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RESEARCH ARTICLE

POTENTIAL USE OF GLYCOGEN CONTENTS AS BIOMARKER OF NICKEL CHLORIDE STRESS ON INDIAN MAJOR CARP *Labeo rohita* (HAMILTON)

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The aquatic habitats are being contaminated with heavy metals due to industrialization and other anthropogenic activities. Aquatic animals inhabiting polluted water bodies tend to accumulate many chemicals in high concentrations even when the ambient environmental contamination levels are low potentially hazardous situation for the entire food chain. The metal works industries release a good amount of heavy metals like mercury, cadmium, manganese, nickel and chromium which ultimately fall in the water bodies. Heavy metals are known to cause alterations in various tissues of fish at the biochemical level. The aim of the present study is to assess the glycogen content in gill, liver, kidney, brain and muscle of the fish *Labeo rohita* exposed to sublethal concentrations of nickel chloride $1/5^{th}$ (high), $1/10^{th}$ (medium) and $1/15^{th}$ (low) of the 96 hour LC_{50} values for the period of 10, 20 and 30 days. The fish exposed to nickel chloride showed a decrease the glycogen level for 10, 20 and 30 days in gill, liver, kidney, brain and muscle. However, no information is on record concerning the three different sublethal concentration of heavy metal, nickel on the glycogen contents of fish. The objective of the present work was to observe the effect of nickel chloride on glycogen levels in gill, liver, kidney, brain and muscle of Indian major carp, *Labeo rohita.*

Key words: Nickel chloride, glycogen, sublethal concentration, *Labeo rohita.*

INTRODUCTION

Environmental pollution by heavy metals has been a matter of growing concern over the last decades. Varieties of human activities, such as mining industries, and various by-products, such as motor car exhaust fumes and fertilizers, are responsible for releasing heavy metals into the environment (Loumbourdis, 1997). Most of the rivers, streams, and ponds in India are severely polluted or serve as "open sewers" because domestic sewage and industrial wastes, either untreated or partially treated are discharged into them. They contain many toxic chemicals that adversely affect aquatic organisms (Anand *et al.*, 2007). Water pollution does not only greatly damage the aquatic ecosystems but even the terrestrial organisms and ecosystems are severely damaged and threatened. The natural resources of water like rivers, ponds, lakes and seas are polluted with a variety of solid and liquid wastes. Every waste is ultimately dumped or emptied in natural water bodies. Most of our rivers have become noxious sewers due to haphazard and extravagant pouring of industrial wastes into them and veritable death traps for aquatic life including fish which is highly nutritious, easily digestible and much sought after food (Garg *et al.*, 2009). As fish fauna serves as a food source, it is essential to know the impact of water pollution on these organisms. Any change in the natural conditions of aquatic medium causes several physiological adjustments in fish (Black, 1955). Heavy metals are the main culprit for undesirable changes in water quality. At higher levels of biological organization heavy metals induce changes in metabolism, biochemistry, physiology, histology, inhibits synthesis of proteins and nucleic acids (Dieuga and Penni, 1989; Wilson and Taylor, 1993). This rapid industrialization and green revolution introduced a large variety of chemicals into the environment. These chemicals create serious ecological problems particularly aquatic pollution (Kharat *et al.*, 2010). A great variety of pollutants affect the majority of water course which receive domestic, industrial and agricultural effluents. The complexity of this situation becomes apparent when toxicity is keenly considered in terms of its ramifications and environmental consequence. The contamination of freshwater with heavy metals such as cadmium and lead has become a matter of great concern over the past decades not only because of their threat to public water supplies but also because of the damage caused to aquatic life especially fishes (Tawari-Fufeyin *et al.*, 2008). Metals are commonly found in the environment, they are present as a natural elements or as a result of anthropogenic activities in different environmental media such as air, water and soil, which constitute an important factor of exposure to animals and human (Louis, 1993). Heavy metals are considered as one of the most important factors which affect fish population, reducing their growth, reproduction and/or survival rate (Mohamed and Saleh, 1996; Saeed, 2000). The sources of

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Nickel are arsenide and sulphide ores. Nickel is used for alloying, as a catalyst in chemical reactors, for battery making and metal plating. It is deposited in rivers and streams through discharges of effluents from mining operations. Its route of exposure to humans, animals, and birds is through drinking contaminated water and dust from the atmosphere. Its health effects include; the disturbance of respiratory system and asthma, birth defects, vomiting and damage to Deoxyribonucleic Acid (DNA) at high concentrations (Ntengwe and Maseka, 2010). Nickel (Ni) occurs as four basic ores namely, arsenide, laterite, silicate and sulphide (Galvin, 1996). Anthropogenic activities (i.e. mining, electroplating & steel plant operations) can result in nickel discharge into water and air (Galvin, 1996). Nickel ions tend to be soluble at pH < 6.5 forming mostly insoluble nickel hydroxides (Dallas and Day, 1993). In aquatic ecosystems, dissolved nickel concentrations are generally between 0.005 and 0.010 mg L-1 (Galvin, 1996). Nickel toxicity is generally low (Khangarot and Ray, 1990) but elevated concentrations can cause lethal effects in the aquatic ecosystems.

