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INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

International Journal of Modern Research and Reviews

ISSN: 2347-8314

Volume 1, Issue 1, pp 73-77 December,2013

ORIGINAL ARTICLE

EFFECT OF CADMIUM CHLORIDE ON THE CARBOHYDRATE METABOLISM OF *Labeo rohita*

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Article History: *Received 17th Dec,2013, Accepted 30th December,2013,Published 31st December,2013*

ABSTRACT

The present study is aimed to investigate glycogen and glucose in tissue and lactic acid in blood in cadmium exposed fish Labeo rohita. The experimental fish were exposed to sublethal concentration (1.87 ppm) of cadmium chloride for a period of 14 days at intervals of 7 and 14 days. The glycogen and glucose level were observed in 7 days and 14 days. The level of glycogen was decreased in the liver and muscle tissue of labeo rohita exposed to cadmium chloride. The level of tissue glucose and blood glucose and lactic acid in the blood were increased. The present study is concluded that the exposure of cadmium chloride altered the carbohydtrate metabolism in the *Labeo rohita*

Keywords: Cadmium chloride, Carbohydrate metabolism, *Labeo rohita*

1.INTRODUCTION

Environmental pollution is caused by the development of industries, technology and an informal settlement does, however, threaten many freshwater ecosystems. Environmental pollution not only causes a decrease in water quality, but it subsequently affects all living organisms in that system. Therefore, it is necessary to not only identify and manage these pollution sources, but also to maintain their effects on the health of aquatic environment. Human activities are mainly responsible for promoting the pollution in the environment by the way of introducing unwanted toxic compounds (Bryan, 1976). Environmental Toxicology is the scientific study of the adverse effect of chemical on living organisms that are present in the environmentHeavy metals enter into aquatic habitats by a number of routes and cause hazardous effect on their morphology and physiology. Heavy metals polluted water may lead to the destruction of beneficial species either indirectly through breaking the biological food chain or directly by affecting the aquatic forms of life.

Cadmium is major contaminants of aquatic environments (Munger *et al.*, 1999) that are toxic towards aquatic organisms (Witeska *et al.*, 1995) even at concentrations found in natural waters (Pelgrom *et al.*, 1994). Metals are present in very low concentrations in natural aquatic ecosystems (Nussey, 1998). The most important heavy metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium (Abel, 1989; Viljoen, 1999). Metal uptake by aquatic organisms is a two-phased process, firstly involving rapid adsorption or surface binding, followed by a slower transport into the cell interior. Transport of metals into the intracellular section may be aided by either diffusion of the metal ion across the cell membrane or by active transport by a carrier protein (Brezonik *et al.*, 1991; Wepener *et al.*, 2001).

Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish(Tulasi *et al.*,1992). The heavy metal in the tissue of fishes may cause various physiological defects and mortality (Torres *et al.*,1987). The fishes which are largely being used for the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Lopes *et al.*,2001).Blood is a 'pathophysiological reflector' of the body and therefore blood parmeters are important in diagnosing the functional status of the animal exposed to toxicants. It plays a decisive role in the regulation of life process. To function properly, the organism must keep its blood composition and constituents relatively constant under optimal conditions (Joshi et al., 2002).

2.MATERILAS AND METHODS

Procurement of experimental animal

The fresh water fish *Labeo rohita* were collected from the fish farm located in Puthur, Nagapattinam District, 15 Km

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away from the Uinversity campus. This fishes were brought to the laboratory and transferred to the rectangular fibre glass tanka (100X175cm) of 500 liters capacity containing chlorine free aerated wellwater.

