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RESEARCH ARTICLE

EFFECT OF ARSENIC ON PROTEIN AND CARBOHYDRATE METABOLISM OF FRESH WATER FISH, *Labeo Rohita*

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ABSTRACT

The present study is aimed to investigate the impact of arsenic on gill and liver tissues of fresh water fish, *Labeo rohita*. In the present study, the protein, amino acids, glucose and glycogen were observed in gill and liver tissues of arsenic treated fish. At the arsenic treatment, the level of protein decreased and amino acids increased and glucose increased and glycogen decreased in the gill and liver tissues of fish. The present study concludes that the toxicity of arsenic alters the protein and carbohydrate metabolism of fresh water fish, *Labeo rohita*.

INTRODUCTION

Environmental Toxicology is the scientific study of the adverse effect of chemicals on living organisms that are present in the environment. Development of industries, technology and informal settlements leads to Environmental pollution threaten many freshwater ecosystems. The wastes of industries are either released into the atmosphere or mixed with large bodies of water. Many industrial and agricultural processes have contributed to the contamination of fresh water system thereby causing adverse effects on aquatic biota and human health (Dautremepuits *et al.,* 2004). It is therefore, necessary to identify and manage these pollution sources, and to maintain their effects on the health of aquatic ecosystems. The heavy metals are constantly polluting natural waters and the adverse effects are manifold on living organisms including economically important fishes. They are also responsible for various disturbances in physiological and biochemical parameters of fishes. Alteration in the chemical composition of the aquatic environment usually affect behavioural, physiological (O' Brein, 1967) and blood flow patterns, cell structures and carbohydrate metabolism (Mcleay and Brown, 1975). Fishes are relatively sensitive to changes in their surrounding environment. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. Natural water reservoirs are traditionally being used for aquaculture and they contribute significantly to total fish production across the globe. Unfortunately, these natural resources are getting polluted with environmental pollutants and contaminants (Kumar *et al.,* 2007).

Arsenic is a natural and ubiquitous element that presents in many environmental compartments. Arsenic contamination in natural water is a world wide problem and has become a challenge for world scientist. Arsenic is being a potent environmental toxic agent and considered as a human carcinogen leads to development of various hazardous effects on human health. Chronic arsenic toxicity due to drinking of arsenic contaminated water has been reported from many countries. Recently, large population in West Bengal in India and Bangladesh has reported to be affected with arsenic (Smith *et al.,* 2000).

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MATERIALS AND METHODS

Labeo rohita was collected from the fish farm located in Pinnalur, 20 km away from the Government Arts College, C.Mutlur. The collected fishes without least disturbance were transported in polythene bags filled half with water without any disturbances. About 100 fishes were put in each bag and water was well aerated, using pressurized air from a cylinder. These modes of transit have proved successful, since there was no mortality in all consignments throughout the course of this study. To evaluate the acute toxicity studies the static renewal toxicity test were conducted according to the methods recommended by American Public Health Association (APHA) (1960). In the present investigation the toxicity of Arsenic trioxide the median lethal concentration (LC_{50}) of Arsenic for 24, 48, 72 and 96 hours were analyzed. The LC_{50} is statistically estimated 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 (Sparague, 1971). The LC_{50} was used, because, the concentration required affecting the response in 50 percent of the test animals is more reproducible than any other value (Pickering and Handerson, 1966).

Preliminary observation showed that beyond 30 ppm of Arsenic trioxide all the test fishes died. Therefore the concentration of arsenic trioxide falling off within 1 to 30 ppm was prepared. Ten number of test fishes were introduced to conform narrow range of concentration viz., 1, 2.5, 5.0, 7.5, 10.0 12.5, 15.0, 17.5, 20.5, 22.0, 25.0, 27.5, and 30.0 ppm of arsenic solutions. The behavioral responses of the fish at various concentration of Arsenic trioxide were observed at regular intervals to ascertain the impact of the arsenic 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 at 24, 48, 72 and 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. The one tenth of the 96 hr LC_{50} values represented the higher and lower sublethal concentration

respectively. 96 hr LC_{50} value for arsenic was found at 1.89 ppm. Hence the one tenth of 96hr LC_{50} value (1.89 ppm) was selected for the present investigation as sublethal concentration for the period of 7 days. The experimental fish were exposed to sublethal concentration of arsenic for a period of 7 days. The control and experimental fish were dissected out at the end of each period of exposure and the selected organs such as gill,and liver were dissected out for biochemical studies.