Nickel is a natural element in the earth's makeup. This must be a factor in assessing its ability to harm the environment. Although trace metals like Ni are essential for normal physiological process, aquatic ecotoxicity testing has shown that NiSO4. 6H2O and NiCl2.6H2O fall into the "harmful" classification where their abnormally high concentrations can become toxic and disturb the homeostasis of an animal (Farkas *et al.,* 2002; Javed, 2003). The aquatic environment, where fish and other aquatic organisms live, is subjected to different types of pollutants which enter water bodies through industrial, domestic and agricultural discharge systems thereby introducing stress to living creatures. Stress is a general and non-specific response to any factors disturbing homeostasis. Stress reaction involves various physiological changes (Ololade and Oginni, 2010). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Farkas *et al.,* 2002; Yousuf and El-Shahawi, 1999; Vinodhini and Narayanan, 2008). Fish can serve as bio-indicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem (Lakra and Nagpure, 2009). Glycogen is the main reserve source of energy for animals during normal metabolism and their content in liver and muscle of fish exposed to chemical substances may indicate the health condition of the fish. During unfavorable environmental situation the normal metabolism is affected which in turn leads to alteration in the glycogen reserve of fish. The observed reduction in glycogen content in the present study indicates the utilization of stored glycogen to meet the high energy requirement under the lindane stress. Similar reduction in glycogen content in *Clarias batrachus* was observed after fish were exposed to pesticide dimethoate and Rogor (Begum and Vijayaraghavan, 1996). Generally depletion of glycogen level under stress condition may indicate an expression of an initial regulatory step resulted an increase in the intermediary metabolism (Kumari and Ahsan, 2010). The effect of heavy metals on the alterations in the biochemical substances of the body is profusely studied by many investigators in fishes. Metal intoxication in fishes usually results in glycogen depletion and is reported in several species of fishes, such as *Heteropneustes fossilis* (Qayyam and Shaffi, 1977); *Sarotheradon mossamibicus* (Akhilender Naidu, 1982); *Channa punctatus* (Sastry and Sunita, 1983) and *Labeo rohita* (Bengery and Patil, 1986). Shukla and Sastry (1990) studied the effects of cadmium on some biochemical and physiological parameters in fish *Channa punctatus*. They showed that these fishes were, hypoglycemic,hypolactemic and the total plasma proteins, the levels of glycogen, lactic acid, pyruvic acid and total proteins in liver and muscles decreased significantly in both acute and chronic exposure. However, no information is on record concerning the different sublethal concentration of heavy metal nickel chloride effect on the glycogen levels of *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 (28mg/L) (Sprague, 1971) and $1/15^{th}$, $1/10^{th}$ and $1/5^{th}$ of the LC_{50} values were 1.86, 2.8 and 5.6mg/L 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, 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).

RESULTS

The glycogen levels in gill, liver, kidney, brain and muscle of *Labeo rohita* exposed to low, medium and high sublethal concentration of heavy metal nickel chloride showed significant decrease when compared to control fish. The decrease in gill, liver, kidney, brain and muscle of *Labeo rohita* glycogen levels were more pronounced at 30 days of exposure periods (Table 1).

DISCUSSION

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). Carbohydrates are the main source of energy that is ingested by the human body

Table 1. Glycogen levels changes (mg/g) in gill, liver, kidney, brain and muscle of *Labeo rohita* exposed to sublethal concentration of nickel chloride

1 Treatments	10 days	20 days	30 days
Gill control	34.27 ± 2.61^{b}	34.69 ± 2.64^{c}	33.85 ± 2.58^{d}
Low concentration	33.19 ± 2.53^{ab}	30.07 ± 2.29^{b}	26.75 ± 2.04^{c}
Medium concentration	31.95 ± 2.43^{ab}	27.49 ± 2.09^{b}	20.68 ± 1.57^{b}
High Concentration	30.23 ± 2.30^{a}	23.74 ± 1.81^{a}	12.32 ± 0.94^{a}
Liver control	82.47 ± 6.28^{c}	83.69 ± 6.37^{d}	84.23 ± 6.42^{d}
Low concentration	79.19 ± 6.03^{ba}	73.65 ± 5.61^{c}	68.15 ± 5.19^{c}
Medium concentration	74.27 ± 5.65^{ab}	60.21 ± 4.59^{b}	54.31 ± 4.14^{b}
High Concentration	69.59 ± 5.30^{a}	52.87 ± 4.03^{a}	43.61 ± 3.32^{a}
Kidney control	28.73 ± 2.19^{c}	29.19 ± 2.22^{d}	29.57 ± 2.25^{d}
Low concentration	27.64 ± 2.11^{bc}	24.12 ± 1.84^{c}	20.65 ± 1.57^{c}
Medium concentration	25.43 ± 1.94^{b}	20.68 ± 1.57^{b}	17.86 ± 1.36^{b}
High Concentration	21.38 ± 1.63^{a}	14.94 ± 1.14^{a}	12.72 ± 0.97^{a}
Brain control	10.28 ± 0.78^{b}	10.54 ± 0.80^{c}	11.12 ± 0.85^{d}
Low concentration	10.02 ± 0.76^{ab}	9.34 ± 0.71^{b}	8.10 ± 0.62^{c}
Medium concentration	9.68 ± 0.73^{ab}	8.62 ± 0.66^{b}	6.74 ± 0.52^{b}
High Concentration	9.14 ± 0.69^{a}	7.18 ± 0.55^{a}	4.96 ± 0.38^{a}
Muscle control	15.64 ± 1.19^{b}	15.92 ± 1.21^{c}	16.58 ± 1.26^{d}
Low concentration	15.10 ± 1.15^{b}	14.16 ± 1.08^{b}	13.12 ± 0.99^{c}
Medium concentration	14.68 ± 1.12^{b}	12.64 ± 0.96^{a}	10.86 ± 0.83^{b}
High Concentration	13.24 ± 1.01^{a}	11.48 ± 0.87^{a}	7.54 ± 0.57^{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)

