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ORIGINAL ARTICLE

EFFECT OF ARSENIC ON DEHYDROGENASES ACTIVITIES LEVEL IN VARIOUS TISSUES OF FRESH WATER FISH, LABEO ROHITA

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ABSTRACT

The present study is aimed to investigate the effect of arsenic on succinate dehydogenase and lactate dehydogenase activities in gill, liver and kidney tissues of fresh water fish, Labeo rohita. The fishes were exposed to sublethal concentration of arsenic on succinate dehydogenase (SDH) and lactate dehydogenase(LDH) activities in fresh water fish, Labeo rohita. The present stydy shows the level of lactate dehydogenase was decreased and succinate activities was decreased due to arsenic

Keywords: Arsenic ,SDH, LDH,*Labeo rohita*, Brain,Gill, Liver Kidney

1.INTRODUCTION

Heavy metals are common persistent pollutants of aquatic ecosystem entering them through numerous diverse anthropogenic and natural sources (More, 1991). In industrial wastewaters, they are usually fond in mixture, which are specific to a particular pollution source [Scorecard, 2005]The heavy metal contamination of aquatic system investigators both in the developed an developing countries of the world [Faromobi *et al*., 2007]. Many industrial and agricultural processes have contributed to the contamination of fresh water system there by causing water system there by causing adverse effects on aquatic biota and human health (Wang, 2002; Dautremepuits *et al*., 2004).

Heavy metals can accumulated in the tissues of aquatic animals and as such tissue concentration of heavy metal can he of public health concern to animals (Kalay *et al*., 1999; Asharf, 2005).As a result of many industrial activities and technological development, the amount of heavy metal lons discharged into streams and rivers by industrial and municipal wastewater have been increasing incessantly. Heavy metal in the environment cause a major threat to animal life (Serencan *et al*., 2008).Besides the natural sources of arsenic contamination in drinking water, use of arsenic contamination in drinking water, use of arsenic containing herbicides, insecticides, rodenticidos, preservatives and by product of fossil fuels are evident to challenge the aquatic environment as well as human kind (Flora *et al*., 1995). Due to arsenic contamination of drinking water, an epidemic of arsenic dermatitis along with hypertension, and skin cancer are formed to human [Saha, 1995; Chowdhury *et al*., 1997]. The mechanism of arsenic toxicity is inhibition of sulfhydryl-group containing cellular enzymes and replacement of phosphate molecules in high energy compounds (Arsenolysis ATSDR, 1990, Hall, 2002). The trivalent arsenic compounds are mone potent in inhibiting enzymes, whereas pentavalent compounds are more involved in arsonists [ATSDR, 1990].
Fishes are being used for the assessment of the quality environment and as such can serve as bio-indicator of environmental pollution [Lopes *et al*., 2001]. Fish is used extensively for environmental monitoring [Lanfranchi *et al*., 2006], because they uptake contaminates directly form water. Generally the ability of fish to metabolize toxicants is moderate; there fore, contaminant loading in fish is well reflective of the state pollution in surrounding environments [Fisk *et al*., 1998].

The brain is an extremely heterogenous organ with a large number of different neuronal and non-neuronal cell types, and extensive morphological differentiation and biochemical compartmenention within the cell [Rana *et al*., 2002]. The gills are the first target organs in the heavy metal accumulation because they are directly in contact with water [Dubale and Shah, 1979]. The gills, which serve as the primary uptake site in the fish for trace metals, represent the most important targets when exposed to elevated levels of ambient metals [New man and Jogoe, 1997]. Liver is one of the most multi faceted and active organ in higher animals. In a vertebrate body, the liver is most important target organ as

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it is the chief metabolic and detoxification center [Bhattacharya and Mukherjee, 1976]. The kidney is the most sensitive organ with respect to overt toxicity following exposure to heavy metal. The kidney is the main excretory organ, is mainly concerned with removar of waste materials [Lawrence and Mc Cabe, 2002].

2.MATERIALS AND METHODS

Procurement of experimental animal

The fresh water fish, *Labeo rohita* were collected from the fish farm located in Puthur,Nagai District, 15 Km away from the Uinversity campus. This fishes were brought to the laboratory and transferred to the rectangular fibre glass tanks (100X175cm) of 500liters capacity containing chlorine free aerated well water.

Acclimatization of animals

The fresh water fish, *Labeo rohita* were acclimatized 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 acclimatization, 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.

Experimental design

The toxicant exposure was done by 24 hour or renewal bioassay system. For analysis sublethal toxicity, 2 groups of 10 fish each were exposed separately and arsenic trioxide (2.73ppm : 10 % 96 hours LC_{50}). Solution prepared in well water. The experimental medium was prepared by dissolving cadmium chloride at 6 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 through out the tenature of the experimental.

Estimation of LC_{50} value

Perior to the commencement of the experiment, 96 hr medium lethal concentration as (96 hr LC_{50}) of mercuric chloride for *Oreochoromis mossambicus* was estimated (Hamilton *et al* 1977). And 24 hrs renewal bioassay system and was found

BIOCHEMICAL STUDIES

After experiment, the fish each from the respective experimental as well as control groups were sacrificed. The brain gills, liver and kidney were isolated from the fish and used for various study.

