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Effects of chromium (VI) on the lipid peroxidation and antioxidant parameters in the gill and kidney tissues of catfish, *Clarias batrachus* (Linnaeus, 1758) (Actinopterygii: Siluriformes)

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Abstract

In the past four decades, as a result of rapid urbanization and industrialization there has been a great variation in the environment in which we lives. The quality of the environment is deteriorating due to the accumulation of several pollutants either in direct way or in indirect way, which ultimately causes several unwanted effects on organisms in general and human beings in particular. Heavy metal pollution is an important pollution amongst the other kind of pollution. Among the various heavy metals, chromium is one of the important aquatic pollutants. The main sources which contribute the pollutant to the environments are hydrogenation of oil industry, paint factories, motor vehicle, aircraft industry, and printing and in some cases the chemical industries too. Its extensive use in electroplating industry is also one of the major factor. Chromium is ubiquitous trace metal and occurs everywhere such as, soil, water, air, and in the biosphere. In the present investigation, an attempt was made to assess the effect on chromium on oxidative stress and antioxidant enzymes in the gill and kidney tissue of *Clarias batrachus*. The fish were exposed to sub lethal dose of 96 hr LC_{50} of chromium for 28 days and tissue lipid peroxidation (LPO), Reduced glutathione (GSH), glutathione peroxidase (GPx), Catalase (CAT), and superoxide dismutase (SOD). The present study of metal treated fish shows the increased level of lipid peroxidation and decreased level of reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) in the Gill and Kidney tissue of fish, *Clarias batrachus.* These observed mean data were found statically significant at $P< 0.05$ student 't' test.

Keywords: Chromium, *Clarias batrachus*, lipid peroxidation (LPO), reduced glutathione (GSH), glutathione peroxidase (GPx), Catalase (CAT), and superoxide dismutase (SOD).

Introduction

Sewage and industrial disposal has greatly increased the addition of heavy metals in the aquatic ecosystems. It influences the productivity and health status of water bodies as abnormal changes in physicochemical conditions and other quality parameters have their impact on diversity. The rapid developments of industrialization and anthropogenic activities have leads to contamination of many ecosystems (Gayathri *et al.*, 2008) especially the aquatic ecosystem, which receives a wide range of pollutants. Effluents released from different manufacturing industries situated along the cities include potentially toxic metals like Cu, Ni, Cr, Pb, Fe and Zn (Singarea and Dhabardeb, 2014). Pollution of aquatic habitats seems to be an inevitable problem of universal nature and the intrusion of various pollutants into the aquatic environment affects the survival growth and reproduction of the biological organisms present in the environment. Heavy metals

are one of the contaminants of fresh water which harbors several aquatic species (Ogamba *et al*., 2016). The various concentration of each heavy metal distributed in different body parts of freshwater fishes. Concentration of these heavy metals exceeded the maximum permissible limit that indicates there is inherent danger in consuming fishes (Joseph *et al*., 2016). The persistence bioaccumulation of these heavy metals causes alterations, in various biochemical profiles of food fish (Garima Pundir, 2016). The manipulation of fish as a biomarker of heavy metals through behavior response, hepatocyte alteration, enzymatic reaction and proteomic studies which have proven to be very useful in the environmental pollution monitoring (Sabullah *et al*., 2015).

