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Full Length Article

Assessment of Antioxidant Defense Mechanism against Oxidative Stress Induced by Sub-Lethal Concentrations of Arsenic in Common Carp (*Cyprinus carpio*)

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Abstract

In the aquatic environment, the industrial and natural spills lead toward high arsenic (As) concentration which affects the life of aquatic organisms. Fish are among those aquatic species which are very sensitive to any waterborne contamination; thus, they serve as bio-indicator to monitor the water quality. In this study, we evaluated the biochemical changes in the enzymatic defense systems in response to the oxidative stress caused by As in fresh water fish, the common carp (*Cyprinus carpio* L.), exposed to different sub lethal concentrations of As (0, 1.25, 1.65, 2.50, and 5.0 mg L^{-1}) for 4 weeks. Data for lipid peroxidation and enzymatic antioxidants activities in liver, gills, muscles and blood were collected. The analytical results indicated that hydrogen peroxide and lipid peroxidation in blood, gills, liver and muscles increased with sub lethal As concentrations. In addition, activity of peroxidase, super-oxidase dismutase and glutathione reductase was gradually increased in blood, liver and muscle with increasing concentrations of As during the exposure period, while CAT activity decreases in blood and gill of fish with increasing the sub lethal As concentrations. In conclusion, activation of antioxidant defense system in fish scavenged the reactive oxygen species and protected fish from the sub-lethal concentration of As. © 2020 Friends Science Publishers

Key words: Bioindicators; Antioxidants; Lipid peroxidation; Arsenic toxicity; Common carp

Introduction

Industrial and natural resources of water have polluted the water body with arsenic (As) compounds. The As is extremely poisonous element and it can cause cancer in living bodies (Wang *et al.* 2006; Kumari *et al.* 2017; Zhang *et al.* 2019). So, water contamination with As is becoming a serious environmental and health issue and its harshness is increasing (Allen and Rana 2004). Animals and plants present in water bodies are infected directly by As in toxic ion (Authman *et al.* 2015). This situation also causes human health risk when they use water polluted with As and via food chain due to bioaccumulation of As in living bodies (Yang *et al.* 2014; Javed 2015).

Using the biomarkers such as oxidative stress is a way to estimate the presence of As in a water body. The end phenomenon is formed by an alteration in the stability between antioxidant defense systems and reactive oxygen species (ROS) of individual (Barata *et al.* 2005). The ROS are comprised of free radical of hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and the superoxide anion (O_2) (Lushchak 2011). The synthesis of OH radicals and increase in H_2O_2 concentration takes place due to deposition of As in the tissues of fish (Atli *et al.* 2006). Various researches proved that introduction to diverse pollutants, which include As, encourages the production of ROS in cell (Ruas *et al.* 2008), provoking an increase in peroxidation of lipids and change in activity of various antioxidant enzymes including glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) (Valko *et al.* 2005; Lushchak 2011).

The antioxidant defense systems have vital role in stabilization of cell and conservation of stern control of free radicals (Halliwell and Gutteridge 1989). In aquatic toxicology, antioxidant actions of enzymes serve as sensitive biochemical pointers and are broadly used to evaluate the fitness of animal (Gul *et al.* 2004). Bhattacharya and Bhattacharya (2007) stated that two sub-lethal dosages of heavy metal on *Clarias batrachus* presented rise in the activities of antioxidant enzymes such as CAT, SOD and GPX. They also recorded a reduction in the activity of glutathione reductase (GR) after one day of imposition of treatments which shows that oxidative damage in fish was caused at initial stages. Superoxide (O^{-2}), which is one of the parental forms of intracellular ROS, is a reactive molecule but it is possible to transform it

to H_2O_2 by SOD and then to oxygen and water by various enzymes such as CAT and GR (Kumari *et al.* 2014). So, observing the variation in antioxidant enzymes activity such as CAT, GR and SOD will be an efficient way of representing oxidative stress and variations in activity. Hence, this study aimed to elucidate the As sub-lethal toxicity on antioxidant defenses (GR, CAT, POD and SOD) and the ability of these enzymes to reduce the oxidative stress in *Cyprinus carpio*.

