World Journal of Fish and Marine Sciences 8 (1): 28-35, 2016
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2016.8.1.101215

Biochemical Studies of Heavy Metals (Zinc, Nickel and Chromium) in the Liver and Ovary of Zebra fish, *Danio rerio*

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Abstract: Oxidative stress, a pathological process relating to over-production of reactive oxygen species (ROS) in tissues leading to stress, is one of the most important general toxicity mechanisms for many xenobiotics. The present study was based to evaluate the effects of Zn, Ni and Cr on the stress biomarkers, catalse (CAT), reduced glutathione (GSH) and lipid peroxidation (LPO) in the liver and ovary of zebra fish. For this study, adult fishes were exposed to 16.19 mg/l, 64.77 mg/l Zn, 24.96 mg/l, 99.82 mg/l Ni and 9.12 mg/l, 36.47 mg/l Cr (20% and 80% of 96-h LC_{50} values, as calculated earlier) continuously for 21 days. A remarkable reduction was observed in the CAT and GSH level in both the tissues, on the other hand there was an enhancement in LPO levels in the liver and ovary for both the concentrations. The effect of Zn, Ni and Cr in the liver and ovary was found to be concentration and time dependent. Thus, the reduction in CAT, GSH and enhancement in LPO indicate that the heavy metals present in the aquatic ecosystem exert their effects through oxidative stress leading to changes at molecular level in zebra fish. However, there is much to learn about the details of this phenomenon and further research is needed to fully elucidate the effect of heavy metals and the environmental risks they pose in the aquatic environment.

Key words: Antioxidant · Stress · Zebra fish · Heavy Metals · Tissue

INTRODUCTION

The rapid increase of industrialization and anthropogenic activity has contaminated the aquatic ecosystem which receives a wide range of pollutant (Pesticides, heavy metals, industrial effluent and domestic sewage). Among these pollutants, heavy metals are most injurious to fish health. These pollutants not only depleted the fish stock but also have threatened the human health by incorporating into food chain. They affect the individual growth rates, physiological functions, mortality and reproduction of non-target organisms including fish [1]. The major concern with heavy metals lies within their acute toxicity and their ability to bio-accumulate in biological systems [2] resulting in a number of deleterious effects such as immune suppression [3] and biochemical changes [4].

Oxidative stress, a pathological process relating to over-production of reactive oxygen species (ROS) in tissues is one of the most important general toxicity mechanisms for many xenobiotics. It is the result of an imbalance between the production of ROS and antioxidant defense in living beings. ROS are induced by substances such as transitional metal, pesticides and petroleum pollutants and causes serious pathology in humans and animals at an early stage of the disease [5]. Oxidative stress may produce enzymatic inactivation and peroxidation of cell constituents especially lipid peroxidation [6]. The resulting damage may alter cell functions, eventually leading to cell death. To cope with continuous generation of ROS from normal aerobic metabolism, cells and tissues contain a series of cellular antioxidant with both enzymatic (Catalase, superoxide dismutase, metallothioniens, glutathione peroxidase, glutathione reductase) and non-enzymatic activities (Vit. A, C and E, carotenes, $Ubiquinol_{10}$).

Catalase (CAT) is a common enzyme found in nearly all the organisms, where it functions to catalyze the decomposition of H_2O_2 to O_2 and H_2O. Reduced glutathione (GSH) is a low molecular weight thiol. It can react directly with ROS species, to detoxify them. Lipid peroxidation (LPO) is a well-established mechanism of

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estimating cellular injury in both plants and animals. It is used as an indicator of oxidative stress in cells and tissues.

Zinc (Zn) is nutritionally essential metal and deficiency causes severe health diseases. Excessive exposure to zinc is relatively uncommon and occurs only at very high levels. Inhalation of freshly formed fumes of zinc has been associated with metal fume fever. Nickel (Ni) is ubiquitous in nature and occurs mainly in the form of sulfide and silicate minerals. Ambient air, as a result of industrial activity, combustion of fossil fuels and waste incineration is known to contain very low level of nickel. Human are exposed through inhalation, ingestion and dermal contact. The Environmental Protection Agency estimates that an average adult consumes 100-300 µg of Ni per day. Chromium (Cr) occurs in different oxidation states ranging from Cr^{+2} to Cr^{+6}. Trivalent and hexavalent forms are of biological significance. Health effects of Cr have been reviewed from time to time. Systemic toxicity occurs from ingestion of high amount of Cr^{+6}. It causes dermal ulcer, asthma and other respiratory disease, DNA damage and optic defect.