Heavy metals are extensively used in industries like electroplating, medicine, pesticide and battery manufacturing, which constitute a serious type of pollution in freshwater and being stable compounds they are not readily removed by oxidation. (Nammalwar, 1985). Most of these metals are characterized by being accumulated in tissues, and lead to the poisoning of fish. These metals can effectively influence the vital operations and reproduction of fish; weaken the immune system, and induce pathological changes Mohammad *et al*, (2015). Chromium is ubiquitous trace metal and occurs in soil, water, air, and in the biosphere. Exposure to chromium may lead to various adverse health effects, such as chromium allergy, contact dermatitis, and organ system-toxicity. Numerous studies have confirmed the carcinogenic potency of chromium compounds in experimental animals (Hughes *et al.,* 1979; Langard and Norseth, 1979; Abbasi *et al.,* 1991; Vincent *et al.,* 1995; Arunkumar *et al.,* 2004; Vutkuru, 2005; Ines Domingues *et al.,* 2010; Kawade *et al.,* 2012). Inhalation exposure in occupational settings is a primary route for chromium -induced toxicity (Banerjee and Banerjee 1988; Stohs, *et al.,* 2000; Bagchi, *et al.,*2002; Aaron *et al.,* 2004; Vutkuru, 2005; Palaniappan and Karthikeyan, 2009; Venkatramreddy Velma *et al.,* 2010; Kumar Parvathi *et al.,* 2011; Judilyn *et al.,* 2013). In aquatic ecosystem, dissolved chromium concentrations are generally ranging between 0.005 and 0.01 mg l^{-1}. The toxicity of Cr to aquatic life has been shown to vary significantly with organism species, p^H and water hardness. Exposure of chromium causes alterations in the histology of various parts (Claramma and Radhakrishnan, 2016). Hence these changes can be used to assess the quality of water contaminated with heavy metal salt(s). Chromium toxicity is generally low (but elevated concentration can cause sub lethal effects (Khangarot *et al.,* 1990; Pamila *et al.,* 1991; Wepener *et al.,* 1992; Bagchi *et al.,* 2000; Coban *et al.,* 2013). Exposure of fish to different chromium concentrations in water reduced their feed consumption and specific growth rate (Tayybah Shaheen and Farhat Jabeen, 2015).

Enzymes are necessary for normal cellular metabolism including that of the gill and kidney, and the degenerative changes due to the combined metal toxicity exhibited in the gill and kidney alter level of a number of its enzymes. For example lipid peroxidation (LPO), Glutothione (GSH), Glutathione peroxidase (GPx) catalase (CAT) and superoxide dismutase (SOD). Exposure of heavy metals causes oxidative stress in fish. due to this, increased LPO, GST level and decreased level of CAT, GSH, SOD and GPx levels in the various tissues Oxidative and antioxidant profiles (Shiv Shankar *et al*., 2015).

These enzymes are biomarkers of acute hepatic damage, thus their bioassay can serve as a diagnostic tool for assessing the functions of the gill and kidney. A limited number of laboratory studies have investigated chromium uptake in fish through aqueous exposures (Tjalve *et al.,* 1988; Sreedevi *et al.,* 1992). However, the biomarkers enzymes assay is still candy on freshwater fish. Hence, the present investigation has been carried out to investigate. the heavy metal chromium on the lipid peroxidation and antioxidant level in the gill and kidney tissue of *Clarias batrachus*.

Materials and Methods

Experimental fish

The *Clarias batrachus* were collected from the fish farm located at Kolathur, near Chennai, 17 km away from the campus. The fish were brought to the laboratory and transferred to the rectangular cement tanks (125 X 100 X 75 cm) of 1000liters capacity containing chlorine free aerated well water and acclimatized to the food and laboratory conditions with 12 hr dark and 12 hr light cycles, p^H range of 6.95 to 7.20 and temperature ranging from 16 to 24 °C for 15 days.

Fish were selected for the experiment from the stock irrespective of the sex. The size selected for the experiments were 80-100mm length and 5-10g of weight fish were divided into two equal groups each comprising of 20 fishes. Each group was kept in separate plastic trough. The first groups were kept as control and were maintained in normal water without

any treatment. The second group was exposed to a sub-lethal concentration of 96 hrs LC_{50} of chromium for 28 days. Solution was renewed once in 24hrs exposure period. The fish from the respective experimental as well as control groups were sacrificed gill and kidney tissues were isolated from the fish and used for the estimation lipid peroxidation and antioxidant parameters.

Estimation of lipid peroxidation and antioxidants in gill and kidney tissues

The isolated gill and kidney tissues of the control and experimental fishes were used for the level of lipid peroxidation in gill and kidney tissues by the method of Nichans and Samuelson (1968), reduced glutathione was determined by the method of Beutler and Kellay, (1963), glutathione peroxidase activity was determined by the method of Rotruck *et al.* (1973), Catalase was determined by the method of Sinha (1972) and Superoxide dismutase activity was assayed by the method of Kakkar *et al.* (1984). The Statistical significance of control and experimental means were analyzed by student 't' test.