Materials and Methods

The fish *C. carpio* (15 ± 2 cm total length and 50 ± 1 g weight) were collected from fish nursery Karor Lali Ehsan, Layyah Pakistan, were shifted to laboratory in polyethylene bags with water that was oxygenated. The fish were acclimatized in aquaria for 15 days under laboratory conditions prior to the experiment. Stone diffusers attached to a motorized air compressor were used to ventilate the aquaria. The pH was maintained between 6.6 and 7.5 and temperature of water was 25 ± 2ºC. The fish were given food containing egg, raw brine shrimp pellets and goat liver twice a day, and were exposed to various As concentrations *viz.*, 1.25, 1.65, 2.50, and 5.0 mg/L. One group was consider as a control treatment where fish were not exposed to As. The 10 fish for each concentration of As test were used. The fish were exposed to As concentrations for 30 days. In the experimental aquaria, water was replaced daily with fresh treatment of As. Five replicates for each concentrations of arsenic were arranged under Complete Randomized Design (CRD). After 30 days, the fish were anesthetized by immersion in 50 mg/L tricaine methane sulphonate (MS-222) solution for 5–10 min before they were killed by transection of the spinal cord.

Sample preparation for bioassay

Every test group of the sample liver, muscle, blood and gill was made according to Habbu *et al*. (2008). By using the iced cold solution of KCl 1.15%, the organs were washed, blotted and weighed. For homogenization, the phosphate buffer (0.1 *M*; pH 7.2) was used. The laboratory acid-washed sand was added to it before placing each organ in mortar and it was followed by blending with mortar and pestle. After blending, the resulted material was centrifuged (at 2500 rpm) for 15 mins. The antioxidant enzymes were determined through UV-visible spectrophotometer from the supernatant stored at -21°C (Habbu *et al.* 2008).

Lipid peroxidation

The data on peroxidation of lipids was calculated using thiobarbituric acid (TBARS) and color reaction for malondialdehyde (MDA) according to procedure by Placer *et al*. (1966). In this method, tissues were homogenized in chilled 0.15 *M* KCl using a Teflon pestle to obtain 10% w/v homogenate. One ml of homogenate was incubated at 37°C (± 0.5) for two hours. To each sample, 1 mL of 10% w/v trichloro acetic acid (TCA) was added. After thorough mixing, the reaction mixture was centrifuged at 2000 rpm for 10 mins. One mL of supernatant was then taken with an equal volume of 0.67% w/v TBA and kept in a boiling water bath for 10 mins, cooled, and diluted with 1 mL of distilled water. The absorbance of the color pink obtained was measured at 535 nm against a blank. The concentration of MDA was read from a standard calibration curve plotted using tetra-methoxypropane (Sigma-Aldrich Co., St. Louis, USA).

Determination of hydrogen peroxide

Ferrous oxidation-xylenol orange method was used to measure hydroperoxide content (HPC) (Jiang *et al.* 1992). To 100 μL of supernatant previously deproteinized with 10% trichloroacetic acid (TCA) was added 900 μL of the reaction mixture [0.25 m*M* $FeSO_4$, 25 m*M* H_2SO_4, 0.1 m*M* xylenol orange and 4 mM butyl hydroxytoluene in 90% (v/v) methanol], incubating for 60 min at room temperature. Absorbance was read at 560 nm against a blank containing only reaction mixture. A type curve was used to interpose result and result was stated as n*M* cumene hydroperoxide/mg protein.