The fresh water fish, *Labeo rohita* were limatized for a minimum period of 15 days in the laboratory conditions at room temperature (28±1ºC) before subjecting them for screening test. These fingerlings were fed with artificial food pellets on alternative days and the water renewed every 24 hours. The tanks were rinsed with potassium permanganate or acroflavine (2mg/l) to prevent fungal attack.the fresh water fish, *Labeo rohita* were critically screened for the signs of disease, stress, physical damage and mortality./the injured, severely diseased, abnormal and dead fishes were discarded. The feeding was discontinued 24 hours before the beginning of the experiment to reduce the excretory products in the test trough as suggested by Arrora *et al*., (1972). During the acclimation, the fishes were reared in tank until there was less than 10 percent mortality in 4 days perior to the beginning of the test as suggested by Anderson (1977). The water in the experimental trough was changed daily and also aeration was stopped to avoid the possible oxidation of the toxicants.

Toxicity Studies

To evaluate the acute toxicity studies the static renewal toxicity test were conducted according to the methods recommended by American Public Health Association (1960). In the present investigation the toxicity of cadmium chloride (LC_{50}) for 96 hours were analyzed. The LC_{50} is statistical estimate to the concentration of toxic material in water that kills 50 per cent of the test species, under experimental conditions during a specific time interval. The LC_{50} was used because the concentration required to affect the response in 50 percent of the test animals is more reproducible than any other value (Pickering and Handerson, 1966).The screening test was conducted to avoid delay and to save time and effort. The object of this test is to obtain approximate indication of the concentration of a substance likely to be hazardous to the test fish and fishes in general in their natural environment. The toxicant concentration used in the present series of tests were approximately the wide range of concentration viz., 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 ppm aqueous solutions were prepared. The tests were conducted in the rectangular plastic troughs. The troughs were cleaned well and dried before conducting experiments. Then the tests were conducted by allowing ten fishes of *Labeo rohita* in each plastic trough containing 10 liters of water with particular concentration of the cadmium chloride. The screening tests was continued to assess the concentration at which all fishes survived for 24 hours and likewise the concentration at which most of the fishes died simultaneously (Bansal *et al.,* 1980).Preliminary observation showed that beyond 30 ppm of cadmium chloride all the test fishes died. Therefore the concentration of cadmium chloride falling of within 1 to 30 ppm were prepared and ten number of test fishes were introduced to confined narrow range of concentration viz., 1,2,3,4,5,6,7,8,9,10 ppm of cadmium chloride solutions. The behavioral responses of the fish at various concentration of cadmium chloride were observed at regular intervals to ascertain the impact of the cadmium toxicity on the organism. Individuals in the test medium, which showed no responses to stimulation and those without opercular movement, were removed quickly to avoid cannibalism among the fish. In all tests, mortalities were recorded 96 hours. The LC_{50} values were determined by following the method of Finney (1971). Sublethal studies are helpful to assess the response of the test organisms under augmented stress caused by metals. 96 hr LC_{50} value for cadmium chloride was found at 1.87 ppm. Hence the one tenth of 96 hr LC_{50} value (1.87 ppm) was selected for the present investigation as sublethal concentration.

Experimental design

The toxicant exposure was done by 24 hour or renewal bioassay system. For analysis sublethal toxicity, 3 groups of 10 fish each were exposed separately and cadmium chloride (8.5ppm: 10 % 96 hours LC_{50}). Solution prepared in well water. The experimental medium was prepared by dissolving cadmium chloride at 1.87 ppm having dissolved oxygen 5.8 ppm, pH7.4, water hardness 30.3mg/l (APHA *et al*., 1992) and water temperature 28± 2 C. Each group was exposed to 50 l of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaric containing 50 l of well water as control. Feeding was allowed in the experimental as well as control groups every day for a period of 3 hours before the reneval of the medium throughout the tenature of the experiment.

The experimental fish were exposed to sublethal concentration (1.87 ppm) of cadmium chloride for a period of 14 days at intervals of 7and 14 days. The control and experimental fish were dissected out at the end of each period of exposure and the selected organs such as gill, liver and kidney were dissected out for bioaccumulation. The blood samples were also collected for haematological parameters. The tissues later were processed for histological and histopathological studies.

