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RESEARCH ARTICLE

HEAVY METAL CHROMIUM INDUCED ALTERATIONS ON GLYCOGEN CONTENT IN KIDNEY, BRAIN AND MUSCLE OF FRESHWATER FISH *Oreochromis mossambicus* (PETERS)

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ABSTRACT

The aquatic medium such as pond, lake, estuary, water reservoirs and rivers surrounding the industrial, agricultural fields and urban areas in India, pose a serious risk for survival of aquatic organisms due to water quality deterioration through industrial effluents, excessive nutrient inputs, acidification, heavy metal, fertilizer contamination and organic pollution. Chromium is known to be a very toxic pollutant introduced into natural waters from a variety of sources including industrial effluents. The aim of the present study is to assess the protein and amino acid content in brain and muscle of the fish *Oreochromis mossambicus* exposed to sublethal concentrations of heavy metal chromium 1/10th (high), 1/15th (medium) and 1/20th (low) of the 96 hour LC_{50} values for the period of 10, 20 and 30 days. The fish exposed to chromium showed a decrease the glycogen levels in kidney, brain and muscle for 10, 20 and 30 days. However, no information is on record concerning the three different sublethal concentration of heavy metal chromium on the glycogen levels of fish. The objective of the present work was to assess the effect of heavy metal chromium on glycogen levels in kidney, brain and muscle of freshwater fish, *Oreochromis mossambicus.*

INTRODUCTION

Nature, nowadays is suffering from a serious problem of environmental pollution. Industrial effluents, a major source of pollution, are found to contain a number of metal ions, which are toxic to aquatic life at higher levels (Geeta Dangi *et al*., 1998). The increasing industrialisation, urbanisation and developmental activities to cope up with the population explosion have brought inevitable water crisis. The industrial wastes containing toxic chemicals are released into the natural water bodies causing severe water pollution (Dagan, 1972). The world population is rapidly increasing day by day. At the same time, many harmful diseases also originate and affect all living beings because of the impurities in our environment, nutrition and human activities. At the same time, Tannery industries are also causing certain amount of pollution in the environment, particularly in the hydrosphere through its discharged effluents. Therefore rivers, lakes, estuaries and ocean get polluted by the effluents. Scientific studies indicate that different pollutants have different effects on living aquatic organisms and on fisheries. Thousands of tons of fish are killed annually in freshwater by discharged chemicals (Kumaraguru, 1995). Water pollution is the addition of something that changes its natural qualities (Coulson and Forbes, 1952). Polluted water becomes unsuitable for drinking purposes. If poisonous substances get dissolved in water it becomes unsuitable not only for aquatic life and also for agricultural operations. Rivers, lakes and ponds are used by people for bathing, washing cloths and even for drinking purposes. This contaminates water with the germs causing diseases, such as cholera, typhoid, dysentery and jaundice. The trace wastes and effluents of industries play a significant role in water pollution. In Tamilnadu, there are 430 tanneries, 8 distilleries, 22 sugar factories, 600 textile mills, 390 chemical industries and 10 fertilizer units which are the major sources of pollution, not only surface water but also in groundwater (Kudesia, 1985). Tanning industry contributes significantly towards exports, employment generation and occupies an important role in the Indian economy while on the other hand; tannery wastes are ranked as the highest pollutants among all the industrial wastes (Soyaslan *et al.,* 2007). Damage to the environment by the hazardous tannery effluent is becoming an acute problem in Indian (Taju *et al.*, 2012). Heavy metals are ubiquitous in the biosphere, where they occur as part of the natural background of chemicals. Anthropogenic activities have also introduced substantial amounts of them into the environment by mobilization from their natural insoluble deposits or environmental sinks (Chiesa *et al.,* 2006). They represent a significant ecological and public health concern due to their toxicity and their ability to accumulate in living organisms. Heavy metals are widespread pollutants of great concern as they are non-degradable and thus persistent. Pollution of the environment by toxic heavy metals is largely as a result of industrial activities such as mining, electroplating,

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leather tanning, etc (Onyancha *et al.,* 2008). Heavy metals are those metals with densities greater than 5 g/cm^3 and atomic number greater than that of calcium. Some form organo-metallic compounds which are more toxic than their elemental or ionic forms in the environment (Craig, 1983). They occur naturally as trace elements while manmade additions sometimes lead to environmental pollution. The use of heavy metals in industrial and agricultural sectors is increasingly becoming a source of concern due to their harmful effects on the environment and human health (Igbo *et al.,* 2014).