Results

The results pertaining to the present experiments are shown in tables 1 and 2; Figures 1-5, the data clearly indicated that the lipid peroxidation and antioxidants level in the gill and kidney tissues of *Clarias batrachus* exposed to chromium (VI) as well as control. The Lipid peroxidation (LPO), Glutathione (GSH), Glutathione peroxidase (GPx), Catalse (CAT) and Superoxide dismutase (SOD) of the gill and kidney tissues of control fish were 0.464±0.002, 1.850±0.079, 0.946±0.002, 1.500±0.037, 0.640±0.018,0.681±0.001, 2.720±0.070, 1.315±0.003, 1.851±0.005 and 0.800±0.031µmole/mg of protein/hr. Whereas in the sub lethal concentration of chromium (VI) treated gill and kidney tissues were, 0.652±0.001, 1.380±0.025, 0.767±0.001, 0.800±0.036, 0.410±0.012, 0.972±0.001, 1.830±0.069 ,1.007±0.001, 0.970±0.003 and 0.440±0.018 µ mole/mg of protein/hr. If compared the control with experimental group, the lipid peroxidation has increased in the experimental group then the control group. The percentage increase was 40.52 and 42.73. The reduced glutathione, glutathione peroxidase, catalase and superoxide dismutase has decreased in the experimental group then the control group. The percentages decreased were -25.41, -18.92, -46.67, -35.94,- 32.72,- 23.42, -47.54 and -45.00. The mean values of lipid peroxidation and antioxidant values of control and chromium treated group was compared for their statistical significance at $P<0.05$.

Table 1. The level of lipid peroxidation and antioxidants in the gill tissue of *Clarias batrachus* exposed to sublethal concentration of chromium (VI).

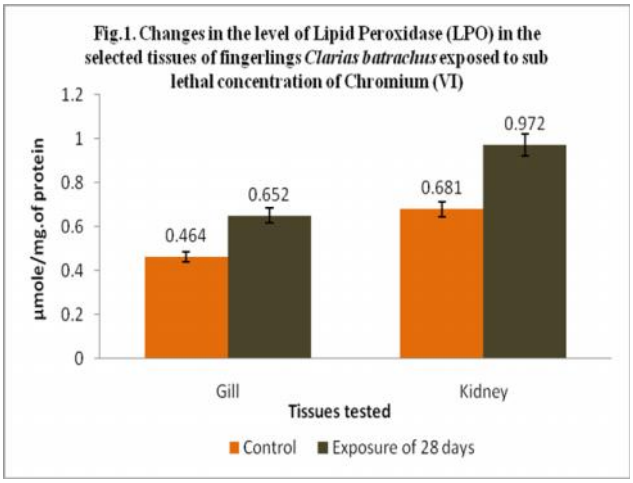
Parameters	Control	Exposure of 28 days	% COC
Lipid peroxidation (LPO) (µmole/mg.of protein)	0.464±0.002	0.652±0.001	40.52
Glutathione (GSH) (µmole /mg.of protein)	1.850±0.079	1.380±0.025	-25.41
Glutathione peroxidase (GPx) (µmole/mg protein)	0.946±0.002	0.767±0.001	-18.92
Catalse (CAT) (Unit/mg.of protein)	1.500±0.037	0.800±0.036	-46.67
Superoxide dismutase(SOD)(Unit/mg.of protein)	0.640±0.018	0.410±0.012	-35.94