Biochemical Studies

Quantitative estimation of blood glucose

Blood glucose was estimated by the modified method of Murrel Leonard and Nace (1958).

Quantitative estimation of tissue glucose and glycogen

Colorimetric micro method of Kemp and Kits Van Heijhingeen (1954) was adopted for the quantitative estimation of glucose and glycogen.

Quantitative estimation of blood lactic acid

Blood lactic acid was determined by the method of Barker and Summerson (1941).

Quantitative estimation of tissue lactic acid

Basker and Summerson (1941) method was followed for the estimation of lactic acid in tissues.

Statistical analysis

Statistical significance was evaluated by using ANOVA followed by Duncan Multible Range Test (DMRT) Duncan (1957).

3.RESULTS

Glycogen level in the muscle

The muscle glycogen exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of cadmium. The muscle glycogen was found to be decreased at 7 and 14 days exposure. The percent change over control in the muscle was –28.14 and –30.49 respectively (Table1).

Glycogen level in the liver

Sub lethal concentration of cadmium chloride treated *Labeo rohita* showed changes in the level of glycogen in the liver. It exhibited fluctuations from the mean control level (Table 1). In the treated fish glycogen content got decreased at 7 and 14 days and the percent change over control were –20.42 and -31.77 respectively (Table 1).

Glucose level in the blood

The blood glucose level exhibited a remarkable change from the mean control level, when the fish were exposed to sub-lethal concentration of cadmium. The blood glucose content was found to be increased at all exposure periods. The percent changes over control were 31.52 and 54.34 at 7 and 14 days of exposure respectively. (Table 2).

Glucose level in the liver

The liver glucose exhibited a significant depletion at all exposure periods of sub-lethal concentration of cadmium. The percent changes over control were found to be –14.14 and –23.98 at 7 and 14 days of exposure respectively. (Table 2).

Lactic acid level in the blood

The blood lactic acid exhibited remarkable changes from the mean control level, when the fish were exposed to sub-lethal concentration of cadmium. The blood lactic acid was found increased at all exposure periods. The percent changes over control were found to be 26.66 and 58.88 respectively. High percent change over control were recorded at 7 and 14 days of exposure (Table 3).

Lactic acid level in the liver

The liver lactic acid showed remarkable changes in the level from a mean control value, when the fishes were exposed to sub-lethal concentration of cadmium chloride. The liver lactic acid level was found to be increased at all exposure periods. The percent changes over control were 36.20 and 45.17 at 7 and 14 days respectively (Table 3).

Table : 1 Levels of glycogen content in muscle and liver of *Labeo rohita* exposed to sub-lethal concentration of cadmium chloride

Tissues	Control	7 days	14days
Muscle	4.46± 0.03	3.16± 0.04*	3.00± 0.03
% COC		-28.14	-30.49
Liver	4.91± 0.04	3.72± 0.03*	3.35± 0.03*
% COC		-20.42	-31.77

Mean ± S.D. of six individual observations,* Significance (p<0.05) Group I compared with group II and III. Values are expressed as (units /mg protein)

Table : 2 Levels of glucose content in blood and liver of *Labeo rohita* exposed to sub-lethal concentration of cadmium chloride

Tissues	Control	7 days	14days
Blood	0.92± 0.02	1.21 ±0.03*	1.42± 0.02*
% COC		31.52	54.34
Liver	3.96± 0.02	3.40± 0.03*	3.01± 0.04*
% COC		-14.14	-23.98

Mean ± S.D. of six individual observations,* Significance (p<0.05) Group I compared with group II and III. Values are expressed as (units /mg protein)

Table : 3 Levels of lactic acid content in different organ of *Labeo rohita* exposed to cadmium chloride

Tissues	Control	7 days	14days
Blood	0.90±	1.14± 0.03*	1.43± 0.01*
% COC	0.01	26.66	58.88
Liver	2.90±	3.95± 0.03*	4.21± 0.03*
% COC	0.02	36.20	45.17

