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Impact of Heavy Metal, Chromium on Protein Metabolism in Brain and Muscle of Freshwater Fish, *Channa striatus* (BLOCH)

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A B S T R A C T

Major adverse effects on environmental quality, ecosystem integrity and human health have often been associated with mismanagement of chemical materials and the eliminations of hazardous substances. Heavy metals from geological and anthropogenic sources are increasingly being released into natural waters. Contamination of aquatic ecosystems with heavy metals has seriously increased worldwide attention. Heavy metals present in effluents discharged by the leather industries constitute the most common group of toxic and non-degradable substances. On reaching the aquatic ecosystem, they pose a serious threat to the biotic living there in especially fish. Fishes have a great significant in the life of mankind, being a most important source of protein and providing other useful products. The aim of the present study was to assess the protein and amino acid levels in brain and muscle of *Channa striatus* was exposed to sublethal concentrations of chromium for the period of 10, 20 and 30 days. The fish exposed to chromium showed a decrease the protein and increase the amino acid levels for 10, 20 and 30 days in brain and muscle. The objective of the present investigation was to observe the effect of chromium on protein and amino acid levels in the brain and muscle of freshwater fish, *Channa striatus.*

Introduction

Environmental pollutants, such as heavy metals, economic poison like pesticides, effluents from industries pose serious risks to many aquatic organisms. The world wide problem is the environmental pollution and most important pollutants are the heavy metals in aquatic network because of their toxicity, accumulation and bio-magnification by aquatic creatures. Domestic, industrial and anthropogenic activities may broadly become the source of natural aquatic systems contamination with heavy metals (Conacher and Mes, 1993; Velez and Montoro, 1998; Afshan *et al.*, 2014). Water pollution by heavy metals, especially chromium pollution from industrial sources can affect aquatic life, all ecosystems and human health directly or through food chain. Heavy metals are introduced into the environment by a wide spectrum of natural sources such as volcanic, erosion and anthropogenic activities including industrial wastes as well as a leakage (Yilmaz *et al.*, 2010). Water pollution is defined as introduction by man, directly or indirectly, of substances or energy to the aquatic environment resulting in deleterious effects such as hazards to human health, hindrance of fish activities and impairment of water quality. A human activity causes contamination of aquatic medium leads to spoil the aquatic life of flora and fauna especially fish. The industrial effluent containing heavy metal like mercury, chromium, lead, cadmium, nickel and arsenic enormously released without treatment nearby river, lake, pond and sea. The aquatic organisms are very much affected due to heavy metal.

Heavy metal pollution of the aquatic medium has long been recognized as a serious environmental problem. Heavy metals reach to the aquatic environment from natural and anthropogenic sources and distributed in the water bodies, suspended solids and sediments during the course of their transportation (Olajire and Imeokparia, 2001; Adeniyi *et al.*, 2005; Aderinola *et al.*, 2009). The consequence of heavy metal pollution can be hazardous to man and it often becomes mandatory to check chemical contaminants in foods from the aquatic environment to understand their hazard levels. Heavy metals like chromium proven to be persistent pollutants. Though present in traces heavy metals tend to bioaccumulate and biomagnifying. Their accumulation in biotic tissues causes toxic effects. Chromium is considered as a heavy metal and pollutant as well as an essential micronutrient. Wastewater pollution by chromium originating from electroplating, dyeing, tannery, hard alloy steel and stainless steel manufacture, has affected the life on earth. Chromium is also used as a catalyst and coating material (Idachaba *et al.*, 2004). Welding, grinding and polishing of stainless steel are among principal ways of introducing chromium into the land environment while other ways of introducing chromium into air and water environments include the burning of fossil fuels and waste incineration (WHO, 1988). This pollution could affect all ecosystems and human health directly or through food chain (Yılmaz *et al.*, 2009). Chromium exists in different oxidation states which have distinct biological effects (Richard, 1991). Hexavalent chromium (Cr VI) is a well known carcinogen metal form for animals and human beings. Cr (VI) compounds readily penetrate into cell membranes via anion transport systems. Fish are often at the top of the aquatic food chain and affected by heavy metals which is present in the waste water from industries (Mansour and Sidky, 2002).