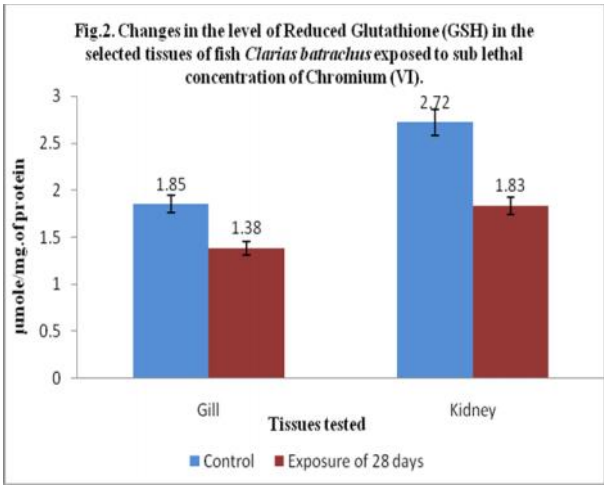
The values are Mean ± S.E of six individual observations, *Significance ($p<0.05$) of student 't' test.

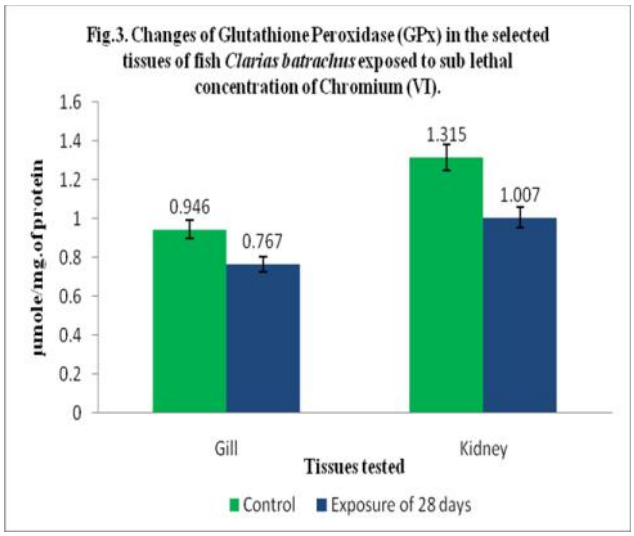
Table 2. The level of lipid peroxidation and antioxidants in the Kidney tissue of *Clarias batrachus* exposed to sublethal concentration of chromium (VI).

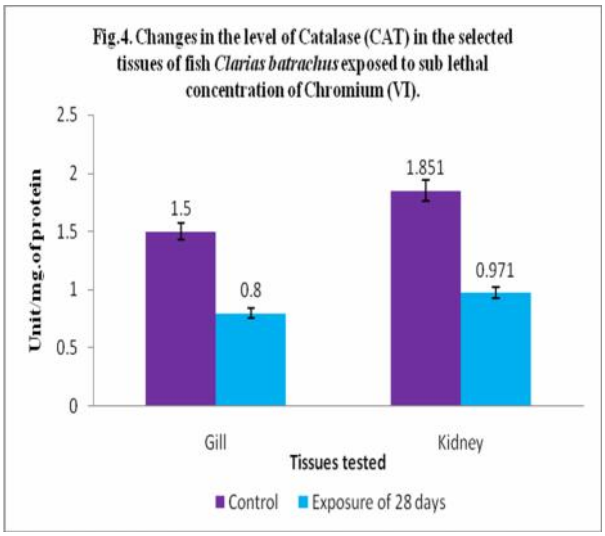
Parameters	Control	Exposure of 28 days	% COC
Lipid peroxidation (LPO) (µmole/mg.of protein)	0.681±0.001	0.972±0.001	42.73
Glutathione (GSH) (µmole /mg.of protein)	2.720±0.070	1.830±0.069	32.72
Glutathione peroxidase (GPx) (µmoles/mg protein)	1.315±0.003	1.007±0.001	-23.42
Catalse (CAT) (Unit/mg.of protein)	1.851±0.005	0.970±0.003	-47.54
Superoxide dismutase(SOD)(Unit/mg.of protein)	0.800±0.031	0.440±0.018	-45