Liver is the main detoxifying organ of fish and is essential for metabolism and excretion of the toxic substances in the body. The gonads are also affected by the pollutants which in turn will affect the reproductive behavior of the fish. Therefore, the biochemical changes were also studied to assess the extent of damage to ovary caused by heavy metals. Although there is little information on the potential hazards of heavy metal (Zn, Ni and Cr) to oxidative stress and antioxidant system of zebra fish and toxicity mechanism still have not been fully elucidated. Considering the above fact and to provide data supporting the usefulness of freshwater fish as indicator of heavy metal pollution, the objective of the study was to examine the toxic effect of sub-lethal concentration of Zn, Ni and Cr on CAT, GSH and LPO in the selected organs (Liver and ovary) of zebra fish, *Danio rerio*. Zebra fish was selected for the present study because they are model organisms for toxicological research and also recommended by the Organization for Economic Co-operation and Development [7].

MATERIALS AND METHODS

The biochemical study was performed to observe the changes in the liver and ovary of zebra fish at different concentrations and different exposure periods. For this experiment, four-month-old matured fish were procured from our stock aquarium and exposed to different concentrations *i.e.* 20% (16.19mg/l Zn, 24.96mg/l Ni and 9.12mg/l Cr) and 80% (64.77 mg/l Zn, 99.82 mg/l Ni and 36.47 mg/l Cr) of the 96-h of LC_{50}, which were calculated from our earlier toxicity test [8]. Fishes were divided into four groups of 50 fish each. All the groups of 50 fishes were exposed to the above different concentrations of Zn, Ni and Cr for 21 days continuously. In the test, aquaria water was replaced daily with fresh treatment of the heavy metals. Fish from the control group were maintained in water free with heavy metals. After the expiry of exposure periods of (7, 14 and 21 days) required number of exposed fishes were taken out from experimental and control groups; their liver and ovary were removed and dissected.

Catalase (CAT) (EC 1.11.1.6) was assayed colorimetrically by the method of Sinha [9]. To 100 µl of the phosphate buffer (0.01 M, pH 7.0), taken in test tube, 100 µl of homogenate was added. To this 100 µl of H_2O_2 was added. The reaction was stopped after 60 seconds by the addition of 2.0 ml of dichromatic acid reagent. The tubes were heated for 10 minutes in a boiling water bath and a green solution of chromic acetate was developed. The absorbance of colour was measured at 570 nm using spectrophotometer. For standard, different concentrations of H_2O_2 ranging 40-160 µ moles were taken in test tubes and reaction mixture was prepared as discussed above. Activity of catalase was expressed as micro-moles (µM) of H_2O_2 utilized/min/mg protein.

Glutathione (GSH) content in the tissues (Liver and ovary) was estimated according to the method of Paglia *et al.* [10]. Tissue (Liver and ovary) was lysed with 2.0 ml of 1g/l EDTA (Ethylene diamine tetraacetic acid) solution and 1.5 ml of precipitating reagent (1.67 g glacial metaphosphoric acid, 0.2 g EDTA, 30 g sodium chloride, distilled water to 100 ml) was added. After mixing, the solution was allowed to stand for five minutes then centrifuged at 3000 rpm for 15 min. 0.50 ml of filtrate was added to 2 ml of disodium hydrogen phosphate (Na_2HPO_4) (0.1M, pH 7.4) and 0.25 ml of DTNB reagent (40 mg) was dissolved in 100 ml of 10 g/l (1%) sodium citrate. A blank was prepared from 1.5 ml of precipitating reagent, 1 ml of distilled water, 2 ml of disodium hydrogen phosphate and 0.25 ml of DTNB reagent. The absorbance of yellow color was read at 412 nm within a minute after adding DTNB. The results were expressed as GSH mg/mg protein.

