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RESEARCH ARTICLE

SHIFTS IN PROTEIN METABOLISM IN LIVER, KIDNEY AND BRAIN OF INDIAN MAJOR CARP, *Labeo rohita* (HAMILTON) UNDER HEAVY METAL, NICKEL CHLORIDE STRESS

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ABSTRACT

Heavy metal pollution is one of the main anthropogenic pollutions causing serious and long lasting damage to all living organisms. The effect of nickel chloride on protein and amino acid contents of liver, kidney and brain of freshwater fish, *Labeo rohita* have been studied. The fish were exposed to sublethal concentration of nickel chloride 1/5th (high), 1/10th (medium) and 1/15th (low) of the 96 hour LC_{50} for the period of 10 and 20 days. All the sublethal concentration of nickel chloride exposed fish for the period of 10 and 20 days showed decrease the protein and increase the amino acid content in liver, kidney and brain of *Labeo rohita*. The significant alterations showed toxic effect of heavy metal nickel chloride at biochemical levels.

INTRODUCTION

Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystem (Oymak *et al.,* 2009). Due to their toxicity, long persistence, bioaccumulative and non-biodegradable properties in the food chain, heavy metals constitute a core group of aquatic pollutants (Uysal *et al.,* 2008). Different kinds of organisms may be used to determine the mechanisms of action of pollutants on specific physiological functions (Gul *et al.,* 2004; Kandemir *et al.,* 2010). Heavy metals are being passed on into aqueous environments through industrial processes, sewage disposal, soil leaching and rainfall. The concentrations of these heavy metals are sublethal or lethal to aquatic organisms when the duration of exposure to these metals are prolonged (Eisler and Gardener, 1973). The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to pollution. With an early use of metals, there was little concern about environmental contamination. However, salts of the metals began to find their way into commercial and industrial applications and then it became evident that metallic salts possess certain biocide properties. Though, many metals play a vital role in the physiological processes of plants, animals and humans, yet excess concentration of metals is harmful. Pollution implies deleterious effects and is usually assessed in relation to biological system (Bhilave *et al.,* 2008). Nickel is introduced into the hydrosphere by removal from the atmosphere by surface run-off by discharge of industrial and municipal waste and also following natural erosion of soils and rocks (Babukutty and Chacko, 1995).

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Nickel concentrations in highly contaminated freshwaters may reach as high as several hundred to 1000 µg/l (Eisler, 1998). In the present study attempt has been made to find out the high, medium and low sublethal concentration of heavy metal, Nickel chloride in respect to protein and amino acid contents changes in liver, kidney and brain of freshwater fish, *Labeo rohita*.

MATERIALS AND METHODS

The fish *Labeo rohita* having mean weight 14-16 gm and length 12 – 14 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1%KMNO4 solution and then kept in plastic pools for acclimatization for a period of seven days. They were fed on rice bran and oil cake daily. The nickel chloride was used in this study and stock solutions were prepared. Nickel chloride LC_{50} was found out for 96 h (32.64 ppm) (Sprague, 1971) and 1/5th ,1/10th and 1/15th of the LC_{50} values were 6.528, 3.264 and 2.176 ppm respectively taken as sublethal concentrations for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from nickel chloride and served as the control. The other 3 groups were exposed to sub lethal concentration of nickel chloride in 10 litre capacity aquaria. The 2nd, 3rd and 4th groups were exposed to nickel chloride for 10 and 20 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein estimation. The nickel chloride was used in this study and stock solutions were prepared. Nickel chloride LC_{50} was found out for 96 h (32.64 ppm) and 1/5th ,1/10th and 1/15th of the LC_{50} values were 6.528, 3.264 and 2.176 ppm respectively taken as sublethal concentrations for this study. Fishes were exposed to sublethal concentrations of nickel chloride separately in plastic troughs and control fishes were also maintained separately. They were fed on ad libitum diet of rice bran and oil cake. The medium was renewed daily with sublethal concentration of the nickel chloride. After the exposure period, *Labeo rohita* were sacrificed and the liver, kidney and brain were removed for biochemical assays. The protein and amino acid content of the tissues were estimated by the method of Lowry *et al.* (1951) and Moore and Stein (1954) respectively. The data so obtained were analysed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

