Effect of Arsenic on Certain Biochemical Parameters in Liver Tissue of an Air Breathing Fish *Channa gachua*

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Abstract

The environment is currently polluted by thousands of chemicals or xenobiotic introduced into the environment by man to meet the demands of the modern era. The pollution is continuous and alarming in flux to aquatic environment worldwide from both naturally occurring and anthropogenic sources. The polluted water may lead to the destruction of the beneficial species either directly effecting aquatic forms of life in directly through breaking the biological food chain such as fish and their habitat and behavioral pattern. The fish as a bio indicator of aquatic medium plays an important role in the monitoring of water pollution because of the sudden death of fish indicates heavy pollution and the effects of exposure to sub lethal levels can be measured in terms of biochemical, physiological and histological responses of the fishes. In the present study, the sub lethal effects of arsenic on various biochemical parameters of *Channa gachua* were studied. The fish was exposed to sub lethal concentration of arsenic for 20 days for chronic toxicity studies. Total protein, amino acid and acetyl cholinesterase, glycogen and lactic acid were observed. The present study showed the protein content was decreased and amino acid content was increased significantly and also Acetyl cholinesterase was increased in the liver tissue of arsenic treated fish, *Channa gachua*. The present study shows the level of glycogen decreased and lactic acid increased in the liver tissue of fish exposed to arsenic. These changes were concentration dependent.

Keywords: Biochemical parameters, arsenic, liver, *Channa gachua.*

Introduction

Arsenic is a trace element and it under goes multiple electron transfer reactions. Arsenic exists in soluble and insoluble forms, organic and inorganic trivalent and pentavalent forms among which the trivalent arsenite is highly toxic. It occurs in the earth's crust along with sulphides and iron pyrites. The sources of arsenic found in environment includes natural and manmade. It is released into the human environment including drinking water through the mining and burning coal, smelting of copper and through industrial effluents. Chemicals containing arsenic are also used in the manufacturing of herbicides and pesticides, leads shots and phosphate detergents and in preservation of wood and hide. Arsenical herbicides and pesticides applied to agricultural soils and vegetation also may be important sources of arsenic contamination of food stuffs respectively. Arsenic is a toxic element for humans and it is commonly associated with serious health disruptions (Brookes, 1998). Total diet as studies carried out in various countries have shown that fish and shell fish are the most significant dietary source, accounting for nearly three quarters of total intake (Dokkun *et al.*, 1989

and Tao *et al.,* 1999). The concentration of arsenic was found in environmental samples, mainly in waters where inorganic form is predominant (Smith *et al.,* 2000, Elci *et al.,* 2008). Arsenic exposure has been related to the appearance of some types of cancer (Tchounwou *et al.,* 2003). A report on an assessment of the cancer risk associated with consumption of oysters caused a panic among consumers in Taiwan (Guo, 2002). Some of these human health effects currently observed in population of South and South eastern Asia, particularly in countries such as Bangladesh and India (Al Ramali *et al.,* 2005). Besides the direct exposure of humans as through drinking contaminated water, this might also be biologically available to aquatic organisms, such as fish which are used as human food there by providing an additional source of nutrition. Arsenic has a considerable tendency to accumulate in bottom sediments (Svobodovo *et al*., 2002). For this reason, issues related to as content in aquatic organisms and sea fish in particular, have attracted considerable attentions. The relevance of this arsenic intake will depend on the concentration of accumulated by the fish (Lai *et al.,* 2001). During recent years, serious concern has been voiced about the rapidly deteriorating state of fresh water bodies with respect to toxic metals pollution. Fishes are often at the top of the aquatic food chain and accumulate large amounts of some metals from the water (Tuzen, 2003).Water pollution leads to fish contamination with toxic metals from many sources such as industrial and domestic wastewater, natural runoff and contributory rivers (Rashed, 2001 and Tariq *et al.,* 1991). Fishes, living in polluted water may accumulate toxic trace metals via their food chains; they assimilate metals by ingestion of particulate material suspended in water, ion exchange of dissolved metals across lipophilic membranes, e.g., the gills, adsorption on tissue and membranes surfaces. The bioaccumulation of metals is therefore, an index of the pollution status of the relevant water body (Alam *et al.,* 2002).