Heavy metals are one of the five major types of toxic pollutants present in fresh water (Mason, 1991). The important environmental pollutants are those that tend to accumulate in organisms, those which are persistent because of their chemical stability or poor biodegradability, and those which are readily soluble and therefore environmentally mobile (Hellawell, 1986). Heavy metals pollutants have been found to be dangerous to the environment (Phillips, 1991). Some of them are essential elements that are required for the normal metabolism of the organism including Cu, Fe and Zn, while the others are non-essential and play no significant role including Cr, Pb and Cd (Sanders, 1997). Their natural effects can be toxic (acute, chronic or sub-chronic), carcinogenic, mutagenic or teratogenic (Young, 2005; EI-Morshedi *et al.,* 2014). Chromium is a transition element and only its trivalent and hexavalent forms have biological significance. Trivalent chromium is more common in nature, while hexavalent chromium is of industrial importance. Hexavalent chromium compounds are carcinogenic and are used in chromium plating, cement and paint production industries, presenting high potential for contamination of aquatic environment (Castro, *et al.,* 2014). Fish represents the higher tropic level in the aquatic food chain. Therefore, persistence of toxic chemical accumulates to a maximum concentration in their body when compared to other organisms in the aquatic environment (Sackmauerova *et al*., 1977). Fish is widely consumed in many parts of the world because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health (Ikem and Egiebor, 2005). Fish are constantly exposed to chemicals in polluted and contaminated waters. Fish have been found to be good indicators of heavy metal contamination in aquatic systems because they occupy different trophic levels and are of different sizes and ages (Burger *et al.,* 2007; Tuzen and Soylak, 2007). The present investigation was to assess the glycogen levels in kidney, brain and muscle of *Oreochromis mossambicus* exposed to three different sublethal concentration of heavy metal chromium.

MATERIALS AND METHODS

The fish *Oreochromis mossambicus* having mean weight 23-28 g and length 11 – 13 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 on rice bran and oil cake daily. The $K_2Cr_2O_7$ (Potassium dichromate) was used in this study and stock solutions were prepared. Chromium, LC_{50} was found out for 96 h (25 ppm) (Sprague, 1971) and $1/20^{th}$, $1/15^{th}$ and $1/0^{th}$ of the LC_{50} values were 1.25, 1.66 and 2.5 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 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 glycogen estimation. The glycogen content in kidney, brain and muscle of *Oreochromis mossambicus* was estimated by the method of Kemp and Kits Van Heijininger (1954). The data so obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

RESULTS

The glycogen contents in kidney, brain and muscle of *Oreochromis mossambicus* exposed to low, medium and high sublethal concentrations of heavy metal chromium showed significant decrease the content of glycogen when compared to control fish. *Oreochromis mossambicus* when exposed to sublethal concentrations of heavy metal chromium was more pronounced at 30 days (Table 1).

Table 1. Glycogen (mg/g) in kidney, brain and muscle of *Oreochromis mossambicus* exposed to sublethal concentrations of chromium

Treatments	10days	20 days	30 days
	Kidney		
Control	31.14 ± 2.36^{c}	31.72 ± 2.41^{d}	30.64 ± 2.33^{d}
Low concentration	30.72 ± 2.33^{bc}	26.54 ± 2.02^{c}	23.25 ± 1.76^{c}
Medium concentration	28.35 ± 2.15^{b}	22.18 ± 1.68^{b}	20.72 ± 1.57^{b}
High Concentration	23.82 ± 1.81^{a}	16.35 ± 1.24^{a}	14.38 ± 1.09^{a}
	Brain		
Control	12.48 ± 0.94^{d}	12.56 ± 0.95^{d}	12.85 ± 0.98^{d}
Low concentration	11.35 ± 0.86^{c}	10.14 ± 0.77^{c}	9.75 ± 0.74^{c}
Medium concentration	9.21 ± 0.70^{b}	7.50 ± 0.57^{b}	7.36 ± 0.55^{b}
High Concentration	5.74 ± 0.43^{a}	$4.38 \pm 0.33z^{a}$	3.88 ± 0.29^{a}
	Muscle		
Control	20.32 ± 1.54^{c}	20.68 ± 1.57^{c}	20.93 ± 1.59^{d}
Low concentration	19.28 ± 1.46^{bc}	18.29 ± 1.39^{b}	17.55 ± 1.33^{c}
Medium concentration	18.12 ± 1.37^{b}	17.35 ± 1.32^{b}	14.74 ± 1.12^{b}
High Concentration	16.26 ± 1.23^{a}	15.52 ± 1.18^{a}	11.38 ± 0.94^{a}