RESULTS

Depletion of protein content of the liver, kidney and brain of *Labeo rohita* exposed to the nickel chloride for 10 and 20 days in $1/5^{th}$, $1/10^{th}$ and $1/15^{th}$ of the LC_{50} values of sublethal concentrations were estimated. Among these, the maximum depletion of protein was observed in liver during 20 days. Generally, depletion in protein content is directly proportional to the exposure period of the toxicant. The obtained biochemical estimation values of the liver, kidney and brain were subjected to statistical analysis and showed significant values at $P<0.05$ (Table. 1). Elevation in the amino acid content in the liver, kidney and brain of the *Labeo rohita* exposed to nickel chloride for 10 and 20 days in $1/5^{th}$,$1/10^{th}$ and $1/15^{th}$ of the LC_{50} values of sublethal concentrations were estimated. Amino acid content in the liver, kidney and brain are shown in Table. 2. Liver of the nickel chloride treated fish shows a gradually increase in amino acid level. Elevation of amino acid was observed at all exposure periods and liver, kidney and brain.

DISCUSSION

The rapid development of industry and especially chemical industry has created serious problems of water pollution. Human destructive influence on the aquatic environment is in the form of sublethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Mason, 1991). Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by organisms, such as fish (Emami Khansari *et al.,* 2005). Liver is the center for metabolism and detoxification in piscine body (Athikesavan *et al.,* 2006). The liver plays an important role in the synthesis of proteins. Liver is the site of metabolism (Harfer *et al.,* 1977). The kidney plays a principal role in the accumulation, detoxification and excretion of nickel and is considered to be a target organ for nickel toxicity (WHO, 1991; Eisler, 1998). The kidney, which is an important organ of excretion and osmoregulation, is indirectly affected by pollution through blood circulation (Newman and Mclean, 1974). The kidney and liver have been proposed as the major target organs for environmental contaminants such as heavy metals, and they are important organs for metabolic waste excretion and heavy metal elimination in fish (Laurent and Dunel, 1980). Fishes are regarded to be the most susceptible bioindicators of all hydrobionts (Hybia, 1982; Hedreyanov and Paoopsu, 1983). The negative effect of heavy metals on fishes is related to the disturbance in their biochemical and physiological processes (Hollis *et al.,* 1990; Vilella *et al.,* 1999). The fish, as a bioindicator species, plays an increasingly important role in the monitoring of water

Table 1. Protein changes (mg/g wet wt. of tissue) in liver, kidney and brain of *Labeo rohita* exposed to sublethal concentration of nickel chloride for 10 and 20 days

Groups	Liver		Kidney		Brain	
	10 days	20 days	10 days	20 days	10 days	20 days
Control	125.34 ± 9.55^{c}	128.76 ± 9.80^{c}	80.73 ± 6.15^{a}	81.59 ± 6.21^{b}	74.47 ± 5.67^{b}	75.28 ± 5.73^{c}
High	92.30 ± 7.03^{a}	88.68 ± 6.75^{a}	74.37 ± 5.66^{a}	70.23 ± 5.35^{a}	65.53 ± 4.99^{a}	58.39 ± 4.99^{a}
Medium	97.08 ± 7.39^{ab}	95.34 ± 7.26^{a}	75.15 ± 5.72^{a}	71.66 ± 5.46^{a}	67.29 ± 5.13^{a}	60.75 ± 4.63^{ab}
Low	106.16 ± 8.08^{b}	109.52 ± 8.34^{b}	78.63 ± 5.99^{a}	76.85 ± 5.86^{ab}	70.15 ± 5.34^{ab}	65.33 ± 4.98^{b}

All the values are mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% level ($p < 0.05$)
Duncan's multiple range test (DMRT)

Table 2. Amino acid changes (mg/g wet wt. of tissue) in liver, kidney and brain of *Labeo rohita* exposed to sublethal concentration of nickel chloride for 10 and 20 days