Lipid peroxidation (LPO) in the liver and ovary was estimated by the method of Placer *et al*. [11]. Malondialdehyde (MDA), an end product of lipid peroxidation, reacts with 2-thiobarbituric acid (TBA) to form pink chromogen. Both the tissues were homogenized in chilled 0.15 M KCl using Teflon pestte to give 10% w/v

homogenate. 1 ml of homogenate was incubated at $37^0 \pm 0.5$ °C in a metabolic shaker for two hours. To each sample, 1 ml of 10 % w/v trichloro acetic acid (TCA) (s.d. Fine-Chem Ltd., Mumbai) was added. After through mixing, the reaction mixture was centrifuged at 2000 rpm for 10 minutes. 1 ml of supernatant was then taken with an equal volume of 0.67% w/v TBA (HiMedia Lab., Pvt. Ltd., Mumbai) and kept in a boiling water bath for 10 minutes, cooled and diluted with 1 ml of distilled water. The absorbance of pink color obtained was measured at 535 nm against blank, which contained distilled water instead of tissue homogenate. The concentration of MDA was read from standard calibration curve plotted using 1, 1, 3, 3' tetra-methoxypropane (Sigma-Aldrich Co., St. Louis, USA). The results were expressed as µM of MDA formed/30 min/mg protein.

Protein content in the liver and ovary of zebra fish was estimated by the method of Lowry *et al*. [12] by using bovine serum albumin as the standard.

Statistical analysis was done by two-way analysis of variance (ANOVA) to test the significance of the data. All data are expressed as Mean (n=6)±standard deviation (SD) and differences were considered significant at $p<0.05$.

RESULTS

Changes in the CAT Activity: Changes in the CAT activity in the liver of zebra fish after 7, 14, 21 days of continuous treatment of heavy metals (Zn, Ni, Cr) is presented in tables 1 and 2. It is evident that there is appreciable changes at all the concentrations of the above heavy metals. The CAT activity in the control group was considered as 100%. The CAT activity was reduced to 52% after Zn exposure, 65% after Ni exposure and 44% in the Cr exposed fishes after treatment of 80% of 96-h LC_{50} for 21 days (Table 1). In the ovary the CAT activity was reduced to 64% in the zebra fish treated with Zn, 73% in the Ni treated group, 66% in the Cr treated group at 80% of 96-h LC_{50} value after 21 days. Analysis of variance confirmed that the inhibition was concentration as well as time dependent ($P<0.05$) (Table 2).

Changes in the GSH Level: The effect of heavy metals (Zn, Ni, Cr) on the GSH level of zebra fish showed significant decrease ($P<0.05$) in the liver of zebra fish, when kept under continuous stress for 21 days. The GSH level in the control was considered as 100%. After Zn exposure the GSH level was reduced gradually to 90% at 20% of 96h LC_{50} after 7 days and 56% at 80% of 96h LC_{50} after 21 days of exposure period. Ni exposed fishes showed 94% reduction after 7 days at 20% of 96h LC_{50} value and 71% after 21 days at 80% of 96h LC_{50} value. Cr treated Zebra fish showed 85% reduction in the liver at 20% after 7 days and 51% reduction after 21 days at 80% of 96h LC_{50} (Table 3).

From table 4 it is clear that the GSH level in the Zn treated zebra fish ovary was reduced to 89% after 7 days at 80% of 96h LC_{50} which was further decreased to 66% after 21 days at 80% of 96 h LC_{50}. Similarly in the Ni treated group it was reduced to 93% at 80% after 7 days and 75% at 80% after 21 days of exposure period. In Cr treated group the GSH level was decreased to 85% after 7 days at 80% of 96h LC_{50} value and 66% at 80% after 21 days of exposure period.