Protein is the most important and abundant biochemical constituent present in the animal body. Hence, Proteins are important in all biological systems. Protein and amino acids are very important nutrients. Protein plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants which enter into the animal body (Ramasamy, 1987). Amino acids are the building blocks of protein which are organic compounds, meaning that they contain carbon and hydrogen bonded to each other. In addition to those two elements, they include nitrogen, oxygen and in few cases sulfur. The basic structure of an amino acid molecule consists of a carbon atom bonded to an amino group which is the ($-NH_2$), a carboxyl group (-COOH) a hydrogen atom, and a fourth group that differs from one amino acid to another and often is referred to as the -R group or the side chain. The R-group, which can vary widely, is responsible for the differences in chemical properties of fetus (Sankar and Jagadeesan, 2006). Acetyl cholinesterase is an enzyme present in various tissues, including muscle and red cells, that breaks down acetylcholine a chemical released by nerves that activates muscle contractions and helps to maintain proper transmission of impulses between nerve cells and between nerve cells and muscles; also called true cholinesterase. Measuring acetyl cholinesterase in amniotic fluid may help confirm a suspected neural tube defect in the foetus Sankar and Jagadeesan (2006). Carbohydrate is an essential energy source for all vital activities of an organism. It is stored in the form of glycogen in animals. Glycogen is broken down into glucose for energy requirements. The stressful condition disturbs the metabolic rate of carbohydrate and thus the level of glycogen; glucose and lactic acid are altered (Srivastava and Singh, 1980; Metelev *et al.,* 1983; Rahman and Shamim 2014). The present study was carried with an aim to investigate effect of the sub lethal dose of arsenic in biochemical parameters in liver tissue of an air breathing fish *Channa gachua.*

Materials and Methods

Live specimens of *Channa gachua* were procured from local fish dealers at Hazaribag (Latitude 25º 59'N and Longitude 85º 22'E) and maintained in large glass aquaria size (90 x 60 x 60 cm) with continuous flow of water. The specimens were fed on chopped goat liver daily during a minimum acclimation period of 20 days in the laboratory. Routine oxygen consumption from air and still water was measured in a closed glass respirometer containing 3 litres of water (initial O_2 content = 6.5 mg O_2/ litre; pH = 7.2) and 0.51 mL of air. The fish were acclimated to the respirometers for at least 12 hours before the readings were taken. The experiments were conducted at 29.0 ± 1.5°C. The pH of the ambient water was measured by an electronic pH meter systronics. The respiratory chambers were thermostated by immersion in a temperature controlled water bath. Fishes from 70-100 grams were used during the experiment. They were checked thoroughly for injury and disease conditions and only healthy fishes were used for this study. After washing with 0.01% $KMnO_4$ solution for 15 min, they were placed in nine plastic pools (300 L) containing non chlorinated water. Prior to the start of the experiment the fishes were acclimatized to the food and laboratory conditions with 12 hours dark and 12 hours light cycles. Fishes were divided into five equal groups each comprising of 30 fishes. Each group was kept in separate aquarium tanks. The first group was kept as negative control; the fishes were maintained in water containing normal water without any treatment. The fishes of two groups were exposed to a sub lethal concentration of 1 ppm concentration of Arsenic added in the water for 30 days respectively. Solutions were renewed once daily after exposure period, animals were sacrificed and the liver tissues were removed, homogenized and stored at −80 °C for further biochemical analysis. Protein content in the tissue was estimated by the method of (Lowry *et al.,* 1951). Total free amino acids and content of the tissue were estimated by the method of (Moore and Stein, 1954). The glycogen content was estimated by Kemp and Kits (1954) and Lactic acid was done by the method of Barker and Summerson (1941). The data were subjected to student "t" test to find out the significance of difference between control and treated values.

Results and Discussion

In *Channa gachua* the gills are pinkish red in colour. Each gill arch is made up of two primary gill filaments. The filaments are beset with the secondary gill lamellae. The gills receive blood from the afferent branchial artery near the origin of gill lamellae. The opening of efferent branchial artery is situated just below the afferent branchial artery. The transverse ligament present between the both arteries. In the present study, attempts have been made to investigate the effects of sub lethal concentration of arsenic on various biochemical parameters of *Channa gachua* in acute and toxicity studies. In the liver tissue of control groups, the protein content was 86.92±1.98 mg/g wt. wt. of tissue. After the mercury exposure the level of protein content was significantly decreased in liver tissue of arsenic exposed fish, as compared to respective control levels (Table 1). The level of protein content was increased in arsenic exposed fish. In the liver tissue of control fish, the acetyl cholinesterase activity was 45.72±0.95 moles of acetylcholine hydrolysed per mg of protein per hr. During the arsenic exposure the activity of acetyl cholinesterase was decreased in the liver tissue of fish. The level of glycogen content in the liver tissue of control fish was 11.99±1.96 mg/g wet wt. of tissue. During the arsenic exposure the level of glycogen decreased in the liver tissue (8.42±0.97mg/g wet wt. of tissue) in the liver tissue of control groups respectively. The lactic acid content was 2.84±1.08 mg/g wt. wt. of tissue. After the arsenic exposure the level of lactic acid content was significantly decreased in liver tissue of arsenic exposed fish, as compared to respective control levels.