SOD activity determination

The method of determining the activity of SOD is dependent upon spectrophotometric calculation of retarding impact of SOD on autoxidation of 6-hydroxyidopamine (6-OHDA) (Heikkila and Cabbat 1976; Crosti *et al.* 1987). When quantity of enzyme reduces the autoxidation of 6-OHDA by 50%, in 1 min at 37°C, then 1U of SOD activity is recognized. Meanwhile, in this reaction, the curvature of rate of autoxidation is steady in the 1–60 seconds, at 490 nm until the 60^{th} s of oxidation, the spectrophotometric calculation was done. In units per milligram protein, results were stated.

CAT activity determination

Aebi's method (1984) was used to measure catalase activity. The principle of the test depends upon rate constant of H_2O_2 determination by CAT enzyme. Usually, it is considered that one unit (IU) of CAT enzyme is equal to the enzyme activity that was recognized in 1 μmoL of H_2O_2 at 37°C in 60 s. By detecting the variation on absorbance of sample and blank for a min, spectrophotometrically at 240 nm, the CAT activity was calculated. Consequences were stated as units per milligram protein.

Determination of soluble protein

Bradford method (1976) was used to determine total soluble

protein content. A 75 μL deionized water and 2.5 mL Bradford's reagent [in 500 mL deionized water, 50 mL H_3PO_4, 25 mL of 96% ethanol and 0.05 g Coomassie blue dye] was added to 25 μL of supernatant. The test tubes shook and rest of 5 min was given before reading absorbance at 595 nm and exclamation on a bovine albumin curve (Sigma-Aldrich, St. Louis).

Statistical analysis

STATISTIX 8.1 was used for statistical analysis of the work. Graphs were presented as means ± standard deviation. By using LSD test, pair wise judgment between experimental and control groups was done to determine the statistical significance of difference between the groups (Pipkin 1984).

Results

The activity of superoxide dismutase (SOD) in blood, liver, gill and muscle of *C. carpio* was significantly affected by As exposure. The activities of SOD were increased significantly by increasing the sub-lethal concentrations of As. In blood and gill tissues, the SOD activities were statistically equal in the all sub-lethal concentrations of As but significantly high than the control group (Fig. 1). In liver, 75% higher SOD activities were found in fish exposed to 2.50 mg L^{-1} sub-lethal concentration of As followed by 67, 64 and 51% SOD activities determined in fish infected with sub-lethal amounts of 5.0, 1.65 and 1.25 mg L^{-1} correspondingly when related to controlled group (Fig. 1). The higher SOD actions were observed in the muscles of fish exposed to 5.0 mg L^{-1}, related with control group. In addition, SOD activities were significantly decreased in the muscles tissues of fish by decreasing the sub-lethal concentrations of As (Fig. 1).

Sub-lethal concentrations of As significantly affected the activities of peroxidase (POD) in muscle, liver, gill and blood tissues of *C. carpio.* A significant increase in POD activities was found in all tissues of fish that were infected with various sub-lethal amounts of As in comparison with the control group of fish. In blood and gill tissues of the fish, POD activities reached to the maximum value when exposed to 1.65 mg L^{-1} sub-lethal concentration of As after that there was sudden decreased in POD activities with increasing the concentration of As (Fig. 2). An increased in POD activity was observed in the liver tissues of the fish with increasing the sub-lethal concentrations of As. Significantly higher POD actions were noted in the fish's liver tissues of infected with maximum 5.0 mg L^{-1} level of sub-lethal concentration of As (Fig. 2). In muscle tissues of the fish, the POD activities were statistically at par in the all sub-lethal concentrations of As but significantly high than the control group (Fig. 2).