Changes in LPO Level: The effect of heavy metals (Zn, Ni, Cr) on lipid peroxidation (LPO) in the liver of zebra fish showed a significant ($P<0.05$) change at different concentrations and exposure periods. Contrary to CAT and GSH, LPO exhibited enhancement after different treatment periods. After 21 days of treatment period of Zn at the lowest (20%) and highest (80%) concentrations, the LPO was increased to 132% and 143% respectively as compared to control. In Ni treated group, there was enhancement of 116% at 20% of 96h LC_{50} and 125% at 80% of 96h LC_{50} after 21 days of treatment period. Cr treated fishes showed a sharp change in LPO as compared to control (100%). At 80% of 96h LC_{50} for 21 days there was drastic increase of 174% in the LPO. With increase in concentration there was significant ($P<0.05$) increase in the LPO showing the concentration dependent enhancement (Table 5).

The effect of heavy metals (Zn, Ni, Cr) in ovary showed a significant ($P<0.05$) change at different concentrations. The LPO in the ovary of Zebra fish exposed to different concentrations of heavy metals for 21 days showed remarkable increase After 7 days of treatment of Zn at the lower concentration i.e., at 20% of 96h LC_{50} and upper concentration i.e., at 80% of 96h LC_{50} after 21 days the LPO increased to 106% and 131% as compared to control. In Ni treated group at lowest concentration (24.96mg/l) LPO was enhanced to 102% after 7 days and at highest concentration (99.82 mg/l) it was enhanced to 119% after 21 days of exposure period. The most significant change was observed at each concentration of Cr after 7, 14 and 21 days of exposure periods. There was gradual increase in the LPO level after 14 to 21 days of heavy metal treatment. There was 117% increase at 20% of 96h LC_{50} after 7 days and 153% increase at 80% of 96h LC_{50} after 21 days of exposure period (Table 6).

Table 1: Effect of heavy metals (Zn, Ni, Cr) on Catalase activity (µM H_2O_2 utilized/min/mg protein) in the liver of zebra fish, *Danio rerio*. ǂ

		Exposure period		
Metals used	Concentration (mg/l)*	7 days	14 days	21 days
Zinc	0.00(control)	153.30±0.18(100)	155.32±0.21(100)	151.32±0.17(100)
	16.19	138.30±0.17(90)	120.02±0.52(77)	89.00±0.56(59)
	64.77	124.17± 0.17(81)	114.00±0.56(73)	78.67±0.23(52)
Nickel	0.00(control)	155.13±0.09(100)	152..48±0.62(100)	150.60±0.14(100)
	24.96	142.60±0.21(92)	126.45±0.23(83)	111.40±0.25(74)
	99.82	133.46±0.21(86)	118.35±0.25(78)	97.88±0.35(65)
Chromium	0.00(control)	156.40±0.25(100)	158.68±0.23(100)	154.39±0.08(100)
	9.12	118.60±0.21(76)	96.38±0.26(61)	79.94±0.44(52)
	36.47	105.40±0.25(67)	83.74±0.72(53)	67.76±0.16(44)

†Values are mean ± SD of six individual observations and significant at P<0.05 (two-way ANOVA). Numerals in parentheses indicate the percent change rounded off to the nearest values in comparison to control value taken as 100%.

*The exposure concentrations used were 20% (16.19 mg/l Zn, 24.96 mg/l Ni, 9.12 mg/l Cr) and 80% (64.77 mg/l Zn, 99.82 mg/l Ni, 36.47mg/l Cr) of 96-h LC_{50} value.

Table 2: Effect of heavy metals (Zn, Ni, Cr) on Catalase activity (µM H_2O_2 utilized/min/mg protein) in the ovary of zebra fish, *Danio rerio*. ǂ

		Exposure period		
Metals used	Concentration (mg/l)*	7 days	14 days	21 days
Zinc	0.00(control)	159.46±0.29(100)	152.53±0.27(100)	155.37±0.23(100)
	16.19	148.29±0.65(93)	126.52±0.59(83)	116.54±0.21(75)
	64.77	140.34±0.25(88)	113.34±0.22(73)	99.44±0.22(64)
Nickel	0.00(control)	155.49±0.22(100)	157.65±0.15(100)	150.82±0.10(100)
	24.96	148.69±0.21(96)	142.04±0.73(90)	122.16±0.59(81)
	99.82	142.62±0.15(92)	134.00±0.56(85)	110.08±0.56(73)
Chromium	0.00(control)	155.31±0.12(100)	156.72±0.16(100)	153.45±0.19(100)
	9.12	141.33±0.12(91)	126.94±0.44(81)	107.42±0.35(70)
	36.47	130.46±0.19(84)	112.83±0.64(72)	101.28±0.13(66)

†Values are mean ± SD of six individual observations and significant at P<0.05 (two-way ANOVA). Numerals in parentheses indicate the percent change rounded off to the nearest values in comparison to control value taken as 100%.