Table 1: Showing biochemical parameters in liver tissue of *Channa gachua* treated with arsenic.

Parameters	Control	20 days treated
Protein (mg/g)	86.92±1.98	73.85±1.68*
Amino acid (mg/g)	2.85±1.54	3.66±1.87*
Acetyl cholinesterase (AChE)	45.72±0.95	36.14±1.92*
Glycogen	11.99±1.96	8.42±0.97*
Lactic acid	2.84±1.08	4.22±1.87

Mean ±S.D of six individual observations; *significance at 5% level

In the present study a reduction in the protein content observed in *Channa gachua* exposed with arsenic. These results suggest that the tissue protein undergoes proteolysis results in an increase in the production of free amino acids. These amino acids are utilized for energy production during stressful situation in the intoxicated fishes. Neff (1985) has reported that decline in protein content may also be related to increased energy cost of homeostasis, tissue repair and detoxification during stress. In the present investigation sub lethal concentrations of arsenic exposed fish *Channa gachua* exposed with arsenic show a decrease in protein content and an increase in amino acid content of liver for 20 days exposure of arsenic. Many investigations have also reported such a change in total protein content of various tissues in different fishes exposed to different heavy metals (Rajamanikam,1992 and Pazhanisamy, 2002). Jana and Bandyopadhyay (1981) have reported such a reduction in protein content when the fish *Channa punctatus* has been exposed to heavy metals such as mercury, arsenic and lead. Protein depletion has been reported in the liver of *Anabas testudineus* exposed to nickel chloride (Jha and Jha, 1995). Decrease in the liver protein level is reported in the fish *Labeo rohita* exposed to arsenic Pazhanisamy (2002) *Channa punctatus* exposed to zinc and phenyl mercuric acetate (Sen *et al.,* 1992 and Karuppasamy, 2000) *Channa punctatus* exposed to arsenic (Jatyajit, 1996) *Channa striatus* exposed to mercury cadmium and lead (Palanichamy and Baskaran, 1995) and *Cirrhina mrigala* exposed to lead acetate (Ramalingam *et al.* 2000). Baskaran *et al.* (1989) have reported the impact of commercial detergent Nirma on feeding energetics and protein metabolism in the fresh water teleost fish *Oreochromis mossambicus*. The decrease in liver and muscle protein has been reported in the sugar mill effluent treated *Channa punctatus* after 96 hours exposure (Avasan and Ramakrishna, 2000). In the present investigation, the decreased level of protein in brain tissue shows that fish exposed to arsenic are subject to stress. Similar results have also been recorded in the protein content of different tissues when the animals are exposed to various pollutants (Palanichamy *et al.,* 1989; Malla and Basha, 1988; Manoharan and Subbiah 1982. Meenakshi and Indra (1998) have noticed depletion in the level of total protein in liver and muscle and an increase in the total free amino acids in blood, liver and muscle of distillery effluent treated *Mystus vittatus*. Anuradha and Raju (1996) have observed the increased level of amino acid content in liver, muscle, kidney and gill tissues of *Anabas scandens* exposed to selenium toxicity. The FAA serves as metabolites for a TCA cycle which have a key role in stepping up the energy requirement respectively.

Acetyl chollinesterase (AchE) activity measurement in fish has been used for monitoring the neurotoxicity of pesticide (Bretaud *et al.,* 2000). AchE, a serine hydrolase catalyzes the breakdown of the neurotransmitter acetyl choline into acetate and choline. This process involves the formation of a substrate enzyme complex, followed by acetylation of the hydroxyl group, the amino acid serine, present within the eastertic side and finally deacetylation. The inhibitory effect on AchE activity indicates that pollutants like insecticide might