Statistical analysis revealed that catalase action within blood, liver, gill and muscle of *C. carpio* were responsive to various sub-lethal concentration of As. The catalase activity was increased in all tissues of fish infected with sub-lethal amounts of As. Levels of catalase activities were statistically equal at all the sub-lethal concentrations of As but higher than control group of the fish in all the tissues (Fig. 3). Glutathione reductase activities were significantly affected by sub-lethal concentrations of As in all tissues *viz* blood, gills, livers and muscles of *C. carpio.* In blood, the activities of glutathione reductase were significantly increased by increasing the level of sub-lethal concentrations of As. Significantly higher glutathione reductase activity was recorded at 5.0 mg L^{-1} sub-lethal concentration that was followed by the concentrations of 2.50, 1.65 and 1.25 mg L^{-1} of As respectively, when comparison to the control group of the fish (Fig. 4). In gill tissues, glutathione reductase activity was increased up to 2.50 mg L^{-1} of As concentration after that there was a decreasing trend with increasing the concentration of As whereas; minimum activity of glutathione reductase was noted in the control group of fish where fish were not exposed to As concentrations (Fig. 4). Related with control group, when noteworthy, the higher glutathione reductase activity was determined within liver and fish's tissues of muscle infected with 2.50 and 5.0 mg L^{-1} of As. In addition, glutathione reductase activities were seriously decreased within liver and fish's muscles tissues by decreasing the sub-lethal concentrations of As (Fig. 4).

Statistical analysis of data indicated that there was significant increase in lipid peroxidation level in various fish's tissues that were infected with the different sub-lethal concentrations of As. In blood and muscles tissues, maximum level of lipid peroxidation was recorded when fish was exposed to 1.65 mg L^{-1} of As that was statistically at par with sub-lethal concentration of 2.5 and 5.0 mg L^{-1} of As when compared with control group of fish (Fig. 5). In gill and liver tissues, 5.0 mg L^{-1} of As induced significantly higher level of lipid peroxidation that was followed by sub-lethal concentration of 2.5 and 1.65 and 1.25 mg L^{-1} of As whereas; minimum lipid peroxidation level was determined in control group of the fish (Fig. 5). The results showed that sub-lethal concentrations of As significantly enhanced the hydrogen peroxide stuffing's in blood, liver, gill and muscles of *C. carpio.* In all tissues of fish, hydrogen peroxide contents were increased with increasing the concentrations of As. Significantly higher level of hydrogen peroxide was recorded in every fish's tissue infected with 5.0 mg L^{-1} when compared to the other sub-lethal concentrations and control group of the fish (Fig. 6).

Discussion

The As is an aquatic ecological pollutant. Tissues of aquatic organism may get deposited by the heavy metals (Farombi *et al.* 2007; Rauf *et al.* 2009) including As. Thus, examination of tissues deposited with toxic metals in the water livings may be a sensible valuation for the health of

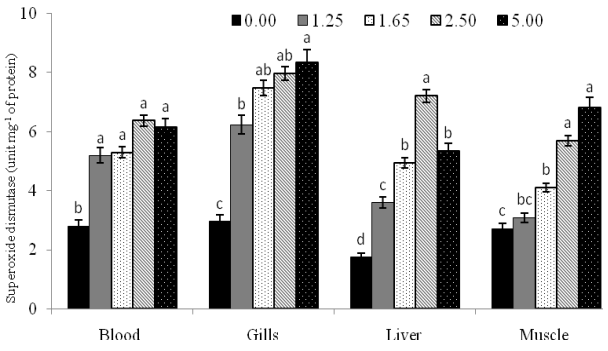

Fig. 1: Effect of various sub-lethal concentrations of arsenic on superoxide dismutase activity in blood, gills, liver and muscles of *C. carpio.* Values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

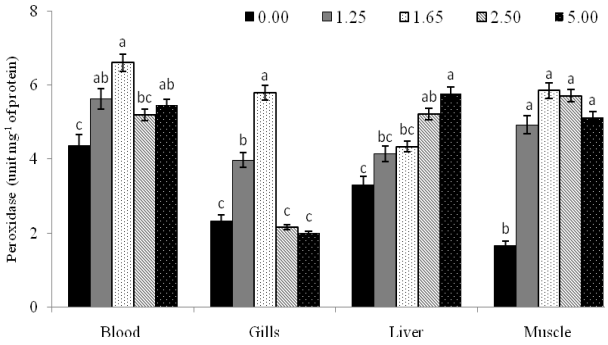

Fig. 2: Effect of various sub-lethal concentrations of arsenic on peroxidase activity in blood, gills, liver and muscles of *C. carpio.* Values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