Measurement of dehydrogenase activity
Preparation of samples

The tissues were isolated from the animal in the cold room and 5 per cent homogenate was prepared in 0.25m sucrose solution and centrifuged at 2500 rpm for 15 minutes to remove cell debris. The supernatant was used for the enzyme assay.

Preparation of succinate dehydrogenase (SDH) reaction mixture

Succinate dehydrogenas was estimated by the method of Nachales *et al*. (1960). In 10.0 ml clean dry test tube the following reaction mixture was added. The reaction mixture consisting of 1.0ml of Na-K-phosphate buffer (0.1M pH 7.4). 0.5ml of sodium succinate (0.1M pH 7.4) and 0.5ml of 0.5 per cent INT {(2-p-iodophyenyl) –3-(P-nitrophenyl) –5-phenyl tetrazolium chloride was added).

Preparation of Lactate Dehydrogenase (LDH) reaction mixture

Lactate dehydrogenase was estimated by the method of Govindappa and Swami (1965). In a 10-ml clean test tube the following reaction mixture consisting of 1.0ml of 0.1M Na-K- phosphate buffer, 0.5ml of 0.1M lithium lactate, 0.5 ml of 5 per cent INT in water was added.

Estimation of Dehydrogenase activity

All the above dehydrogenase reactions were initiated by the addition of 1.0 ml tissue homogenates. The samples were incepted at 37°C for one hour and the reactions were stopped by the addition of 6.0ml of acetic acid. The formazan formed was extracted with 6.0ml of toluene by keeping the tubes evernight in a reingerator at 5°C. The colour was read at 495 nm in double beam spectrophotometer. The dehydrogenase activity was expressed in u moles formazan formed / mg protein / hour.

Statistical analysis

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

3.RESULTS

Level of succinate dehydrogenase in brain tissue

In the brain tissue of normal fish, the level of succinate dehydrogenase was 32.11±1.07 μmole formazone formed/mg of protein/hr. During the sublethal concentration of arsenic, the level of succinate dehydrogenase was decreased upto

16.72±1.72 μmole formazone formed/mg of protein/hr when compared to control. The percent change over control was –15.39(Table 1).

Level of succinate dehydrogenase in gill tissue

The level of succinate dehydrogenase was 46.35 ±1.21 μmole formazone formed/mg of protein/hr. in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the decreased trend of succinate dehydrogenase (20.72±1.32 μmole formazone formed/mg of protein/hr). The percent change over the control was –55.30(Table 1).

Level of succinate dehydrogenase in liver tissue.

In the normal liver tissue, the level of succinate dehydrogenase content was 39.18±1.02 μmole formazone formed/mg of protein/hr when the fish exposed to arsenic, the level of succinate dehydrogenase content was decreased upto 18.63±01.19 μmole formazone formed/mg of protein/hr. The percent change over control was –52.45(Table 1).

Level of succinate dehydrogenase in kidney tissue

The level of succinate dehydrogenase present in the kidney tissue of normal fish was 30.76±1.62 μmole formazone formed/mg of protein/hr. The level of succinate dehydrogenase was decreased upto 16.46±1.27 μmole formazone formed/mg of protein/hr when the fish exposed with sub lethal concentration of arsenic. The percent change over control was-43.98(Table 1).

Table 1Level of succinate dehydrogenase in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic

Tissues	Control	21 days	% COC
Brain	32.11±1.07	16.72±1.72	-15.39
Gill	46.35±1.21	20.72±1.32	-55.30
Liver	39.18±1.02	18.63±1.19	-52.45
Kidney	30.76±1.62	16.46±1.27	-43.98

Mean ± S.D. of six individual observations;* Significance ($p<0.05$) Group I compared with group II; Values are expressed as (μ mole formazone formed/mg of protein/hr.)

Level of lactate dehydrogenase in brain tissue

In the brain tissue of normal fish, the level of lactate dehydrogenase was 20.16±1.22 μmole formazone formed/mg of protein/hr. During the sublethal concentration of arsenic, the level of lactate dehydrogenase was increased upto 28.81±1.72 μmole formazone formed/mg of protein/hr when compared to control. The percent change over control was 42.90(Table 2).

Level of lactate dehydrogenase in gill tissue

The level of lactate dehydrogenase was 31.19 ±1.72 μmole formazone formed/mg of protein/hr. in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the increased trend of lactate dehydrogenase (52.62±1.07 μmole formazone formed/mg of protein/hr). The percent change over the control was 68.70(Table 2).

Level of lactate dehydrogenase in liver tissue.

In the normal liver tissue, the level of lactate dehydrogenase content was 26.28±0.98 μmole formazone formed/mg of protein/hr when the fish exposed to arsenic, the level of lactate dehydrogenase content was increased upto 41.11±1.27 μmole formazone formed/mg of protein/hr. The percent change over control was 56.43(Table 2).