*The exposure concentrations used were 20% (16.19 mg/l Zn, 24.96 mg/l Ni, 9.12 mg/l Cr) and 80% (64.77 mg/l Zn, 99.82 mg/l Ni, 36.47mg/l Cr) of 96-h LC_{50} value.

Table 3: Effect of heavy metals (Zn, Ni, Cr) on GSH level (GSH mg/mg protein) in the liver of zebrafish, *Danio rerio*. ǂ

		Exposure period		
Metals used	Concentration (mg/l)*	7 days	14 days	21 days
Zinc	0.00(control)	4.75±0.15(100)	3.66±0.56(100)	4.49±0.29(100)
	16.19	4.25±0.12(90)	2.95±0.64(81)	3.25±0.12(72)
	64.77	3.68±0.25(78)	2.59±0.34(71)	2.51±0.22(56)
Nickel	0.00(control)	3.35±0.25(100)	3.23±0.09(100)	3.31±0.65(100)
	24.96	3.14±0.07(94)	2.78±0.53(86)	2.67±0.25(80)
	99.82	2.96±0.65(88)	2.66±0.49(82)	2.35±0.17(71)
Chromium	0.00(control)	4.54±0.22(100)	4.43±0.19(100)	3.75±0.15(100)
	9.12	3.87±0.63(85)	3.36±0.12(76)	2.75±0.14(68)
	36.47	3.31±0.17(73)	2.66±0.67(60)	1.91±0.68(51)

†Values are mean ± SD of six individual observations and significant at P<0.05 (two-way ANOVA). Numerals in parentheses indicate the percent change rounded off to the nearest values in comparison to control value taken as 100%.

*The exposure concentrations used were 20% (16.19 mg/l Zn, 24.96 mg/l Ni, 9.12 mg/l Cr) and 80% (64.77 mg/l Zn, 99.82 mg/l Ni, 36.47mg/l Cr) of 96-h LC_{50} value.

Table 4: Effect of heavy metals (Zn, Ni, Cr) on GSH level (GSH mg/mg protein) in the ovary of zebra fish, *Danio rerio*. ✝

		Exposure period		
Metals used	Concentration (mg/l)*	7 days	14 days	21 days
Zinc	0.00(control)	3.71±0.54(100)	3.23±0.14(100)	5.49±0.46(100)
	16.19	3.49±0.51(94)	2.87±0.67(89)	4.28±0.13(78)
	64.77	3.30±0.18(89)	2.39±0.25(74)	3.62±0.08(66)
Nickel	0.00(control)	5.47±0.35(100)	4.28±0.17(100)	3.29±0.20(100)
	24.96	5.31±0.61(97)	3.94±0.64(92)	2.89±0.64(88)
	99.82	5.11±0.56(93)	3.72±0.22(87)	2.47±0.22(75)
Chromium	0.00(control)	3.43±0.24(100)	3.46±0.28(100)	4.55±0.32(100)
	9.12	3.08±0.71 (90)	2.98±0.66(86)	3.42±0.23(75)
	36.47	2.92±0.68(85)	2.49±0.28(72)	3.00±0.56(66)

†Values are mean ± SD of six individual observations and significant at P<0.05 (two-way ANOVA). Numerals in parentheses indicate the percent change rounded off to the nearest values in comparison to control value taken as 100%.

*The exposure concentrations used were 20% (16.19 mg/l Zn, 24.96 mg/l Ni, 9.12 mg/l Cr) and 80% (64.77 mg/l Zn, 99.82 mg/l Ni, 36.47mg/l Cr) of 96-h LC_{50} value.