Human beings have been exposed to heavy metals through food chain. Industrialization of the world has dramatically increased the overall environmental 'load' of heavy metal toxins to the point that our societies are dependent upon them for proper functioning. Now a day, heavy metals are abundantly available in our environmental condition due to increased use of these compounds in the industries. It is very difficult to avoid exposure to harmful heavy metals that are so prevalent in our environment. Human consuming more fish that may be collected from polluted area. The heavy metal enters through food chain especially fish to reach human beings and may cause metabolic diseases, nervous disorder, headache, vomiting and cancer. Heavy metal chromium contributes to a variety of adverse health effects. Hence, the present investigation was to assess the protein and amino acid content in brain and muscle of *Channa striatus* exposed to sublethal concentrations of chromium.

Materials and Methods

The fish *Channa striatus* having mean weight 18 - 22 g and length 13 – 15 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 two weeks. They were fed twice daily i.e. morning and evening on boiled chicken eggs approximately 4% of fish body weight divided into two equal meals daily. The chromium was used in this study and stock solutions were prepared. Chromium, LC_{50} was found out for 96 h (34 ppm) (Sprague, 1971) and $1/20^{th}$ (1.7 ppm), $1/15^{th}$ (2.27 ppm) and $1/10^{th}$ (3.4 ppm) 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 chromium and served as the control. The other 3 groups were exposed to sub lethal concentration of chromium, 10 litre capacity aquaria. The 2^{nd}, 3^{rd} and 4^{th} groups were exposed to chromium, for 10, 20 and 30 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein and amino acid estimation. The protein and amino acid content in brain and muscle of *Channa striatus* were estimated by the method of Lowry *et al.*, 1951 and Moore and Stein (1954) respectively. The data obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

Results and Discussion

Protein level in brain

The protein contents were observed in the control brain to be 82.83 ± 5.94, 83.64 ± 6.00 and 84.21± 6.05 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with low (78.51 ± 5.61, 73.69 ± 5.25 and 68.69 ± 4.87), medium (75.65 ± 5.40, 69.11 ± 4.90 and 65.33 ± 4.61) and high (73.89 ± 5.26, 66.75 ± 4.71 and 60.62 ± 4.25) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively (Fig 1).

Protein Level in Muscle

The protein contents were observed in the control muscle to be 76.71 ± 5.48, 77.63 ± 5.54 and 77.79 ± 5.56 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with low (74.69 ± 5.32, 71.83 ± 5.10 and 66.63 ± 4.71), medium (73.12 ± 5.20, 69.65 ± 4.91 and 64.67 ± 4.56) and high (70.85 ± 5.03, 67.71 ± 4.79 and 60.59 ± 4.25) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively. The decrease in the protein content of the liver was more in 30 days of exposed fish compared to 10 and 20 days (Fig 1).

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Amino Acid Level in Brain

The amino acid contents were observed in the control brain to be 3.60 ± 0.27, 3.62 ± 0.27 and 3.57 ± 0.27 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly increased when the fish *Channa striatus* exposed with low (3.97 ± 0.30, 4.58 ± 0.35 and 6.06 ± 0.46), medium (4.34 ± 0.33, 4.92 ± 0.37 and 6.58 ± 0.50) and high (5.08 ± 0.39, 5.77 ± 0.44 and 7.94 ± 0.60) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively. In sublethal concentrations of heavy metal chromium exposed fish, *Channa striatus* the amino acids showed a gradual increase from 10 to 30 days. The maximum increase was noticed in high sublethal concentration of heavy metal chromium at 30 days of exposure periods (Fig 2).

Amino Acid Level in Muscle

The amino acid contents were observed in the control muscle to be 3.42 ± 0.26, 3.40 ± 0.26 and 3.43 ± 0.26 mg g^{-1} wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly increased when the fish *Channa striatus* exposed with low (3.96 ± 0.29, 4.44 ± 0.34 and 4.98 ± 0.38), medium (4.40 ± 0.33, 4.77 ± 0.36 and 5.34 ± 0.40) and high (4.57 ± 0.31, 5.51 ± 0.42 and 6.66 ± 0.51) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively. In sublethal concentrations of heavy metal chromium exposed fish, *Channa striatus* the amino acids showed a gradual increase from 10 to 30 days. The maximum increase was noticed in high sublethal concentration of heavy metal chromium at 30 days of exposure periods (Fig 2).