Level of lactate dehydrogenase in kidney tissue

The level of lactate dehydrogenase present in the kidney tissue of normal fish was 18.11±1.67 μmole formazone formed/mg of protein/hr. The level of lactate dehydrogenase was increased upto 25.22±1.62 μmole formazone formed/mg of protein/hr when the fish exposed with sub lethal concentration of arsenic. The percent change over control was 39.26(Table 2).

Table 2Level of lactate dehydrogenase in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic

Tissues	Control	21 days	% COC
Brain	20.16±1.22	28.81±1.72	42.90
Gill	31.19±1.72	52.62±1.07	68.70
Liver	26.28±0.98	41.11±1.27	56.43
Kidney	18.11±1.67	25.22±1.62	39.26

Mean ± S.D. of six individual observation;* Significance ($p<0.05$) Group I compared with group I; Values are expressed as (μ mole formazone formed/mg of protein/hr.)

4.DISCUSSION

The succince acid dehydrogenase (SDH) is an important enzymes of kreb's cycle whose qualitative changes are significant during certain pathological conditions. It is the oxidative enzyme, which was drastically affected by the action of heavy metal [Harper *et al*., 1978]. The lactate dechdonogenase (LDH) is an important role I the carbohydrate metabolism which converts the lactate to private. It is generally associated with cellular metabolic activity and inhibition in enzyme activity [Gupta and Sastry, 1981].

Heavy metals are pervasive components of the aquatic environment. The extent of physiological damage resulting from high level of heavy metal combination of fresh water which harmful to organisms. [More *et al*., 2005]. Heavy metals are known for their strong action on biological tissues. Metal ions once absorbed into body are capable of reacting with a variety of active binding sites and then disturbing the normal physiology of an organism which may tend to death of organism [More *et al*.,2005]. Mackee and Wolf, (1963) stated that the important poisoning effect on enzyme system depends on capacity of toxicants to react with ligands essential for normal functioning of enzyme system. Metals can form metal complexes or coordination compounds mainly with sulthydryls groups and to a lesser extent with a amino phosphate carboxylase, imidiozole and hydroxyl radicals of essential biological proteins.The harmful pollutants may cause injury to organism and the damaged tissues shall dysfunction, which results in quantitative altered enzyme activity. Thus enzyme bioassay can provide diagnostic means to assess a change to pollutants.

In the present study, the decreased level of succinic acid denydrogenaseand increaselevel of lactic acid dehydrogenase observed in train, gill, liver and kidney tissues of labeo rohita exposed to arsenic. Behari *et al*., (1978) have reported that chromium might be preset rated into the tissuecells affecting the mitochondria dehydrogenises labeo rohita. Mary Chandravathy and Reddy, 1994 have reported that the increased LDH and decreased SDH in the gill and liver tissues of Anabass candens exposed to leadnitrate. Sharma (1999) has reportedthat significant decrease in the activity of liver SDH suggests that anerobic metabolism is favored over aerobic oxidastion of glucose through kreb's cycle in order to migrate the energy crisis for survival. Microslous (1973) has reported that depletion in the level of hepatic SDH in rats exposed to Malathion Ahamed *et al*., (1978)noted inhibition a SDH due to exposure of pesticides More *et al*., (2005 have reported that the level of SDH decreased in Lamellidens marginclis exposed to heavy metal.

Basha mohideen and paramesh war Rao, (1979) have reported that the depletion of SDH activity usually denotes the various pathological conditions. Since the osmoregulatory mechanism are also dependent a large scale of SDH. Decrease in SDH activity indicates a inhibited mitochondrial oxidation of succinct, which may lead to a drop in energy production. Since heavy metal exposure is a serious mind of adverse effect and is classified as stress, mitochonciral alterations are bound to take place on a very large scale which are exhibited in the total disruption of all biochemical process including enzymatic reactions.

James *et al*., (1992) reported that the decreased level of SDH onsidereably within elevation of LDH activities in stressed animals. They also suggested hat the stressed animals are meeting their energy requirements through anaerobic oxidation. Radhaiah, (1985)has observed the increased permeability of cell and hecrosis are usually characterized by rise in LDH activity. The enzyme lactic acid dehydrogenase catalyses the inter conversion of lactate and private in the glycol tic pathway and occurs as a tetra menmolecules.

Almeida *et al*., (2001) reportedthat the increased LDH activity observed in oreochromis holiticus exposed to chadmium. Gosh, (1987) reported the increased LDH activity in liver tissue of Channa punctatus exposed to sub lethal concentration of metaystox and in clariasd batracus exposed to phosphamidon respectively. Martin *et al*., (1983) have stated that the LDH activity is increased during stress condition favouring anaerobic respiration to meet the energy demand. Jagadeesan (1994) has observed the increased LDH activity in the animal exposed to mercury. Vasilos *et al*., (1976) the depression and SDH and and elevation of LDH activities indicate dependence of anaerobic metabolism in the arsemic exposed fish to meet the extra energy requirements. Thus there is a shiftin energy metabolism of the fish from aerobiosis to anaerobiosis and this may be due to outcome of mitochondrial disruption.

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