Estimation of Total Protein in tissues

The total protein content in tissues was estimated by the method of Lowry *et al.* (1951). The CO-NH groups in the protein molecules reacted with the copper sulphate in alkaline medium to give a purple colour which was read at 620 nm. The tissues were isolated from the experimental animals and then homogenized in cold 10% TCA solution. The homogenized tissues were centrifuged for 15 minutes at 3000rpm. The supernatant was discarded and the precipitate was taken and then dissolved in 1.0ml of 0.1N NaOH. From this, 0.5ml of supernatant (0.5ml of serum incase of serum separated from blood) was mixed with 4.0ml alkaline copper reagent. This was allowed to standard at room temperature for 10 minutes. Then 0.5ml of folin-ciocalteau reagent was added and mixed well. The absorption of blue colour developed was read in an UV Spectrophotometer (Bausch and Lamb) at 620nm. Standards in the concentration range of 20-100µg were treated in a similar manner along with blank containing 1.0ml of distilled water. The protein content was expressed as mg/dl for serum and mg/g wet wt. for tissue.

Estimation of total free amino acids in tissues

Total free amino acids in the tissue were estimated by the method of Moore and Stein (1954). The tissues were isolated in ice, quickly weighed in an cold room and immediately homogenized in cold 10 percent TCA. The homogenate contains 10 mg of tissues). One ml of the clear supernatant was taken into a clean test tube and 2.0 ml of ninhydrin reagent was added. The mixture was cooled immediately under running tap water and the intensity of the color was read at 570 nm in a UN-visible spectrophotometer (Jasco, model 650). Tyrosine was used to construct the standard graph and the values were expressed mg/g wet weight of the tissues.

Estimation of tissue glucose and glycogen

Kemp and Kits van Heijningen (1954) were employed for the quantitative estimation of glycogen and glucose. The tissues were isolated and homogenized in 5.0 ml of 80% methanol and centrifuged at 3,000 rpm for 15 minutes. The supernatant containing free glucose was decanted into a calibrated test tube. The residue was set apart for the quantitative estimation of glycogen.

Estimation of Glycogen

The residue left after methanol extraction was homogenised in 5.0 ml of deprotenizing solution (5-0 ml of TCA and 100 mg of $AgS0_4$ in 100 ml of distilled water) and heated at 100° C over a water bath for 15 minutes. The mixture was cooled and made up to 5.0 ml with deproteinizing solution once again and later centrifuged at 2,000 rpm for 10 minutes. The clear supernatant was collected for the estimation of glycogen.

Estimation of glucose

To the decanted solution approximately 10.0 mg of activated animal charcoal powder was added. The methanol was allowed to evaporate by warming the solution over a water bath for 30 minutes. Deproteinizing solution (100 gm. of TCA in 100ml of distilled water) was added to the residual aqueous solution to bring the total volume to 5.0 ml. The suspension was centrifuged at 2,000 rpm for 15 minutes and the clear supernatant was used for the estimation of free glucose.

Quantitative estimation of glycogen and glucose

1.0 ml of the respective sample was taken in a separate test tube and 3.0 ml of concentrated sulfuric acid was added to it. The mixture was heated in a boiling water bath for 6.0 minutes and subsequently cooled in running tap water. The intensity of the colour developed was measured in a UV Spectrophotometer against the reagent blanks (3.0 ml of concentration sulfuric acid) at 520 nm. The quantitative of glucose and glycogen present in the respective samples were read form the standard graph drawn previously form known quantities of the sample. The glucose and glycogen values are expressed as mg/ g wet weight of tissues.

Statistical analysis

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

RESULTS

Level of total protein

The level of total protein was 54.17 ± 1.25 mg/g well wt of tissue I in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the decreased trend of protein (22.39±1.67mg/g wet wt. of tissue).In the normal liver tissue, the level of protein content was 75.21 ±1.11.mg/g wet wt. of tissue when the fish exposed to arsenic, the level of protein content was decreased upto 49.29±1.17mg/g wet wt. of tissues (Fig.1).