The rapid industrialization is one of the major causes of water pollution. The discharges of untreated and partially treated wastewater from various industries like chemical, pesticides, fertilizer, pulp and paper and sugar etc., have polluted the aquatic bodies such as river, pond and ditches. In India about two tones wastewater is discharged into aquatic bodies annually from industries (Shaffi, 1981). Discharges of industrial wastewater, cause serious consequence for fisheries resulting in impairment of important function such as respiration and osmoregulation (Kumaraguru, 1995; Yadav *et al.*, 2007). Toxicants produce many physiological and biochemical changes in freshwater organisms by influencing their activities. Alterations in the chemical composition of the natural aquatic environment usually affect behavioral and physiological systems of the inhabitants, particularly those of the fish (Radhaiah *et al.*,1987). Fish mortality due to toxicant exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure (Ram *et al.*, 2009). The detection of abnormal activity is based on comparisons of the responses of exposed fish, either with activity measured during a baseline or pre-exposure period or observations of fish under a control treatment (Richmond and Dutta, 1992). The natural physiological functioning of an organism gets disturbed on exposure to toxicant stress. It induces its effect first at cellular or even at molecular level, but ultimately causes physiological, pathological and biochemical alterations. It is, therefore necessary to focus attention on changes in biochemical composition of organisms, which are constantly under pollutant threat (Venkata Rathinamma and Nagaraju, 2013). Pollutants enter the fish body through a number of routes: directly through the digestive tract due to consumption of contaminated water and food or non-dietary routes across permeable membranes such as the gill or skin (Burger *et al.*, 2002; Banaee *et al.*, 2013).

Fishes are major sources of protein. They constitute major components of most aquatic habitats acting as bio-indicator of heavy metal levels in the aquatic environment (Banaee *et al.*, 2013). 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). Protein are of the most important and complete group of biological material comprising of nitrogenous constituents of the body and performing different function, proteins are involved in several major physiological events. Therefore the assessment of protein content can be considered as a diagnostic tool to determine the physiological phases of organisms.

The amino acids are the basic building block of all proteins. Those amino acids which exist in free from in tissues and not bound to proteins are called as free amino acids. Normally during diseased conditions in plants and animals there will be a change in the total free amino acids composition. Hence the estimation of total free amino acid gives as indication about the physiological and health condition of animal (Bais and Lokhande, 2012).

The present investigation, in the brain and muscle protein content had decreased whereas amino acids content had increased at all periods of exposure when *Channa striatus* was exposed with sublethal concentrations of chromium for the periods of 10, 20 and 30 days exposure. The free amino acid pool was increased in the tissues of the fish during exposure to lihocin (Abdul *et al.*, 2010), while the elevated free amino acid contents were utilized for energy production by supplying them as keto acids into TCA cycle through aminotransferases to contribute energy needs during toxic stress. Increased free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.*, 1996).

The decrease the protein and lipid might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins which are important cellular constituents of cell membranes and cell organelles present in the cytoplasm (Harper, 1963). Increase in free amino acid in protein synthesis (Singh *et al.*, 1996). It is also attributed to lesser use of amino acids (Seshagiri, Rao *et al.*,1987) and their involvement in the maintenance of an acid base balance (Moorth *et al.*, 1984; Natarajan, 1985) suggested that stress conditions induce elevation in the transamination pathway. The decreases in the total protein level and increases in the total free amino acid level in liver and muscle suggest the high protein hydrolytic activity due to elevation of protease activity (Zodape, 2011). Simillarly Senthil Elango and Muthulingam (2014) suggested that declining trends of protein and elevated levels of amino acid in brain and muscle of *Oreochromis mossambicus* was exposed to sublethal concentrations of chromium. The protein content decreased in the liver, brain and kidney tissues of *Channa punctatus* during lihocin treatment (Abdul *et al.*, 2010).

Satyaparameshwar *et al.*, (2006) suggested that *Lamellidens marginalis* exposed to sublethal concentration of chromium showed decrease the protein and RNA where as amino acid level was elevated. The decreased protein level was observed in the kidney tissue of *Catla catla* at sublethal concentration of chromium (Vincent *et al.*, 1995).

Muscle rich in proteins, forms mechanical tissue intended for mobility and do not participate in metabolism. Liver being the centre for various metabolisms is also rich in proteins. In the gill, liver, kidney, heart and brain tissues of the exposed fish, the total protein content was found to be reduced (Sobha *et al.*, 2007). Protein and glycogen contents were decreased in gill, muscle and intestine of Zebra fish, *Danio rerio* exposed to sublethal concentration of mercuric chloride (Vutukuru and Kalpana, 2013).