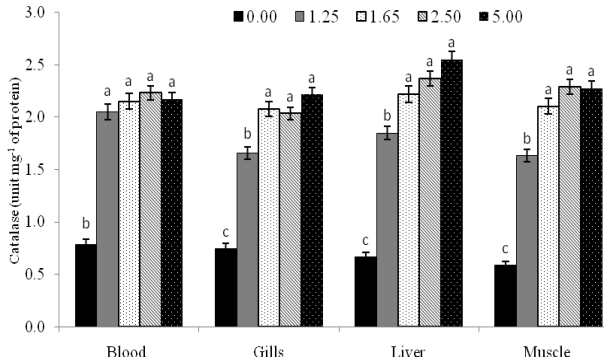

Fig. 3: Effect of various sub-lethal concentrations of arsenic on catalase activity in blood, gills, liver and muscles of *C. carpio.* Values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

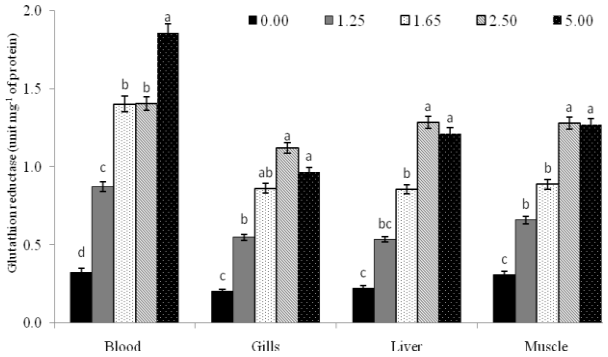

Fig. 4: Effect of various sub-lethal concentrations of arsenic on glutathione reductase activity in blood, gills, liver and muscles of *C. carpio.* Values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

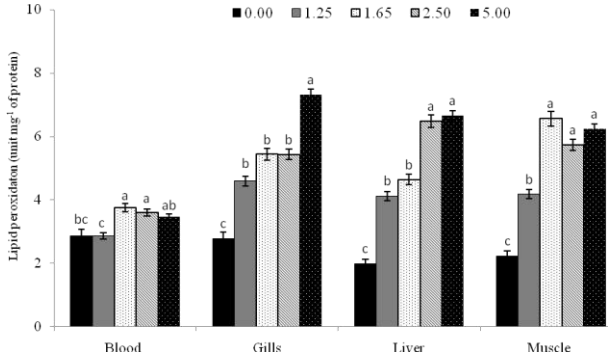

Fig. 5: Effect of various sub-lethal concentrations of arsenic on lipid peroxidation in blood, gills, liver and muscles of *C. carpio.* Values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

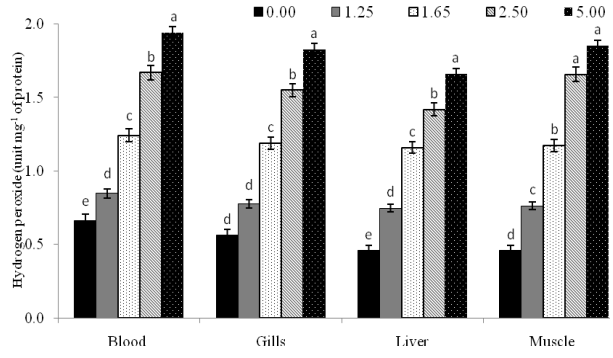

Fig. 6: Effect of various sub-lethal concentrations of arsenic on hydrogen peroxide in blood, gills, liver and muscles of *C. carpio.* Values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

