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RESEARCH ARTICLE

EFFECT OF ARSENIC ON CERTAIN BIOCHEMICAL PARAMETERS IN LIVER TISSUE OF *Tilapia mossambica*

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ABSTRACT

In the present study, the sub-lethal effects of arsenic on various biochemical parameters of *Tilapia mossambica* were studied. The fish was exposed to sub- lethal concentration of arsenic for 15 days for chronic toxicity studies. In the present study, total protein, amino acid and acetylcholinesterase, glycogen and lactic acid were observed. The present study showed the protein content was decreased and amino acid content was increased significantly and also Acetylcholinesterase was increased in the liver tissue of arsenic treated fish, *Tilapia mossambica*. 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.

INTRODUCTION

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 of as, accounting for nearly three quarters of total intake (Dokkum *et al.,* 1989,Tao *et al.,*1999). The concentration of as 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 (Ranbis *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 Southeastern Asia, particularly in countries such as Bangladesh and India (AlRamali, 2005). Besides the direct exposure of humans to as through drinking contaminated water, the As might also be biologically available to aquatic organisms, such as fish which are used as human food thereby providing an additional source of as. Arsenic has a considerable tendency to accumulate in bottom sediments (Svobodovo,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 as intake will depend on the concentration of As accumulated by the fish (Lai *et al.,* 2001).During recent years, serious concern has been voiced about

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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, e.g., industrial and domestic wastewater, natural runoff and contributory rivers (Rashed, 2001,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 (Alam *et al.,* 2002). The bioaccumulation of metals is therefore, an index of the pollution status of the relevant water body (Mastoi *et al.,* 2008). Protein is the most important and abundant biochemical constituent present in the animal body.

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 a few cases, sulfur. The basic structure of an amino-acid molecule consists of a carbon atom bonded to an amino group ($-NH_2$), 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 fetus (Sankarsamipillai and Jagadeesan, 2006). Acetylcholinesterase 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 acetylcholinesterase in amniotic fluid may help confirm a suspected neural tube defect in the fetus (Sankarsamipillai 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). The present study was carried with an aim to investigate the sub-lethal effect of arsenic in biochemical parameters in liver tissue of *Tilapia mossambica.*

MATERIALS AND METHODS

The fresh water fish *Tilapia mossambica* were collected from fish farm at Puthur, Tamil Nadu, India. The collected fish were acclimated to laboratory condition for 15 days. 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 (500 L) containing non-chlorinated water. Prior to the start of the experiment, the fishes were acclimatized to the food and laboratory conditions with 12 h dark and 12 h light cycles, pH range of 6.95 to 7.60 and temperature ranging from 16 to 24 °C for 15 days.

Fishes were divided into three equal groups each comprising of 36 fishes. Each group was kept in separate plastic 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 analyses.

Estimation of biochemical study

Protein content in the tissue were 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 enzyme acetylcholinesterase was assayed by Metcalf (1951), The glycogen content was estimated by Kemp and Kits Van Heijhingen (1954), and Lactic acid was done by the method of Barker and Summerson (1941)

Statistical analysis

The data were subjected to student "t" test to find out the significance of difference between control and treated values.

RESULTS

In the present study, attempts have been made to investigate the effects of sub-lethal concentration of arsenic on various biochemical parameters of *Tilapia mossambica* 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).

Table 1 shows the amino acid content in the brain tissue of fish. The level of protein content was increased in arsenic exposed fish. In the liver tissue of control fish, the acetylcholinesterase activity was 45.72±0.95 µ moles of acetylchoine hydrolysed/mg of protein/hr. During the arsenic exposure the activity of acetylcholinesterase was decreased in the liver tissue of fish (Table.1).

The level of glycogen content in the liver tissue of control fish was 11.99±1.96mg/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). The In the liver tissue of control groups, 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).

