. The decreased levels of GSH are previously reported in fish exposed to some disinfectants (20,29).

In conclusion, the results showed that exposure to different concentrations of formalin caused a significant alteration in the metabolism of rainbow trout, as shown by the antioxidant status parameters. It was also demonstrated that its antioxidant defence system was deal with formalin exposure, as evidenced by the oxidative damage.

References

1. U.S. Environmental Protection Agency. Health and Environmental Effects Profile for Formaldehyde. EPA/600/x-85/362. 1988. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH.

2. World Health Organization. 1989. Environmental Health Criteria for Formaldehyde. Volume 89. World Health Organization, Geneva, Switzerland.

3. Blair A, Stewart P, O'Berg M, Gaffey W, Walrath J, Ward J, Bales R, Kaplan S, Cubit D. Mortality among industrial workers exposed to formaldehyde. J Natl Cancer Inst 1986; 76:1071-84.

4. Blair A, Saracci R, Stewart PA, Hayes RB, Shy C. Epidemiologic evidence on the relationship between formaldehyde exposure and cancer. Scand J Work Environ Health 1990; 16:381-93.

5. Freestone J, Bentley A. Case of formaldehyde poisoning. Br J Pharm Pract 1989;11:20-1.

6. Kilburn KH. Neurobehavioral impairment and seizures from formaldehyde. Arch Environ Health 1994; 49:37-44.

7. Sharma M, Shrivastav AB, Sahni YP, Pandy G.. Overviews of the treatment and control of common fish diseases. Inter. Res. J. Pharma. 2012; 3(7): 123–7.