human and animal standards (Kumar and Banerjee 2012a). It is reported that after introducing the As, a great concentration of As deposition in blood, brain, muscle, skin, gills, and liver tissues of the catfish were observed (Kumar and Banerjee 2012b). Actions of enzyme are understood as biochemical pointers and extensively used to evaluate the organism's health in toxicology of water (Gul *et al.* 2004). It is due to excess production of ROS induced by the metal

toxicity (Flora *et al.* 2005). Oxygen species that are reactive can result in serious damage or injury to tissue of liver, gills, skin, muscle, brain and blood (Patlolla and Tchounwou 2005). In current study, exposure of the *C. carpio* to sub-lethal concentrations of As was found to cause an increased level of lipid peroxidation in liver, gills, skin, brain, muscle and blood tissues, which is an indicative of oxidative stress in infected animals related to control group (Wang *et al.* 2004). The rise in lipid peroxidation is because of retarding outcome on mitochondrial system of transport of electron resulting in stimulated formation of intracellular ROS (Stohs *et al.* 2001). A minor increase in ROS production in exposed faunas to arsenite was sufficient to encounter a vital rise in peroxidation of lipid (Zarazúa *et al.* 2006). The results indicate that as the concentration of As increases, fish tissues are prone to redox reactions, that generate free radicals specifically ROS *i.e*., H_2O_2 (Patra *et al.* 2011). The highest H_2O_2formed by the incomplete reduce oxygen, may induce alteration and may change some physiological responses of fish (Varanka *et al.* 2004; Brucka-Jastrzebaska 2010).

ROS formed due to oxidative stress is neutralized by antioxidant resistance scheme (Gumustekin *et al*. 2005). The action of antioxidant enzymes can be boosted or repressed under heavy metals concentrations depending upon concentration and the extent of the applied stress, along with the vulnerability of infected species (Kumari *et al.* 2017). In this, the enzyme action of SOD was enhanced in blood, liver, gill and muscles of *C. carpio* in comparison to control group. The SOD enzyme is recognized for providing cyto-protection against damage induced by ROS due to conversion of superoxide radicals (O^{2-}) produced in mitochondria and peroxisomes to H_2O_2 (Olagoke 2008). Increased level of SOD under heavy metal toxicity in blood, brain, muscle, skin, gills and liver results in decreased oxidative stress within tissues due to harmful actions of the superoxide (Bharti *et al*. 2012). This indeed proved in the study because when infected fishes were related to the control, there was an increase in activity of CAT due to the As metals. Same phenomena of an increase in CAT activity was stated by Otitoloju and Olagoke (2011), Saliu and Bawa-Allah (2012) and Fatima and Ahmad (2005) that as increasing concentration of heavy metals enhanced the activity of CAT. In present study, GR level has been significantly increased in liver, gills, skin, blood & muscle tissue of *C. carpio* exposed to sub-lethal amounts of As. The reduction of arsenate to arsenite is due to electron donation by Glutathione. ROS is generated by As cell metabolisms, however mechanisms are not well known. Toxic metals' collaboration with the metabolism of GR is an important part of response of different toxic metals (Hultberg *et al.* 2001). Tissues are also defended from oxidative stress by it (Jifa *et al.* 2006). In this study, after 30 days, the action of GST was reduced in the tissues of fishes' liver that were infected with heavy metals when related to control. This consequence is an agreement of conclusions of Saliu and Bawa-Allah (2012) and Otitoloju and Olagoke (2011).

Conclusion

The findings of the current study reveal that As creates harmful effects by generating the ROS that damage the cells of organs by lipid peroxidation. However, it was counter balanced by the production of antioxidants such as SOD, POD and GRX in organs tissues of carp fish with increasing the sub lethal As concentrations. It was concluded that defensive nature and the adaptive mechanism of cells against free radical play an important protective role to cope with As stress.

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