(Caffall and Mohnen, 2009). Glucose is the major enegy source in the body. Glycogen is the storage form of glucose and glycogen is stored in skeletal muscles and liver. If glucose intake exceeds than it is utilized in the body it is converted into fat (Asif *et al.,* 2011). 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).

The results of the present study showed that the sublethal concentrations of heavy metal nickel chloride significantly altered the glycogen levels in gill, liver, kidney, brain and muscle of *Labeo rohita* after 10, 20 and 30 days exposure. The glycogen levels were decreased in the gill, liver, kidney, brain and muscle of *Labeo rohita* when exposed to sublethal concentrations of nickel chloride may be glycogenolysis takes place by the action of heavy metal nickel chloride. A fall in glycogen levels clearly indicates its rapid utilization to meet the enhanced energy demands in pesticide treated individuals through glycolysis or hexose monophospahte 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 aminoacids as observed by Gaiton *et al.,* (1965). 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). 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, 1993). Prolonged environmental stress in fish makes adaptation difficult and creates weakness in fish. Weakness is characterised 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. Cadmium decreased the glycogen reserves in Heteropneustes fossilis by stimulating glycolytic enzymes like lactate dehydrogenase, pyruvate dehydrogenase and succinate dehydrogenase (Sastry and Subhadra, 1982). Reduction in glycogen level is thought to be the result of greater stress the organs experienced during the process of detoxification of active moieties and their metabolites (Singh and Singh, 2002). Several reports are

available on the effect of muscular exercise on liver glycogen energy reserves in fish, which get depleted (Black *et al.*, 1960, 1962; Chaudhary and Nath, 1985; Nath and Kumar, 1987; McLeary and Brown, 1975). Liver glycogen levels are depleted during acute hypoxia or physical disturbances in the fish (Heath and Fritechard, 1965).

Decrease in carbohydrates is probably due to glycogenolysis and utilization of glucose to meet increased metabolic cost as suggested by Viswarajan *et al*. (1988) in *Oreochromis mossambicus* under the stress of tannic acid. Decrease in liver glycogen may also be due to acute hypoxia (Heath and Pritchard, 1965). Decrease in glucose and glycogen content in gill tissue has been observed in *Anabas testudineus* fingerlings when exposed to mercuric chloride (Jagadeesan, 1990). 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). Shoba Rani *et al*. (2000) have also observed the decline in gill glycogen content in *Tilapia mossambica* exposed to sodium arsenite. Stressful situation in fish elicits neuroendocirine response which in turn induces disturbances in carbohydrate metabolism (Mazeand *et al.*, 1977) and this lend support to the present results in declined glycogen levels in the gill, liver, kidney, brain and muscle of *Labeo rohita* when exposed to sublethal concentration of nickel chloride. The decrease in glycogen reserve in the muscle and liver tissues of fish under heavymetal toxicity has been demonstrated to change with species (Sastry and Rao, 1984; Naidu *et al.*, 1984). This change might stem from the metabolic differences between species and environmental concentrations of heavy metals and duration, which the fish are exposed to. Diwan et al. (1979) have reported that exposed fish *C. batrachus* and *H. fossilis* to the industrial effluents having high load of the heavymetals like Hg, Zn, Cd, Co, Cr, Pb, Cu and Fe. It was reported that there was a significant decrease in glycogen level in liver, muscles and gills whereas in heart and kidney there was a slight decrease in the glycogen level in the muscles of *H. fossilis* was not significant. Quayyum and Shaffi (1977) reported a decrease in the glycogen level of liver, muscles and kidney in *H. fossilis* when exposed to Hg. Virk and Sharma (2003) assessed the effects of acute toxicity of nickel and chromium on fingerlings of the *C. mrigala*. After 45 days of exposure significant decline in the protein and carbohydrate content of gills was observed. Heavy metals thus may produce damage to an organ, inhibition of enzymes activity and significant alterations in various metabolic activities. In conclusion, this study showed that nickel chloride altered the carbohydrate metabolism in *Labeo rohita* by affecting the levels of glycogen in gill, liver, kidney, brain and muscle due to impairments in energy requiring vital processes.

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