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

DISCUSSION

Carbohydrates are stored as glycogen in fish tissue and organs like the muscle and liver in order to supply the energy needs when there are hypoxic conditions, intensive stocking and a lack of food (Cicik and Engin, 2005; Wendelaar-Bonga, 1997). It has been demonstrated that liver glycogen levels decreased in *Oncorhynchus mykiss* as a result of the activation of glycolytic enzymes via catecholamines under lack of food and hypoxic conditions (Vijayan and Moon, 1992). The carbohydrate metabolism of the fish used in the present experiment might also have been affected by the lack of food since they were not fed during the experiments. It was also found that heavy metals could create stress in fish (Richard *et al.,* 1998) and that cadmium could decrease glycogen reserves in the American eel (*Anguilla rostrata*) by increasing the production of catecholamines from the adrenomedulla (Gill and Epple, 1992). Prolonged environmental stress in fish makes adaptation difficult and creates weakness in fish. Weakness is characterized by decreases in liver glycogen and serum cortisol levels, which subsequently create a series of alterations in the metabolism and shorten the life span of organisms (Heath, 1995). Some investigations also showed that heavy metals could decrease the glycogen reserves in fish (Levesque *et al.,* 2002) by affecting the activities of enzymes that play a role in the carbohydrate metabolism. Glycogen, a large and branched polymer of glucose, is the storage form of carbohydrate for virtually every organism from yeast to primates. The major glycogen stores in mammalian vertebrates exist in liver and muscle, smaller amounts of glycogen being present in kidney, intestine and several other tissues. Classically, it is thought that the glycogen stored in liver, kidney and intestine can be made accessible to other organs by virtue of their possession of an enzyme glucose 6-phosphatase (Vornanen *et al.,* 2011). Glycogen levels are found to be highest in liver, as it is the chief organ of carbohydrate metabolism in animals, followed by muscle. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant through glycolysis or hexose monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones, which contribute to glycogen synthesis (Sobha *et al.*, 2007).

Biochemical responses of aquatic organisms to contaminants usually represent the first measurable effects of contaminant exposure, and accordingly are advantageous for use in monitoring programs (Hinton, 1994). The results of the present study showed that the sublethal concentrations of heavy metal chromium significantly decreased the glycogen levels in kidney, brain and muscle of *Oreochromis mossambicus* after 10, 20 and 30 days exposure. The glycogen levels were decreased may be due to glycogenolysis taken place by the action of heavy metal chromium. A fall in glycogen levels clearly indicates its rapid utilization to meet the enhanced energy demands in pesticide treated individuals through glycolysis or hexose monophosphate pathway (Cappon and Nicholes, 1975). Decreased glycogen synthesis is attributed to inhibition of enzyme glycogen synthesis (Stamp and Lesker, 1967). The decreased carbohydrate level is also attributed to the conversion of carbohydrates into amino acids as observed by Gaiton *et al*. (1965). Many workers reported a similar trend of decrease in carbohydrate (Venkatramana *et al*., 2006; Saradhamani and Selvarani, 2009). Alteration of carbohydrate metabolism is observed in *Tilapia mossambicus* exposed to arsenic toxicity (Shobha Rani *et al.,* 2000) in *Labeo rohita* exposed to arsenic trioxide (Pazhanisamy, 2002) and in *Mystal guili* exposed to lead (Kasthuri and Chandran, 1997). Cadmium decreased the glycogen reserves in *Heteropneustes fossilis* by stimulating glycolytic enzymes like lactate dehydrogenase, pyruvate dehydrogenase and succinate dehydrogenase (Sastry and Subhadra, 1982). The decrease in glycogen reserves in the muscle and liver tissues of fish under heavy metal toxicity has been demonstrated to change with species (Sastry and Rao, 1984; Naidu *et al.,* 1984). Veeraiah *et al.* (2013) addressed that the glycogen levels were decreased in gill, liver, kidney, brain and muscle of *Labeo rohita* exposed to indoxacarb. Bedii and Kenan, (2005) observed that the glycogen contents were decreased in liver and muscle of Cyprinus carpio exposed to sublethal concentration of cadmium. Glycogen and protein levels were decreased in *Palaemonetes pugio* was exdposed to chromium (Ciftci *et al.,* 2012). The glycogen levels were decreased in liver, muscle, kidney and gill of *Labeo rohita* exposed to potassium dichromate for the periods 10, 20 and 30 days (Dhanalakshmi *et al.,* 2012). Zodape (2010) revealed that the glycogen content reduced in *Labeo rohita* when exposed to chromium. Gummadavelli et al., (2013) reported that glycogen levels were declined in gill, liver and kidney of *Cyprinus carpio* exposed to heavy metals. Decrease in glucose and glycogen content in gill tissue has been observed in *Labeo rohita* fingerlings when exposed to mercuric chloride (Jagadeesan and Mathivanan, 1999). The decreased level of glucose and glycogen contents in the liver, muscle, intestine, kidney and brain of *Channa punctatus* exposed to phenyl mercuric acetate (Karuppasamy, 2000). Stressful situation in fish *Channa punctatus* elicits neuroendocirine response which in turn induces disturbances in carbohydrate metabolism (Sastry and Siddiqui, 1982) and this trend supports to the present results in declined glycogen levels in the kidney, brain and muscle of *Oreochromis mossambicus* when exposed to sublethal concentrations of chromium. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicants through glycolysis or hexose monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. In conclusion, thus the chromium intoxication has disturbed the normal functioning of kidney, brain and muscle with alterations in the fundamental biochemical mechanism of fish. This study showed that heavy metal chromium altered the carbohydrate metabolism in *Oreochromis mossambicus* by affecting the levels of glycogen in kidney, brain and muscle due to impairments in energy requiring vital processes.

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