Table 5: Effect of heavy metals (Zn, Ni, Cr) on LPO (µM of MDA formed/30 min/mg protein) in the liver of zebra fish, *Danio rerio*. ✝

		Exposure period		
Metals used	Concentration (mg/l)*	7 days	14 days	21 days
Zinc	0.00(control)	10.75±0.13(100)	10.21±0.11(100)	11.27±0.25(100)
	16.19	11.72±0.14(109)	12.26±0.13(120)	14.87±0.67(132)
	64.77	13.26±0.14(123)	13.66±0.56(135)	16.12±0.07(143)
Nickel	0.00(control)	11.55±0.37(100)	11.44±0.34(100)	12.45±0.20(100)
	24.96	12.02±0.42(104)	12.25±0.12(107)	14.44±0.20(116)
	99.82	13.05±0.74(113)	13.74±0.14(120)	15.56±0.32(125)
Chromium	0.00(control)	13.75±0.15(100)	13.43±0.19(100)	13.97±0.64(100)
	9.12	17.22±0.09(125)	17.69±0.67(132)	21.09±0.56(151)
	36.47	19.39±0.23(141)	21.34±0.14(159)	24.30±0.15(174)

†Values are mean ± SD of six individual observations and significant at P<0.05 (two-way ANOVA). Numerals in parentheses indicate the percent change rounded off to the nearest values in comparison to control value taken as 100%.

*The exposure concentrations used were 20% (16.19 mg/l Zn, 24.96 mg/l Ni, 9.12 mg/l Cr) and 80% (64.77 mg/l Zn, 99.82 mg/l Ni, 36.47mg/l Cr) of 96-h LC_{50} value.

Table 6: Effect of heavy metals (Zn, Ni, Cr) on LPO (µM of MDA formed/30 min/mg protein) in the ovary of zebra fish, *Danio rerio*. ✝

		Exposure period		
Metals used	Concentration (mg/l)*	7 days	14 days	21 days
Zinc	0.00(control)	11.46±0.27(100)	12.37±0.29(100)	11.38±0.20(100)
	16.19	12.15±0.53(106)	13.98±0.67(113)	14.49±0.18(127)
	64.77	12.72±0.22(111)	15.35±0.25(124)	14.92±0.64(131)
Nickel	0.00(control)	12.44±0.34(100)	12.42±0.23(100)	12.56±0.27(100)
	24.96	12.69±0.67(102)	13.17±0.53(106)	13.81±0.63(110)
	99.82	13.55±0.22(109)	14.18±0.09(114)	14.97±0.67(119)
Chromium	0.00(control)	13.32±0.17(100)	13.53±0.32(100)	10.44±0.22(100)
	9.12	15.58±0.32(117)	16.91±0.68(125)	15.47±0.19(148)
	36.47	16.79±0.13(126)	19.23±0.19(142)	15.97±0.65(153)

†Values are mean ± SD of six individual observations and significant at P<0.05 (two-way ANOVA). Numerals in parentheses indicate the percent change rounded off to the nearest values in comparison to control value taken as 100%.

*The exposure concentrations used were 20% (16.19 mg/l Zn, 24.96 mg/l Ni, 9.12 mg/l Cr) and 80% (64.77 mg/l Zn, 99.82 mg/l Ni, 36.47mg/l Cr) of 96-h LC_{50} value.

DISCUSSION

During the present study, CAT activity was decreased after 21 days of exposure to Zn, Ni and Cr and the values obtained were significantly lower than control. Decrease in CAT activity could be due to decrease in the rate of reaction because of the excess production of H_2O_2. It may be due to the flux of superoxide radicals, which have been shown to inhibit CAT activity. When the concentration of H_2O_2 in the cell exceeds the physiological level, CAT takes over the protective function. Sub-lethal concentrations of all the three metals induce oxidative stress in zebra fish and could be an adaptive response to protect the fish from the heavy metal-induced reactive oxygen free radical toxicity. We presumed that the reduction in CAT is probably related to the enhancement of LPO. The same decrease of CAT was observed in the liver of adult zebra fish exposed to silver nanoparticles [13]. Cruz *et al*. [14] observed the simultaneous reduction in the CAT activity in the tissues of zebra fish larvae in comparison to the control.

