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RESEARCH ARTICLE

ARSENIC INDUCED BIOCHEMICAL CHANGES IN THE BRAIN TISSUE OF FRESH WATER FISH, Catla catla

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ABSTRACT

In the present study, an attempt has been made to analyze the changes in the biochemical parameters in the brain tissue of Catla catla for 21 days. The sublethal concentration of arsenic alters such as total protein, amino acid, glycogen, glucose acetylcholine and acetyl cholinesterase in the brain tissue. Protein, glycogen and acetyl cholinesterase were decreased .Amino acid, glucose, and acetylcholine were increased in the brain tissue due to toxicity of arsenic.

INTRODUCTION

Heavy metals are distributed throughout the environment and it is derived from industrial processes, agricultural activities, burning of fossil fuels and weathering of geologic formation (WHO, 1989). Heavy metals are the major environmental pollutants when discharged into estuarine system can be accumulating in aquatic biota. Heavy metals from natural and anthropogenic sources continuously enter the aquatic ecosystem where they pose serious threat because of their toxicity, Long persistence, Bioaccumulation and biomagnifications in the food chain (Sankar Samipilai and Jagadeesan, 2006). Arsenic is a toxic element for humans and it is commonly associated with serious health disruptions (Brooks, 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 (Dakkun et al., 1999; Tao and Bolger,1996). 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 is widely distributed in the environment including water resources and animal tissues and occurs as a variety of organic and inorganic compounds (Webb, 1996).

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The concentration of arsenic in the environments is of great concern as thus element is recognized as a cumulative poison to animals. Arsenic is mainly released in to the environment through intestinal process during the preparation of base metals and thermal power generation. The arsenic and its compounds are used as pesticides herbicides, insecticides and fungicides (Webb, 1996). The animals are exposed to inorganic arsenic through drinking well water, food, air and are occasionally exposed occupationally through arsenic fumes or dust (NRC, 1999). Arsenic is normally in the pentavalent inorganic arsenate form in drinking water, but upon consumption by animal, it rapidly undergoes metabolic conversion that includes reduction of arsenate to arsenates. In developing countries arsenic contamination of ground water remains a crucial water ground water remains a crucial water quality problem in particular, in developing countries. Acute and chronic poisoning of arsenic has occurred as a result of consumption of high level of arsenic contaminated well water, and causes numerous disease including specific causes numerous disease including specific cancers (Kitchin, 2001), Hypertension (Chen, 1995). 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, 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). Hence, this investigation was aimed to study the changes in biochemical studies in the brain tissues of *Catla catla* exposed to arsenic.

MATERIALS AND METHODS

Chemical

Heavy metal arsenic has purchased from High Media Chemicals, India Private Limited, Mumbai, India.

Experimental fishes

The fresh water fish *Catla catla* were collected from fish farm at Puthur, Tamil Nadu, India. The collected fish were acclimated to laboratory condition for 21 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.

Experimental design

Fishes were divided into two equal groups each comprising of 20 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 Arsenic (0.1ppm) added in the water for 21 days respectively. Solutions were renewed once daily after exposure period, animals (n=20/group) were sacrificed and the brain was removed, homogenized and stored at −80 °C for further biochemical analyses. After experiment, the fish each from the respective experimental as well as control groups were sacrificed. The brain was isolated from the fish and used for various study.

Biochemical Studies

Protein content in the tissues 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). Kemp and Kits van Heijningen (1954) were employed for the quantitative estimation of glycogen and glucose. The tissue ach content was estimated by the method of hestrin as described by Augustinson (1957). Acetylcholinesterase (AchE) activity was estimated by the following method of Metcalf (1951) and Statistical significance was evaluated by using ANOVA followed by Duncan Multiple Range Test (DMRT) Duncan (1957).

