Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

Int. J. Modn. Res. Revs.

Volume 2, Issue 11, pp 517-521, November, 2014

ORIGINAL ARTICLE

LEAD ACETATE INDUCED GLYCOGEN LEVEL ALTERATIONS IN GILL AND KIDNEY TISSUES OF FRESHWATER FISH *CYPRINUS CARPIO* (LINN.)

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Article History: *Received 2nd October,2014, Accepted 31st October, 2014, Published 1st November,2014*

ABSTRACT

The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. The aim of the present study was to assess the glycogen levels in gill and kidney of the fish *Cyprinus carpio* exposed to sublethal concentrations of heavy metal lead acetate 1/15th (low), 1/10th (medium) and 1/5th (high) of the 96 hour LC_{50} values for the period of 10, 20 and 30 days. The fish exposed to lead acetate showed a decrease the glycogen levels in gill and kidney for 10, 20 and 30 days. However, no information was on record concerning the three different sublethal concentrations of heavy metal lead acetate on the glycogen level of fish. The objective of the present work was to assess the effect of heavy metal lead acetate on glycogen levels in gill and kidney of freshwater fish, *Cyprinus carpio*.

Keywords: lead, glycogen, sublethal concentration, *Cyprinus carpio*.

1.INDRODUCTION

The modern industries are making use of various heavy metals such as iron, steel, copper, nickel, platinum and lead. Among the different types of pollutions, chemical pollution appears to be the major type which threatens the living systems very extensively. Among the different habitats aquatic environment is the major target of pollution (Afsar *et al*., 2012). Most of the heavy metals are natural constituents of the aquatic environment. Some of them are biologically essential, but some metals like cadmium, lead and mercury are highly hazardous to aquatic biota and normally occur in low concentration (Mali, 2002). Heavy metals contamination of aquatic environment has drawn increasing attention as it may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. These metals tend to accumulate in organisms and have been found to have a variety of adverse effects on fishes. Higher concentrations of lead, cadmium and mercury were toxic to fishes (Atta *et al*., 2012; Salah *et al*., 2013). Dangerous pollutant that can be absorbed by fish when exposed to elevated levels in an aquatic environment is Lead (Pb). Absorption of lead occurs by different ways through gills and skin or by ingestion of contaminated water and food; .and may lead to high mortality rate or cause many biochemical and histological alterations in survived fish (Coetzee, 1998; Mokhtar *et al*., 2013).

The primary source of lead exposure to animals are contaminated soils, lead paints that remain on older structures, water from plumbing systems that contain lead, and lead based products, especially batteries, used crankcase oil and linoleum (Waldner *et al*., 2002). The lead containing gasoline fumes from automobile exhausts constitute the chief and wide spread source of lead contamination in urban environments. A major source of lead to waterfowl and other wildlife is spent lead shot, bullets, cartridge, and lead sinkers used in sport fishing (De Francisco *et al*., 2003). Lead as an industrial pollutant and immunotoxicant, has the potential to adversely affect human and animal health. It induces a broad range of physiological, biochemical and neurological dysfunctions in human (Nordberg *et al*., 2007). Exposure to low level of lead during early development was found to produce longlasting cognitive and neurobehavioural deficit, persistent immune changes, reduced fertility, a delay in sexual maturity, irregular estrus and reduced number of corpora lutea in human and experimental animals (Mobarak,2008).Recent and notable reports have indicated that lead can cause neurological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes in the animals (Park *et al*., 2006;

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Berrahal *et al*., 2007; Reglero *et al*., 2009; Abdallah *et al*., 2010; Mobarak and Sharaf 2011; Alkahemal-Balawi *et al*., 2011).

The aquatic environment is subjected to different types of pollutants which enter water bodies with industrial, domestic and agricultural waste waters and affect severely the aquatic organisms (El-Moghney and Mohamed, 2003). In last few decades increase in population density, heavy industrialization and agricultural activities have resulted in more and more wastes entering in freshwater resources (Chavan and Muley, 2014). Contamination of freshwater with a wide range of pollutants has become a matter of concern over last few decades (Vutukuru, 2005). Heavy metals released from domestic, industrial and other man made activities may contaminate the natural aquatic system extensively (Velez, 1998). Heavy metals have devastating effects on ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al*., 2007). Heavy metals and chemicals are toxic to animals and many cause death or sublethal pathology of liver, kidneys, reproductive system, respiratory system or nervous system in both invertebrate and vertebrate aquatic animals (Wilbur, 1969).