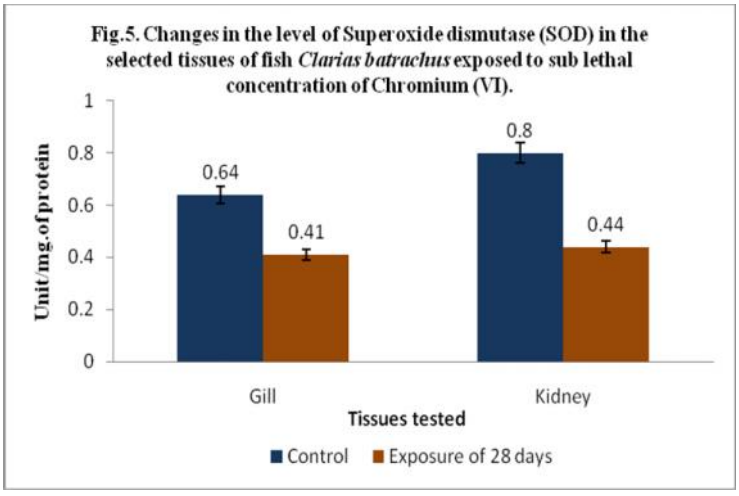
The values are Mean ± S.E of six individual observations, *Significance ($p<0.05$) of student 't' test.











Discussion

Heavy metal promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. The ROS enhances the peroxides and reactive hydroxyl radicals (Miller *et al*, 1991; Hussan *et al*, 1999). These lipid peroxides and hydroxyl radicals may cause cell membrane damage and thus destroy the cell. Heavy metal increases the rate of formation of reactive oxygen species including superoxide anion radical O2 and hydroxyl radical (OH) through a chain reaction (Yamanaka *et al.*, 1991). In the present study, the free radical scavenger enzymes GPx, CAT, and SOD were reduced in the sub lethal concentration of

chromium toxicity in *Clarias batrachus*. The inhibition of these fress radical scavenger enzymes might be due to interaction of sublethal concentration of chromium directly with metal ion, which is dependent on subcellular origin. It is possible that chromium in the tissue interacts with the metal moiety and produces inhibition of enzyme activity (Chandravathy and Reddy, 1999). Similarly, Rana *et al*., (1996) and Romeo *et al.*, (2000) reported that cadmium increases the formation of lipid peroxidation in rats and fishes. Several studies have been shown that there is increase in the formation of oxygen free radicals or reactive oxygen species (Stohs and Bagchi, 1995). The present results showed the decrease in GSH levels, which can explain the increased ROS concentration and of LPO levels in chromium exposed fish. The similar results have been observed in the Indian catfish (*C. batrachus*) after exposure to low concentration of Arsenic (Battacharya and Battacharya, 2007). The decrease in the activities of these two enzymes can inhibit the citric acid cycle and thereby decrease the generation of reducing equivalents such NADH and NADPH, which impairing ATP production (Ramanathan *et al.*, 2003) and oxygen reduction to form water. Moreover, Metal can affect NADH dehydrogenase and cytochrome oxidase. The significant decline in the activity of these two enzymes would result in the inhibition of electron flow from NADPH to oxygen, augmenting the chance of ROS generation and lowering oxygen consumption. (Rmanathan *et al.*, 2003; Battacharya and Battacharaya, 2007) Soundararajan *et al.*, 2009) reported that the alterations of GSH, SOD and Catalase in liver tissues of *Tilapia mossambica* treated with Arsenic and suggested that liver is an active site for synthesis of these antioxidant enzymes. Basha and Rani (2003) also noted significant elevations of SOD and catalase activities in liver and kidney from day 7 onward, andthese activities were maintained until day 15 and then decreased slightly on day 30 of exposure. Basha and Rani (2003) suggested that upregulation of enzyme production might be a defense mechanism, providing first line of defense against metal toxicity before the induction of metallothionein synthesis. Fattorini and Regoli (2004) observed remarkable accumulation of Arsenic in the branchial crown of *Sabella spallanzanii* with dimethylarsinate (DMA) as the main Arsenic metabolite, while in another polychaete species, *Arenicola marina*, Arsenic is accumulated mostly in the inorganic forms (Geiszinger *et al.*, 2002). It can be stated that chromium affects the antioxidant responses in *Clarias batrachus* in terms of increased lipid peroxidation which could impair ATP production, and triggering oxidative damage.

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