Groups	Liver		Kidney		Brain	
	10 days	20 days	10 days	20 days	10 days	20 days
Control	4.78 ± 0.36^{a}	4.81 ± 0.36^{a}	2.54 ± 0.19^{a}	2.60 ± 0.20^{a}	2.38 ± 0.18^{a}	2.40 ± 0.18^{a}
High	8.92 ± 0.68^{d}	12.36 ± 0.94^{d}	4.25 ± 0.32^{d}	6.32 ± 0.48^{d}	3.86 ± 0.30^{d}	4.55 ± 0.35^{d}
Medium	6.76 ± 0.52^{c}	8.55 ± 0.65^{c}	3.72 ± 0.28^{c}	4.58 ± 0.35^{c}	3.12 ± 0.24^{c}	3.70 ± 0.28^{c}
Low	5.84 ± 0.45^{b}	6.24 ± 0.47^{b}	3.08 ± 0.24^{b}	3.69 ± 0.28^{b}	2.75 ± 0.21^{b}	3.36 ± 0.26^{b}

All the values are mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% level ($p <0.05$)
Duncan's multiple range test (DMRT)

pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicates heavy pollution; the effects of exposure to sublethal levels of pollutants can be measured in terms of biochemical, physiological or histological responses of the fish organism (Mondon et al. 2001). Changes in age and species distribution in a stock fish population are general indicators of water pollution, but there are also responses specific to a single pollutant or a group of contaminants. Biochemical markers are biochemical responses induced in the presence of a specific group of contaminants that have the same mechanism of toxic activity (Iroka and Drastichova, 2004). Proteins are the building units of the body and are also the most abundant macromolecules in the cells constituting half of their dry weight (Jaleel *et al.,* 2008). They regulate and integrate various physiological and metabolic processes (Jaleel *et al.,* 2007) in the body. Further, the protein content of the fish body mainly determines the quality and food value of its flesh. The maximum concentration of protein in the liver clearly shows the importance of liver in the body metabolism as well as its role as the major storehouse of various metabolites (Guyton and Hall, 1996). Further, a higher content of the protein in the liver may be attributed to the fact that liver synthesizes most of the plasma protein (Lehninger, 1983). Proteins are involved in major physiological events therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are highly sensitive to heavy metal poisoning (Jacobs *et al.,* 1977). In the present study exhibited the levels of protein significantly decreased and an elevation of amino acids in liver, kidney and brain of freshwater fish, *Labeo rohita* exposed to high, medium and low sublethal concentration of nickel chloride. Liver recorded more protein content when compared to kidney and brain. Liver showed higher protein content which might be due to greater concentration of enzymes. Many investigators have also recorded such reduction in the protein content in the kidney which could possibly be due to protein break down leading to increased amino acid pool of tissue (Radhiah *et al.,* 1987). Depletion of protein content has been observed in the muscle, intestine and brain of the fish *Catla catla* as a result of mercury chloride toxicity (Martin Deva Prasath and Arivoli, 2008). When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted (Neff, 1985).

Rao (1989) has reported decreased protein content in kidney of *Catla catla* after exposure to endosulfan. The different concentration of malathion, thiodon and ekalux significantly reduced the total protein in liver of *O. mossambicus* (Palanichamy *et al.,* 1986). Eva (1990) has observed that an increase in amino acid content both in liver and intestine of *Anabas testudineus* when exposed to sublethal concentration of Cuman L. Balaji and Chockalingam (1990) have reported that the increase in amino acid content in liver of *Channa punctatus* when exposed to sublethal concentration of dairy effluent. Appreciable decrease in protein level of liver, muscle, intestine, gill and blood of *Heteropneustes fossilis* was noticed after the exposure of fish to nickel for 30, 60 and 90 days (Prasanta Nanda *et al.,* 2000). Jha (1991) has observed protein depletion in liver, intestine and gonads of fresh water murrel, *Channa punctatus* under the stress of heavy metal, lead. Natarajan *et al.* (1983) have observed that the Metasystox exposed *Channa striatus* showed increase in amino acid content in the kidney, gill and liver and also further they have reported that the increase in amino acid may be due to proteolysis and were routed through gluconeogenesis for increasing the energy supply to cope up with the pesticide stress. A reduction in the protein content in the present investigation in *Labeo rohita* suggests that the tissue protein undergoes proteolysis, which results in an increase in the production of free amino acids. These amino acids are utilized for energy production during stressful situation in the intoxicated fishes. Many investigators have also recorded such a reduction in protein content in fishes exposed to different toxicants (Karuppasamy, 1990 and 2000; Rao, 1989). It is evident that proteins are degraded to meet the energy requirements during nickel chloride exposure. It can be concluded that in *Labeo rohita* exposed to nickel chloride at sublethal concentration causes energy crisis and alter protein metabolism.

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