interfere in the vital processes like energy metabolism of nerve cells (Nath and Kumar, 1999). AchE inhibition and an accumulation of ACh in the tissues of sumithion treated fish *Channa gachua* have been observed by Kaundinya and Ramamurthy(1978). Basha and Sailbala (1989) have observed a steep decline in AchE activity with a concomitant elevation in AchE content in different tissues like gill, kidney, brain, liver and different types of muscles in *Cyprinus carpio* following 10 days exposure to malathion. The decrease in brain AchE is found to be inversely proportional to the increase in Ach content in methyl parathion treated tadpoles of frog, *Rana cyanophiclits*. Ravi and Selvarajan (1990) have reported an increase in the levels of amine in the brain region of *Labeo rohita* and *Cyprinus carpio* exposed to phosalone. Sevgiler *et al.* (2004) have reported a significant correlation between increase in lipid peroxidation and inhibition of AchE activity in liver. They have further stated that etoxazole mediated lipid peroxidation may be related to its anticholine esterase action. Increased lipid peroxidation caused by etoxazole indicates that this compound induces the generation of reactive oxygen species, creating oxidative damage in the cell membrane. Yang and Dettbarn (1996) in their study with disisopropyl fluorophosphates have suggested that AchE inhibitor induced cholinergic hyperactivity has initiated the accumulation of free radicals leading to lipid peroxidation, which may be the initiator of AchE inhibitor induced cell injury. Nachmanson and Feld (1947) have reported that the animal dies when AchE activity of the brain is inhibited by 95 percent. Coppage (1972) have observed 79 percent reduction in AchE activity in the esturine fish *Lagodon rhomboids* exposed to 48 hours median lethal concentrations (92±g/L) of malathion. Carbohydrate is an essential energy source for all vital activities of an organism. It is stored in the form of glycogen in animals. Glycogen is broken down into glucose for energy requirements. The stressful condition disturbs the metabolic rate of carbohydrate and thus the level of glycogen; glucose and lactic acid are altered (Srivastava and Singh, 1980; Metelev *et al.*, (1983). The toxic substances are absorbed into the body and transported to various organs through blood. The blood glucose is a sensitive biochemical indicator of stress. Exposure of fishes to different types of toxic substance is known to elicit changes in the biochemical constituents and thereby altering the metabolic pathways. In the present study the level of glycogen content and lactic acid was increased in the liver tissue of fish exposed to arsenic. Changes in the glycogen level of liver have been noticed by many investigators. Mcleay and Brown (1974) have recorded a considerable decrease in glycogen content of bleached kraft pulp mill effluent. Baskaran *et al.*, (1989) have noticed the depletion on the hepatic glycogen content in *Oreochromis mossambicus* when exposed to textile dye effluent. Depletion in the glycogen content of liver and muscle has been observed in *Rasbora daniconius* exposed to pulp and paper mill effluent (Vijayaram and Vasugi; 1989). *Channa gachua* exposed to sub lethal concentration of arsenic shows an overall increase in the blood glucose at all periods of exposure thereby indicating that the glycol genolysis takes place in the liver, where by the reserved glycogen is being slowly converted into glucose. The hyper glycemic condition in the present study correlated with the observations of some researcher's *viz.*, the juvenile Coho salmon on *Oncorhynchus kisutch* treated with sub lethal concentration of neutralized unbleached kraft mill effluent Mcleay (1973). Similar results were made by Vijayram and Vasugi (1989) in paper and pulp mill effluents. Similar elevated blood glucose levels have been noticed in *Heteropneustes fossilis* exposed to textile mill effluent Nisha and Shukla (1986). Lactic acid is formed through glycolysis under anaerobic condition of glucose catabolism. In the present study it showed an increase in the lactic acid content of liver and blood at all the hours of effluent treatment. Accumulation of lactic acid is more in liver and blood of fishes exposed to raw effluent. It is likely that the lactic acid formed in the muscle and other tissue during glycolysis, might have been transported to liver *via* blood accounting for the hyper lactamia in blood and liver. Because of the absence of the enzyme glucose 6-phosphatase in the

muscle which is necessary for the conversion of lactic acid into glucose, the lactic acid produced in the tissue is transported to the liver through blood (Shanmugam, 1980). Since liver is the metabolic site the lactic acid transported from the tissue to liver is utilized for the resynthesize the of glucose and glycogen through Cori cycle contributing to the increase in the level of lactic acid in liver and blood at all periods of study. Burton *et al.* (1972) have observed the heavy accumulation of lactic acid in liver of rain brown trout *Salmo gairdneri* exposed to zinc. Rahman and Shamim (2014) reported that impact of zinc sulphate on bio chemical parameter in *Channa gachua* reported that the decreased glycogen concentration in the liver could be due to its enhanced utilization as an immediate source to meet the energy demand under metallic stress 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. Depleted glycogen level under other heavy metals stress is also supports our findings with other workers. The increased in glucose level of tissue while decrement in tissue glycogen in exposed fish *Channa gachua* makes it clear that glycogen reserves are being used to meet the stress. To summarize these results indicate that the heavy metal at sub lethal and lethal concentrations altered the bio chemical composition of the test fish due to utilization of biochemical energy to counter act the toxic stress due to heavy metals present in effluents.

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