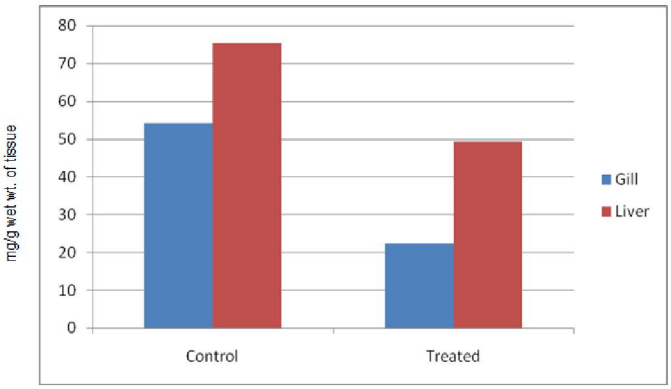

Fig.1. Level of protein in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic

Level of amino acid

The level of amino acid was 4.21±1.56 µg/g wet wt. of tissue in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the increased trend of amino acid (9.85±1.78µg/g wet wt. of tissue).

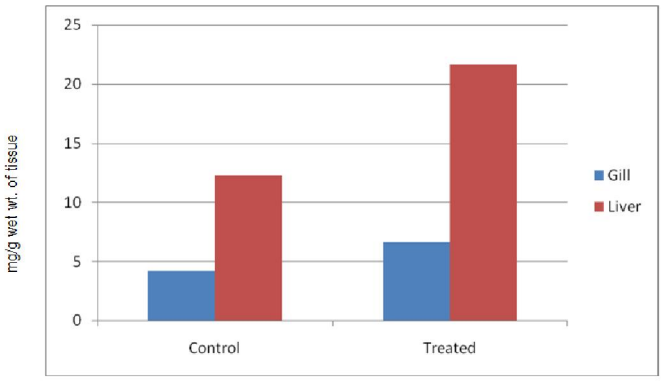

Fig.2. Level of amino acid in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic

In the normal liver tissue, the level of amino acid content was 12.26 ±1.59µg/g wet wt. of tissue. When the fish exposed to arsenic, the level of amino acidcontent was increased upto 21.69±1.72µg/g wet wt. of tissues (Fig.2).

Level of glucose

The level of glucose was 3.42 ±1.52 mg/g well wt of tissue I in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the increased trend of glucose (4.86±1.02mg/9 wet wt. of tissue). In the normal liver tissue, the level of glucose content was 8.35±1.16. when the fish exposed to arsenic, the level of glucose content was increased upto 12.86±1.08 g/g wet wt. of tissues. (Fig.3).

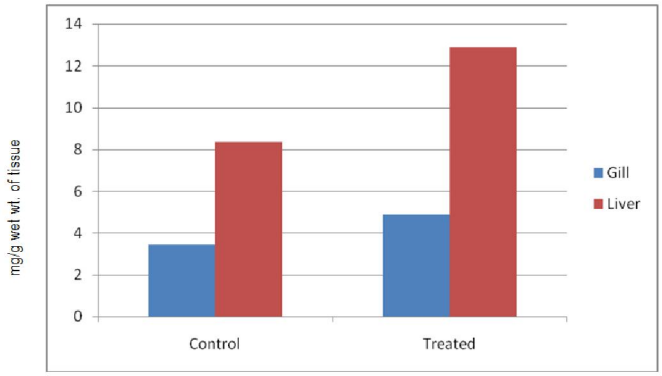

Fig.3. Level of glucose in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic

Level of glycogen in gill tissue

The level of glycogen was 5.22 ±1.52 mg/g well wt of tissue I in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the decreased trend of glycogen (2.91±1.58mg/g wet wt. of tissue).In the normal liver tissue, the level of glycogen content was 9.65±1.25. When the fish exposed to arsenic, the level of glycogen content was decreased upto 4.35±1.76 g/g wet wt. of tissues. (Fig.4)

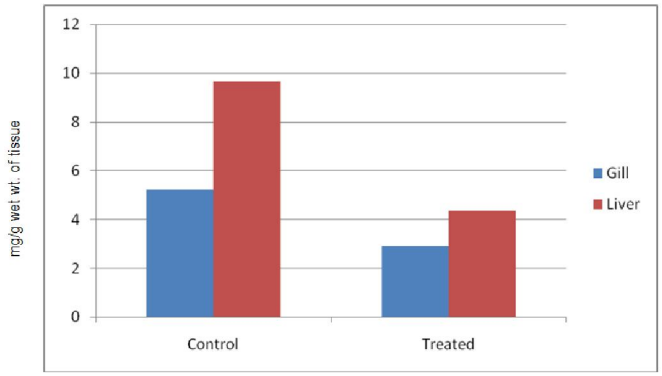

Fig.4. Level of glycogen in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic

DISCUSSION

Protein is the most important and abundant biochemical constituent present in the animal body especially synthesizing microtonal detoxifying enzymes which helps to detoxify the toxicants which enter into the animal body (Ramasamy, 1987). The amino acid and the building blots of protein. Free amino acids would also serve as precursors for energy production under stress and for the synthesis of required proteins to face the stress. The assessment of the protein and total amino acid content can be considered as a diagnostic tool to determine the physiological phase of the cell. There are number of amino acids present in the animal body and these very in accordance with the number and sequence of amino acids (Linder, 1985). The heavy metals are known to elicit changes in the biochemical constituents of fish there by altering the metabolic pathway (Sarkar and Medda, 1993). Toxic exposure of organisms interferes with organ integrity at the biochemical level and unlimitedly gives rise to affect at the individual levels (Smolders *et al.,* 2002). The heavy metal and pesticides are found to influence the biochemical composition of fishes (Shakoori *et al.,* 1997). From this point of view, the present study has been designed to observe total protein and amino acids in the gill, liver and kidney tissues of fish, *Labeo rohita* exposed with arsenic for 7days of exposure In the present study, the level of total protein and amino acids were observed at subletal concentration of arsenic in fish *Labeorohita.*The decrease in liver and muscle protein has been reported in the sugar mill effluent treated *Channapunctatus* after 96 hr exposure (Avash Maruthi and Ramakrishna Rao, 2000). Almeida *et al.* (2001) have reported a decrease in total protein concentrations in liver and white muscle of *Oreochromismossambicus* exposed to sublethalconcentrations of cadmium. The protein levels were found to be depleted in all the tissues after pesticide exposure. The decrease in protein might be due to their degradation and also to their possible utilization for metabolic purposes (Digvijay Singh and Ajay Singh, 2002). Tilak *et al.* (2003) have observed the decrease in total protein and glycogen and the increase in the LDHamino acids in the gill, liver, kidney and muscle tissues of freshwater fish, *Channa punctatus* exposed to sublethal and lethal concentrations of fenvalerate. Carbohydrates are an important sources of energy required for the various metabolic activities of the living organisms, the energy being derived as a result of oxidation. Carbohydrate is one of the chief sources of energy. They are mainly in the form of polysaccharides (starch) and disaccharides (sucrose, lactose and maltose) which are hydrolysed into monosaccharides such as glucose, fructose and galactose by the enzymes of digestive tract. Chowdhury *et al.* (2004) reported the similar result on *Oncorhychusmykiss* 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.

In the present investigation, *the* glucose and glycogen shows a remarkable changes gill and liver on exposure to cadmium chloride, irrespective of the duration of exposure. The lactic acid formed in the muscle and other 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). Kamalaveni *et al*, (2003) observed similar changes the increased LDH activity and lactic acid in during the toxic stress. The present study showed the level of glycogen decreased and glucose increased in the gill and liver tissue of *Labeo rohita* exposed to arsenic trioxide. This results indicates and extensive utilization of energy stores. Patil and Dhande, (2000) reported that a fall in glycogen in the fishes exposed to heavy metal. Dezwaan and Zendee, (1989) have observed the reeducation in tissue glycogen content due to decrease in synthesis or break down as consequence of toxic stress. Samuel and Satry, (1989) reported the level of glycogen decreased in *Channa punctatus* exposed to moncrotophos. Bakthavathsalam and Srinivasa Reddy (1985) have reported the similar fluctuation in *Anabas testudineus* exposed to disyston. Colley *et al.,* (2001) reported that the glycogen content reduced in the liver tissue of Oncorhynchusmykiss exposed to dietary effluent. Anita Susan *et al.* (2010) reported the variations in the distribution of biochemical constituents in the five major tissues viz., liver, muscle, kidney, brain and gill of the two carps, *Labeo rohita* and *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of the technical grade pyrethroid, Fenvalerate. They also reported that depression in the levels of glycogen and increase in glucose level in different tissues observed were discussed in the light of metabolic stress caused due to the exposure to the toxicant. Satyaparameshwar *et al.* (2006) observed

that sublethal toxicity of copper sulphate on glycogen in selected tissues of freshwater mussel, *Lamellidens marginalis*. The decrease in glycogen appears to be a shift in the carbohydrate metabolism from aerobic to anaerobic type due to toxicity of copper.

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