Mean ± S.D. of six individual observations,* Significance (p<0.05) Group I compared with group II and III. Values are expressed as (units /mg protein)

4.DISCUSSION

Changes in glycogen content in the tissues

Glycogen plays an important role in the carbohydrate metabolism. Glycogen content in the tissues is one of the sensitive biochemical indicators, which reflect changes in the normal activity of various functional systems (Metelev et al, 1983). When the fishes were exposed to sub-lethal concentration of cadmium, depletion of glycogen content was evident at 7 and 14 days. Carbohydrate metabolism was disturbed when fish were exposed to environmental stress. Earlier studies have shown that rapid depletion in the muscle and glycogen occurred when the fish were to pollutants (Murthy and Priyamada Devi, 1982). Nakano and Tamlinson (1967) and McLeay and Brown (1974) have reported that during the actual or potential stress, catecholamines were secreted in high quantities, which deplete the glycogen reserve in fish. Thus, marked glycogeneolysis in liver causes hyperglycemia and hyperlactemia in blood. Hence, in *Labeo rohita* the depletion of the muscle glycogen content may be due to the glycogenolysis. A marked decrease in liver glycogen indicates an extensive utilization of energy stores. Perhaps, this stepped up utilization is to meet the extra demands of energy necessitated by the quick and brisk movements, which the animal shows in its behavioural response due to cadmium stress. The reduction in stored glycogen content in the liver and muscle of cadmium treated *Labeo rohita* indicates that during the exposure periods of stress, the demand for the extensive utilization of energy is met by glycogen. The liberated glucose mobilized from liver glycogen are transported to other organs through blood to meet the energy requirements necessitated by the accelerated movements of the fish under stressful situations to adopt themselves in the cadmium toxic medium. This is indicated in the hyperglycemic condition in the blood of cadmium treated fish. Several investigators have observed similar changes in the glycogen level of liver. McLeay and Brown (1974) have recorded a considerable decrease in glycogen content of liver when *Oncorhynchus kishtch* exposed to bleach kraft mill effluent. Baskaran *et al*., (1989) have noticed the depletion in the hepatic glycogen content in *Oreochromis mossambicus* when exposed to textile dye effluent. Bakthavathsalam and Srinivasa Reddy (1985) have reported the similar fluctuation in *Anabas testudineus* when exposed to disyston. Cooley *et al.,* (2001) reported that the liver glycogen of *Oncorhynchus mykiss* is decreased with higher concentration of dietary effluent. Sastry and Subhadra (1982) have reported the hepatic glycogen depletion in Indian cat fish *Heteropneutes fossilis* when exposed to ethyl acetone. Srinivasa Neera *et al.,* (2002) observed that the glycogen of muscle and liver are decreased gradually with the higher concentration of Zinc.

Changes in blood glucose level

Glucose acts as an indicator of external environmental stress (Davis et al, 1993). The blood sugar level represents a dynamic balance between the rate at which the sugar enters the blood from the liver and the rate at which it is being removed by the body tissue from the blood (Nemscok and Boross, 1990). At sub-lethal concentration, the level of blood glucose in *Labeo rohita* increased during all periods of exposure thereby indicating that the glycogenolysis take place in the liver, whereby the reserved glycogen is being slowly converted into glucose. Many toxicologists have observed similar increase in the blood glucose level, when the fish were introduced into the toxic medium. McLeay (1973) reported that the juvenile Cohosalmon *Oncorhynchus kisutch* when treated with sublethal concentration of mineralized unbleached kraft mill effluent for 12 hours, showed a remarkable increase in blood glucose level.