According to Arunchalam *et al.* [15] the specific activity of CAT declined significantly in the liver of *Catla catla* in response to the treatment with Cr for 21 days. Similar decrease pattern was also observed by other researchers [16-19]. The same concentration-dependent decrease in the activity of CAT was also observed by Saliu and Bawa-Allah [20] in the liver of Post juvenile *Clarias gariepinus* treated with lead and zinc. The reduction may be associated with the direct binding of metal to –SH group on the enzyme molecule. The inhibition of the CAT level could be due to the flux of superoxide radicals, resulting in H_2O_2 increase in the cell as reported by Begam and Sengupta [21].

The term "*Antioxidant*" is used to define the cell's own protective mechanisms. The reduced glutathione (GSH) antioxidant system is the main protective mechanism of the cell and is a crucial factor in the development of the immunity by the immune cells. Glutathione (L-gamma-glutamyl-L-cysteinylgyl-cine) can spontaneously, or with the help of peroxidase, easily deliver the H^+ necessary for the reduction of the reactive oxygen radicals. In both cases, the active element is the SH (Thiol) group of cysteine which when performing its antioxidant activity is oxidized to cystine or cysteine disulfide. It is the ratio cysteine/cystine that defines the redox state, which is the major determinant of the optimal function of the cell. Cysteine is the only factor of GSH synthesis in the cell. Reduced glutathione has sulfide as a functional groups that can capture unpaired electrons and is thus capable of removing harmful free radicals. GSH depletion might increase the risk of the oxidative stress. A considerable decline in GSH level in the tissues (Liver and ovary) under the present experimental study may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from Zn, Ni and Cr. Thus, the increase or decrease of enzyme activity is related to the intensity of cellular damage in zebra fish after heavy metal exposure. The apparent decrease in the GSH level suggests an adaptive and protective role of this biomolecule against oxidative stress induced by heavy metals. According to Zhang *et al*. [22] under severe stress, suppression in the levels of GSH may occur, due to the reduction capacity of synthesis. Previous investigators have also reported the significant concentration and time dependent decrease in the level of GSH [4, 20, 23]. The decreased level of GSH in the liver and ovary shows that protection against the ROS is highly required.

LPO has been used as a biomarker to measure the xenobiotics-induced oxidative stress, which was originally defined as the disequilibrium between pro-oxidants and antioxidants in biological systems. Once this imbalance appears cellular macromolecules may be damaged by the predominant free radicals. In the present study the level of LPO was elevated after the treatment of heavy metal (Zn, Ni, Cr). Many other workers [24, 25] observed a concentration-dependent increase in the level of LPO which is in favour of our result. According to Adeogun *et al*. [26] increased LPO in *Clarias gariepinus* exposed to industrial effluent is the result of oxidative stress and causes several degenerations. Impairment of enzymatic antioxidant system may favour accumulation of free radicals that may be responsible for increased LPO due to heavy metal exposure. Battacharya and Battacharya [27] also observed a significant increase in the LPO in the catfish (*Clarias batrachus*) after exposure to low concentration of Arsenic. The elevated level of LPO in the liver and ovary of zebra fish in response to the exposure to Zn, Ni and Cr suggests that there is increased production of ROS. Increased ROS production may, thus be associated with the metabolism of the above metals leading to the peroxidation of membrane lipids of both the tissues. El-Gazzar *et al*. [28] also reported the enhancement of LPO in the gill and liver of Nile *Tilapia* and *Oreochromis* treated with cadmium.

CONCLUSION

The results of the present study led to conclude that heavy metals (Zn, Ni and Cr) disturb the normal functioning and alters the fundamental biochemical mechanisms in zebra fish. Therefore, Zebra fish can be used as bio-indicators of metals in the environment by studying the induction of oxidative stress. It is also suggested that these types of toxicological studies are highly required to monitor the aquatic system and asses the toxic effect of heavy metals on aquatic organism particularly the fish.

ACKNOWLEDGEMENT

The study was supported by University Grant Commission, New Delhi [Project No. F. 42-620/2013 (SR)]. Thanks are also extended to Prof. Rajendra Singh, Head of the Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur for providing the laboratory facilities.

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