Fig.1 Protein levels changes (mg/g) in brain and muscle of *Channa striatus* exposed to sublethal concentrations of chromium

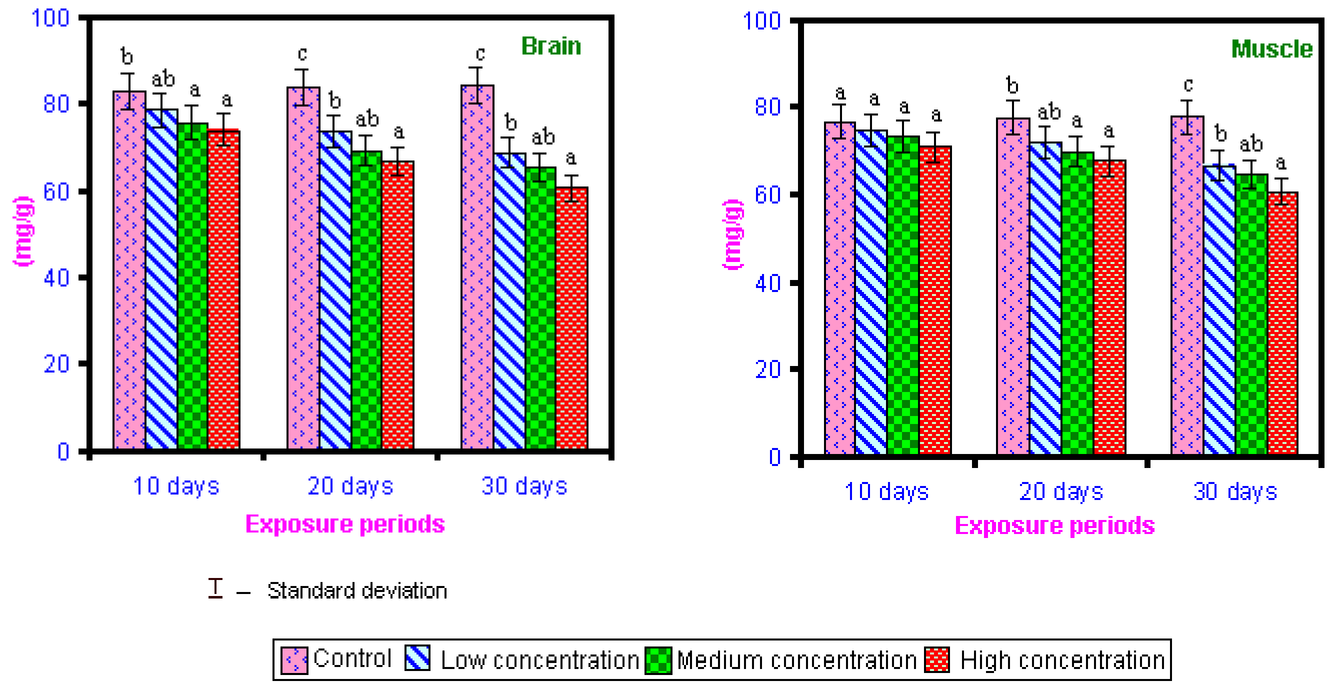
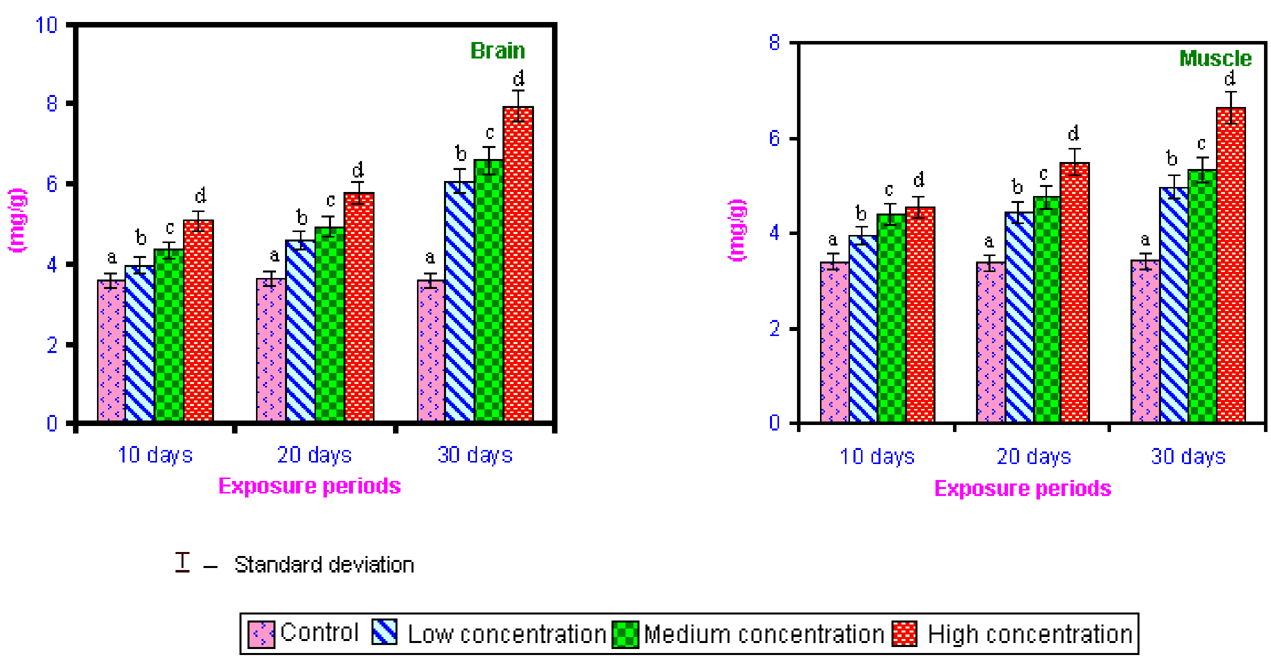