Gupta and Rajbanshi (1979) reported hyperglycemia in Indian cat fish *Heteropneutes fossilis* when exposed to sub-lethal concentration of rogor. Koundinya and Ramamurthi (1979) has reported the increased glucose level in *Sarotherodon mossambicus* with the higher concentration of organophosphate pesticide. Bakthavathsalam and Srinivasa Reddy (1985) noticed similar increase of blood glucose level in Anabas *testudineus* when exposed to sub-letahl concentration of disyston. Das and Mukherjee (2000) have reported that the fish *Labeo rohita* the liver glycogen is converted into blood glucose, when exposed with quinalphos. (Chowdhury, *et al.*, 2004) reported the similar result on *Oncorhychus mykiss* when exposed to cadmium. In the present study, the blood glucose level increased at all periods of exposure. From the above result, it may be assumed that the blood glucose derived from glucogenolysis was utilised chiefly for the release of energy, at the time of adaptation of the animal to the toxic environment.

Changes in liver glucose level

Glucose is one of the most important biochemical substances, which gives immediate energy to an organism. In the treated fish, liver glucose content was found decreased in all periods. The decline in the glucose level shows that the fish due to the heavy environmental stress has utilized it. Radhakrishnaiah *et al.*, (1992) have observed similar changes in the liver glucose level in *Labeo rohita* when exposed to copper. Sastry and Subhadra (1982) have reported that the liver glucose level decreased in the Indian cat fish *Heteropneutes fossilis* when exposed to zinc for 15 to 30 days. This sudden fall in the glucose level indicates the rapid supply and utilization of liver glucose. Ferrando and Andrew-Moliner (1991) have reported that in the fish European eel *Anguilla anguilla* the glucose level in liver decreased when exposed to lindane. Desai Himadri Sekhar *et al.*, (2002) reported that the *Channa punctatus* showed the increase of liver glucose when under the nickel stress.

Changes in lactic acid content of blood and liver

The important of lactic acid study in an animal is evident by its roll in several interrelated activities including mortalities, following hyperactivity (Black, 1960). Lactic acid is formed through glycolysis under anearobic condition of glucose catabolism. In the present investigation, *Labeo rohita* shows a remarkable increase in the lactic acid content in blood and liver on exposure to cadmium chloride, irrespective of the duration of exposure. The lactic acid formed in tissues during glycolysis might have been transported to the liver through blood accounting for hyperlactimia of blood and increased liver lactic acid level. In the absence of the enzyme, glucose-6-phosphatase in the muscle which is necessary for the conversion of lactic acid to glucose. The lactic acid produced in the muscle is transported to the liver through blood (Ambika Shanmugam, 1980). Yasmeen *et al.*, (1989) have noticed similar observation in *Ababus scandens* when exposed to thiodon. Shobharani and Venkateshwaralu (1991) have observed similar increase of lactic acid level in the liver of C*larias batnachus* when exposed with malathion. Bakthavathsalam and Srinivasa Reddy (1985) has reported the fluctuation in the level of lactic acid in *Anabus testudineus* when exposed to Lindane. The decrease in liver and muscle glycogen at different time intervals in the treated *Tilapia mosssambica* has led to hyperlactimia. Hyperlactimia occurs following muscular exertion and hepatic glycogen is the source of blood lactic acid (McLeay and Brown, 1975). Kamalaveni *et al*, (2003) observed similar changes the increased LDH activity and lactic acid in during the toxic stress.

In the cadmium toxic medium, *Labeo rohita* exhibits an increase in muscular activity due to the abnormal behavioural changes under the toxic stress. The oxygen supply for muscular activity is not sufficient enough to meet the oxidative requirements to fulfill the tremendous energy requirements under cadmium stress, Therefore, glucose in the muscle tissue under anaerobic condition converted to lactic acid, which turn in increase the blood lactic acid on *Labeo rohita* during the cadmium chloride exposure.

It is evident from the present investigation that the glycogen level decreases and the blood glucose level increases significantly in the experimental animal indicating that the cadmium interferes with the carbohydrate metabolism. In conclusion, it could be stated that *Labeo rohita* treated with cadmium, elicited a severe hypoxia resulting in the utilization of stores glycogen by way of anaerobic glycolysis to meet energy demands under cadmium stress.

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