DISCUSSION

In the present study, A reduction in the protein content observed in *Tilapia mossambica* 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, sublethal concentrations of arsenic exposed fish *Tilapia mossambica* exposed with arsenic show a decrease in protein content and an increase in amino acid content of liver for 15 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; Pazhanisamy, 2002). Jana and Bandyopathyay (1981) have reported such a

Table 1. Level of biochemical parameters in liver tissue of *Tilapia mossambica* treated with arsenic

Parameters	Control	15 days treated
Protein (mg/g wte wt. of tissue)	86.92±1.98	73.85±1.68*
Amino acid (µg/g wet wt.of tissue)	2.85±1.54	3.66±1.87*
Acetylcholineesterase (AchE) (µ moles of acetylchoine hydrolysed / mg of protein/hr)	45.72±0.95	36.14±1.92*
Glycogen (mg/g wet wt. of tissue)	11.99±1.96	8.42±0.97*
Lactic acid (mg/g wet wt. of tissue)	2.84±1.08	4.22±1.87*

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

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 testudines* 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; Karuppasamy, 2000) *Channa punctatus* exposed to arsenic (Jatyajit Hota, 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*. (1991) have reported the impact of commercial detergent (Nirma) on feeding energetics and protein metabolism in the freshwater teleost fish *Oreochromis mossambicus*.

The decrease in liver and muscle protein has been reported in the sugar mill effluent treated *Channa punctatus* after 96 hr exposure (Avash Maruthi and Ramakrishna Rao, 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 Reddy and Bashamohidran, 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*. The remarkable increase in the free amino acid may represent proteolysis in the tissues to meet the demands for energy requisites in addition to the carbohydrates and fat. Increase in amino acid content in liver is observed in *Mystus vittatus* exposed to median lethal concentration of mercuric chloride (Jagedeesan, 1994) and in *Mystus vittatus* exposed to sublethal and median lethal concentration of copper (Rajamanickam,1992). 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. Acetylchollinesterase (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 acetylcholine 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 esteratic 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).In the present study, 15 days exposure period of lead has resulted the inhibition of AchE activity level in the brain of *Tilapia mossambica* a decrease in AchE activity level has led to the accumulation of acetylcholine in the brain of fish (Josh *et al.,* 1982). AchE inhibition and an accumulation of ACh in the tissues of sumithion treated fish *Tilapia mossambica* have been observed by Koundinya and Ramamurthi (1978). Bashamohideen and Sailbala (1989) have observed a steep decline in AchE activity with a concomitant elevation in ACh 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 cyanophlicits*. Accumulation of ACh and inhibition of AchE activity levels in liver, muscle, gill and brain have been reported in *Tilapia mossambica* exposed to fenvalerate (Ghosh, 1990). 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 Dettab (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 *et al*. (1975) have observed 79 percent reduction in AchE activity in the esturine fish *Lagodon rhomboids* exposed to 48 hours median lethal concentrations (92µg/l) of malathion. Quinolphos, another organophosphorus insecticide also produces a highly significant inhibition of AchE activity in the brain and a reduction in RBC of female guinea pigs (Dikshith *et al.,* 1980).

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 (Motelev *et al.,* 1983). 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 has been noticed by many investigators. Mcleay and Brown, (1975) 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 have been observed in Rasbora daniconirus exposed to pulp and paper mill effluent (Vijayaran and Vasugi, 1989).

Tialpia mossambica exposed to sublethal concentration of arsenic shows an overall increase in the blood glucose at all periods of exposure, thereby indicating that the glycogenolysis takes place in the liver, where by the reserved glycogen is being slowly converted into glucose. The hyperglycemic condition in the present study correlated with the observations of some researcher's *viz.,* the juvenile Cohosalmon on *Corhynchus kisutch* treated with sub-lethal concentration of neutralized unbleached kraft mill effluent (Mcleay, 1973). Similar results were mode by Vijayram, and Vasugi, (1989) in paper and pulp mill effluents. Similar elevated blood glucose levels have been noticed in *Meteropheustes 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 *Cyprinus carpio* 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 (Ambika shanmugam, 1980). Since liver is the metabolic site, the lactic acid transported from the tissue to liver is utilised for the resynthesis the of glucose and glycogen through cori cycle (Mayer Bodensky, 1947) 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 bow trout *Salmo gairdneri* exposed to zinc.

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