Organisms may develop a great number of strategies to minimize adverse and toxic effects produced by chemical stress. In a first stage of the antistress response, organisms may adopt behavioral changes, such as avoidance responses; or biochemical changes, such as increased synthesis of detoxification enzymes and binding ligands, increased biotransformation processes and increased elimination rates (Walker *et al*., 2001). But when these compensatory mechanisms are overcome, the damage may progress causing the death of the organism. In this way, if the stressor persists, the death of individuals may threaten their population numbers, and finally the entire community (Depledge and Rainbow, 1990; Moriarty, 1990; Walker *et al*., 2001; Ansaldo *et al*., 2006). All the antistress responses start at the subcellular level, and usually consist of the disruption of normal metabolic pathways. These responses imply an energetic cost that interferes with the energetic budget available for other vital processes, such as growth and reproduction (Widdows and Donkin, 1992). Some of these responses may be considered specific, since they are produced by one or a few substances with closely related structures or properties. Other more general or non-specific effects, are due to the energetic costs involved in the defense strategies and may be produced by a large number of substances (Haux and Forlin, 1988; Hugget *et al*., 1992). Glycogen level is one of the parameters that reflects the energetic and reserves status of an organism. Moreover, glycogen is used rapidly when organisms are under stress, and levels of this energy reserve have been suggested as useful biomarker of general stress (Hugget *et al*., 1992; Vasseur and Cossu-Leguille, 2003).

Lead has no known role in biological systems (Gaber, 2007). Lead (Pb+2) is a particular concern in this aspect because fish are able to bioaccumulate it in the body tissues due to reduce human food safety, especially protein source. Pb^{2+} is a non-essential metal and contemporary contaminant throughout the world. Moreover, Pb^{2+} is often used in varieties of industrial applications and products such as battery productions, chemicals, pigments and paints (Cavas, 2008). Kumaraguru (1995) reported that thousands of tons of fish are killed annually in freshwater by discharged different varieties of chemicals. Fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Vosyliene and Jankaite, 2006 and Farombi *et al*., 2007). Fish readily absorb dissolved metals and may serve as indicators of the extent of pollution (Shukla *et al*., 2007). Tissue changes in test organisms exposed to a toxicant are a functional response of organisms that provides information on the nature of the toxicant (Mathur and Gupta, 2008). The present investigation was to assess the glycogen levels in gill and kidney of *Cyprinus carpio* exposed to three different sublethal concentrations of heavy metal lead acetate.

2.MATERIALS AND METHODS

The fish *Cyprinus carpio* having mean weight 22-25 gm and length 10 – 12 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 lead acetate was used in this study and stock solutions were prepared. Heavy metal lead acetate LC_{50} was found out for 96 h (27.50 ppm) (Sprague, 1971) and $1/15^{th}$, $1/10^{th}$ and $1/5^{th}$ of the LC_{50} values were 1.83, 2.75 and 5.50 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 heavy metal lead acetate and served as the control. The other 3 groups were exposed to sub lethal concentration of heavy metal lead acetate 10 litre capacity aquaria. The 2nd, 3rd and 4th groups were exposed to heavy metal lead acetate 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 of the tissues 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).

3.RESULTS

The glycogen content declined substantially in gill and kidney of *Cyprinus carpio* exposed to low, medium and high sublethal concentration of heavy metal lead acetate for the periods of 10, 20 and 30 days when compared to control. The maximum glycogen content reduction was observed in high sublethal concentration on 30^{th} day exposure (Table 1 and 2).

4.DISCUSSION

Carbohydrates are considered to be first among the organic nutrients degraded in response to stress conditions imposed on animals. Even though protein is the major source of energy in animals, stress or severe hypoxia causes depletion of stored carbohydrates (Rani *et al*.,1999). Carbohydrates in the tissues of aquatic animals exist as glycogen. It is well-known that the glycogen serves as energy reserve for various metabolic processes (Vijayavel *et al*., 2006). Liver Glycogen found to be decreased as it is the chief organ of carbohydrate metabolism. The tissue-specific decline in Glycogen may be due to its speedy consumption to assemble the energy demands for more strapping activity under toxic symptom (Padma priya and Avasan maruthi., 2013).