8. Elia AC, Anastasi V, Dörr AJ. Hepatic antioxidant enzymes and

total glutathione of *Cyprinus carpio* exposed to three disinfectants, chlorine dioxide, sodium hypochlorite and peracetic acid, for superficial water potabilization. Chemosphere 2006; 64:1633-41.
9. Marjani A, Golalipour MJ, Gharravi AM. The effects of subacute exposure of peracetic acid on lipid peroxidation and hepatic enzymes in Wistar rats. Oman Med. J. 2010; 25(4):256-60.
10. Tkachenko H, Kurhaluk N, Grudniewska J. Oxidative stress biomarkers in different tissues of rainbow trout (*Oncorhynchus mykiss*) exposed to disinfectant-CIP formulated with peracetic acid and hydrogen peroxide. Arch. Pol. Fish. 2014; 22(3):207-19.
11. Mişe Yonar S, Saglam N, Yöntürk Y, Aytemur A, Kosar A. Formaldehit uygulanan gökkuşağı alabalığı (*Oncorhynchus mykiss*)' nda bazı hematolojik ve antioksidan parametrelerin araştırılması. J. FisheriesSciences.com 2014; 8(4):317-3.
12. APHA (American Public Health Association). Standard methods for the examination of water and wastewater. 16th ed. Washington DC: American Public Health Association; 1985.
13. Placer ZA, Cushman L, Johnson BC. Estimation of products of lipid peroxidation (malonyl dialdehyde) in biological fluids. Anal Biochem 1966, 16: 359–64.
14. Aebi H. Catalase. In: Methods in Enzymatic Analysis. Bergmeyer HU. (eds.), Academic Press, New York, 1983, pp. 276–286.
15. Beutler E. Red cell metabolism. In: A manual of Biochemical Methods. Beutler E. (eds.), New York, Grune Strottan, 1975, pp.71-73.
16. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959; 82:70–7.
17. Lowry OH, Rosenberough NJ, Farr AL, Randal RJ. Protein measurement with Folin phenol reagent. J Biochem 1951; 193:265–75.
18. Beaumont MW, Butler PJ, Taylor EW. Exposure of brown trout, *Salmo trutta*, to a sublethal concentration of copper in soft acidic water: effects upon muscle metabolism and membrane potential. Aquat Toxicol 2000; 51:259–72.
19. Sağlam N, Ispir U, Yonar E. The effect of therapeutic bath of malachite green on some haematological parameters of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792). Fresen Environ Bull 2003; 12(10):1207-10.
20. Vutukuru SS, Chintada S, Madhavi KR, Rao JV, Anjaneyulu Y. Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish, *Esomus danricus*. Fish Physiol Biochem 2006; 32:221–9.
21. Santos R, Dias H, Fujimoto R.Acute toxicity and histopathology in ornamental fish amazon blue spotted corydora (*Corydoras melanistius*) exposed to formalin. An Acad Bras Cienc 2012; 84(4):1001-7.
22. Kochhann D, Pavanato MA, Llesuy SF, Correa LM, Riffel APK, Loro VL, et al. Bioaccumulation and oxidative stress parameters in silver catfish (Rhamdia quelen) exposed to different thorium concentrations. Chemosphere 2009; 77:384–91.
23. Mişe Yonar S, Sakin F, Yonar ME, Ispir Ü, Kirici M. Oxidative stress biomarkers of exposure to deltamethrin in rainbow trout fry (*Oncorhynchus mykiss*). Fresen Environ Bull 2011; 20(8):1931-5.
24. Mişe Yonar S. Toxic effects of malathion in carp, *Cyprinus carpio carpio*: Protective role of lycopene. Ecotoxicol Environ Saf 2013; 97:223–9.
25. Amin KA, Hashem KS. Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (*Clarias gariepinus*): antioxidant defense and role of alpha-tocopherol. BMC Vet Res 2012; 8:45.
26. Zhou DX, Qiu SD, Zhang J, Wang ZY. Reproductive toxicity of formaldehyde to adult male rats and the functional mechanism concerned. Sichuan Da Xue Xue BaoYi Xue Ban 2006; 37(4):566-9.
27. Borg DA, Trombetta LD. Toxicity and bioaccumulation of the booster biocide copper pyrithione, copper 2-pyridinethiol-1-oxide, in gill tissues of *Salvelinus fontinalis* (brook trout). Toxicol Ind Health 2010; 26(3):139-50.
28. Monteiro DA, Rantin FT, Kalinin AL. Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxa, *Brycon amazonicus* (Spix and Agassiz, 1829). Ecotoxicology 2010; 19;105–23.
29. Yonar ME, Mişe Yonar S. Changes in selected immunological parameters and antioxidant status of rainbow trout exposed to malachite green (*Oncorhynchus mykiss*, Walbaum, 1792). Pestic Biochem Phys 2010; 97(1):19-23.
30. Sreejai R, Jaya DS. Studies on the changes in lipid peroxidation and antioxidants in fishes exposed to hydrogen sulfide. Toxicol Int 2010; 17(2): 71-7.
31. van der Oost R, Beyer J, Vermeulen NP. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Phar 2003; 13:57-149.
32. Mişe Yonar S, Yonar ME, Yöntürk Y, Pala A. Effect of ellagic acid on some haematological, immunological and antioxidant parameters of rainbow trout (Oncorhynchus mykiss). J Anim Physiol An N 2014; 98(5):936-41.
33. Trivedi MH, Sangai NP Renuka A. Assessment of toxicity of copper sulphate pentahydrate on oxidative stress ındicators on liver of gold fish (*Carassius auratus*). Bull Environ Pharmacol Life Sci 2012; 1(9):52-7.
34. Moreno I, Pichardo S, Gómez-Amores L, Mate A, Vazquez CM, Cameán AM. Antioxidant enzyme activity and lipid peroxidation in liver and kidney of rats exposed to microcystin-LR administered intraperitoneally. Toxicon 2005; 45:395–402.
35. Sakin F, Ispir Ü, Mişe Yonar S, Yonar ME, Taysi R. Effect of short-term cypermethrin exposure on oxidant-antioxidant balance in the whole body of rainbow trout fry (*Oncorhynchus mykiss*). Fresen Environ Bull 2011; 20(10a): 2806-9.
36. Ural MS, Yonar ME, Mişe Yonar S. Protective effect of ellagic acid on oxidative stress and antioxidant status in *Cyprinus carpio* during malathion exposure. Cell Mol Biol 2015; 61(5): 58-63.
37. Dorval J, Hontela A. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). Toxicol Appl Pharm 2003; 192:191–200.
38. Yonar ME. Protective effect of lycopene on oxidative stress and antioxidant status in *Cyprinus carpio* during cypermethrin exposure. Environ Toxicol 2013; 28(11):609-16.
39. Yonar ME, Mişe Yonar S, Çoban MZ, Eroglu M. Antioxidant effect of propolis against exposure to chromium in *Cyprinus carpio*. Environ Toxicol 2014; 29(2):155-64.