Fig.2 Amino acid (mg/g) in brain and muscle of Channa striatus exposed to sublethal concentrations of chromium



Many investigators have also recorded such a reduction in protein content in fishes exposed to different toxicants. A reduction in the protein content in the present investigation in *Channa striatus* exposed to sublethal concentrations 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. Anitha *et al.,* (2014) reported that freshwater fish, *Labeo rohita* treated with sub lethal concentrations of Fosmite shows depletion of total protein content in brain, muscle, gill, liver and kidney. The total protein and glycogen content reduced in muscle, brain, liver, kidney, gill and gut of *Labeo rohita* exposed to sublethal concentration of phenthoate (Somaiah *et al.,* 2014). The two sublethal concentration of azodrin exposure results show that decrease in the level of protein content in brain, muscle, ovary, intestine, gills and liver (Janardana Reddy *et al.,* 2016). Protein, glycogen and lipid contents were decreased in gill, liver, kidney and muscle of *Labeo rohita* after acute and chronic exposure to textile mill effluent (Nikalje *et al.,* 2011). 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). Protein and glycogen contents were decreased whereas amino acid and glucose levels increased in gill, liver, kidney and muscle of *Cirrhinus mrigala* exposed to sublethal concentration of nickel for the periods of 7, 14, 21 and 28 days (Parthipan and Muniyan, 2013). The protein content was decreased in liver, brain and kidney of *Channa punctatus* during sublethal concentration of triazophos (Naveed *et al.,* 2010). The level of total protein content was found to decrease in the liver and muscle tissues of fish, *Labeo rohita* were exposed to arsenic (Zodape, 2010). Declining trends in protein, lipid and glycogen contents whereas amino acid level and protease activity was elevated in liver and muscle of the fish, *Labeo rohita* exposed to sublethal concentration of zinc (Zodape, 2011). Protein and lipid content of vital organs like gill, liver, muscle and kidney depleted in exposed to chromium (Arillo *et al.,* 1982; Sastry and Sunitha, 1984; Ambrose *et al.,* 1994). Moorthikumar and Muthulingam (2010) noticed that decline in the protein and elevated contents of amino acid in liver, kidney and brain of *Labeo rohita* under heavy metal, nickel chloride stress. It is evident that proteins are degraded to meet the energy requirements during sublethal concentrations of chromium. The depletion in protein level was due to diversification of energy to meet the impending energy demand when the fish *Channa striatus* exposed to sublethal concentrations of heavy metal chromium.stress. The reduction in protein content in the present study indicates that the tissue protein undergoes proteolysis resulting in the production of free amino acids leads to disturbances in the physiological activity of the fish *Channa striatus*.

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