Table 1. Glycogen (mg/g) in gill of *Cyprinus carpio* exposed to sublethal concentrations of heavy metal lead acetate

	10 Days	20 Days	30 Days
Control	37.45 ± 2.93^{b}	37.87 ± 2.96^{c}	37.03 ± 2.90^{d}
Low concentration	36.37 ± 2.89^{ab}	33.25 ± 2.61^{b}	29.93 ± 2.36^{c}
Medium concentration	35.13 ± 2.75^{ab}	30.67 ± 2.41^{b}	23.86 ± 1.89^{b}
High Concentration	33.41 ± 2.62^{a}	26.92 ± 2.13^{a}	15.50 ± 1.26^{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)

Table 2. Glycogen (mg/g) in kidney of *Cyprinus carpio* exposed to sublethal concentrations of heavy metal lead acetate

	10 Days	20 Days	30 Days
Control	31.91 ± 2.51^{c}	32.37 ± 2.54^{d}	32.75 ± 2.57^{d}
Low concentration	30.82 ± 2.43^{bc}	27.30 ± 2.16^{c}	23.83 ± 1.89^{c}
Medium concentration	28.61 ± 2.26^{b}	23.86 ± 1.89^{b}	21.04 ± 1.68^{b}
High Concentration	24.56 ± 1.95^{a}	18.12 ± 1.46^{a}	15.90 ± 1.29^{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)

Binukumari and Vasanthi (2014) reported that carbohydrates are very important reserved food materials of all organs of living cells and sources of energy for fishes. Carbohydrates form an important organic constituent of animal tissues. It is one of the important macromolecule, which comes first to reduce fish from enduring stresses caused by any xenobiotic by providing energy. Cicik and Engin (2005) suggested that carbohydrates are stored as glycogen in fish tissue and organs like muscle and liver in order to supply the energy needs when there are hypoxic conditions, intensive stocking and a lack of food. The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition (Reddy *et al*., 1993).

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).

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). Stressful situation in fishes elicit neuroendocrine response, which in turn induce disturbance in carbohydrate metabolism. Both catecholamines and adrenocorticosteroids are secreted in increased amounts due to stress stimuli and elicit marked changes in carbohydrate energy reserves of fishes (Larson,1973).

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 findings showed a significant decrease in glycogen levels in gill and kidney of *Cyprinus carpio* exposed to sublethal concentrations of heavy metal lead acetate at 10, 20 and 30 days. 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). Many workers reported a similar trend of decrease in carbohydrate (Venkatramana *et al*., 2006; Saradhamani; Selvarani, 2009 and Thenmozhi *et al*., 2011). De Boeck *et al*., (2010) addressed that *Carassius auratus* exposed to sublethal concentration of copper shows liver glycogen was decreased when compared to control. Decrease the levels of glycogen in gill, liver, kidney, testis and ovary of freshwater fish, *Channa gachua* when exposed to sublethal concentration of chromium (Salahuddin and Khola, 2013). Venkataramana *et al*., (2006) reported that depletion of glycogen in heart muscle of gobiid fish, *Glossogobius giuris* when exposed to malathion. Bantu *et al*., (2013) suggested that glycogen content was decreased in the muscle of freshwater fish exposed to chlorantraniliprole. Glycogen level was decreased in liver and muscle of *Oreochromis niloticus* exposed to malathion (Al-Ghanim, 2012). Adeyem and Adewale (2013) addressed that the glycogen content was decreased in liver and muscle of African cat fish, *Clarias gariepinus* was exposed to lead and cypermethrin. In conclusion, thus the lead acetate affect the normal function of fish because the toxicant disturbed the cells leads to decline the glycogen contents in gill and kidney of fish, *Cyprinus carpio* for overcoming the lead acetate toxicity. The affected fish were not suitable for human consumption.

5.ACKNOWLEDGEMENT

The authors wish to thank the authorities of Manonmaniam Sundaranar University and Dr. N. Indra Professor and Head, Department of Zoology, Annamalai University for providing the facilities to carry out the research work.

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