RESULTS

In the brain tissue of normal fish, the level of protein was 56.18 ± 1.07 (mg/g wet wt.) of tissues. During the sublethal concentration of arsenic, the level of protein was decreased upto 20.65 ± 1.86 (mg/g wet wt.) of tissues when compared to control. In the brain tissue of normal fish, the level of amino acid was 6.79±1.10 (µmg/g wet wt. of tissues). During the sublethal concentration of arsenic, the level of amino acid was increased upto may occur as a result of impairment of energy production or inhibition of enzymes involved in the synthesis and 9.85±1.88 (µg/g wet wt. of tissues) when compared to control (Table 1). In the brain tissue of normal fish, the level of glycogen was 5.98 ± 1.82 (mg/g wet wt. of tissues). During the sublethal concentration of arsenic, the level of glycogen was decreased upto 2.11±1.66 (mg/g wet wt. of tissues) when compared to control. In the brain tissue of normal fish, the level of glucose was 5.21±1.65 (mg/g wet wt. of tissues). During the sublethal concentration of arsenic, the level of glucose was increased upto 7.15±1.82 mg/g wet wt. of tissues when compared to control (Table 1). In the brain tissue of normal fish, the level of acetylcholine was (32.66±0.86 µmole/g wet wt.). of tissues. During the sublethal concentration of arsenic, the level of acetylcholine was increased upto 41.85±0.62 (µmole/g wet wt. of tissues) when compared to control. In the brain tissue of normal fish, the level of acetylcholinesterase was 5.12±0.62 (µmole of acetylcholine hydrolyzed/mg.of protein/hr.). During the sublethal concentration of arsenic, the level of acetylcholinesterase was decreased upto 4.06±0.27 (µmole of acetylcholine hydrolyzed/mg. of protein/hr) when compared to control (Table 1).

DISCUSSION

Proteins are important organic constituents of the animal cells. It plays a vital role in the process of interactions between intra and extra cellular media being a part of cell membrane and enzymes (Ramalingam, 2002). The amino acid and the building blot of protein. 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). In the present study, the level a protein decreased and the level of amino acid increased in the brain tissue when the fish exposed with arsenic trioxide for 21 days. This result suggests that the decreased level of protein might be due to their catabolism to liberate energy during the stress of arsenic toxicity. Similarly, Jana and Bandyopathway (1981) have reported the reduction in protein content in *Channa punctatus* exposed to arsenic and lead.

Reddy *et al*., (1998) have reported that the fall in protein level during heavy metal exposure may be due to increased catabolism and decreased anabolism of protein. Jha and Jha, (1995) have reported that the level of protein content decreased in liver tissue of anabu testudineus exposed nickel chloride. Ramalingam *et al*., (2000) reported that protein content was decreased in *Cirrhinas mrigala* exposed to lead acetate. Palanichamy and Baskaran (1995) have reported a reduction in the level of protein in the muscle and liver tissue of *Channa striatus* exposed to mercury, cadmium and lead. James *et al*., (1991) observed a reduction in protein content in liver, gill and muscle tissue to *Oreochaomis mossambicus* exposed to zinc and cadmium. Almeida *et al*., (2001) have reported that a decrease in protein content in liver and muscle of *Oreochromis niloticus* exposed to cadmium. The decrease in protein might be due to their degradation and also to their possible utilization for metabolic purposes (Sing and Sing, 2003).

In the present study, the level of amino acid content is increased in brain tissue of *fish* exposed to arsenic trioxide for 21 days. This is mainly a consequence of higher catabolic activity of protein to meet the high energy demand by breaking down the protein into free amino acids. Seshagiri Rao *et al*., (1983) have reported that an increased level of amino acid content in the tissues of *Sarothoradon mossambicus* when exposed to benthiocarp toxicity. They also reported that the enhanced level or amino acids result of on intensive proteolysis in the respective tissues. Karuppasamy (1999) has observed the level of amino acids was in creased in liver, muscle, kidney, brain and gill tissue of *Channa punctatus* exposed to phenyl mercuric acetate.

by hydrolyzing to excitatory transmitted acetylcholine (Mitatrovic and Dettbarn, 1996).

In the present study, the level of acetylcholine increased and acetylcholinesterase decreased in brain tissue of *Catla catla* exposed to arsenic. This result indicates that arsenic block the active center of enzyme and also drastically inhibits its de novo synthesis. The toxic effect of heavy metals on the necrotronsmitter may result from their action or sub cellular process such as interference with mechanism regulating calcium distribution in nerve terminals and anabolic effect that may occur as a result of impairment of energy production or inhibition of enzymes involved in the synthesis and

Table 1. Level of biochemical parameters in the brain tissue of Catla catla treated with arsenic

Parameters	Control	Arsenic treated
Total protein (mg/g wet.wt.of tissue)	56.18±1.07	20.65±1.86
Amino acid (µg/g wet.wt of tissue)	6.79±1.10	9.85±1.88
Glycogen (mg/g wet wt. of tissue)	5.98±1.82	2.11±1.66
Glucose (mg/g wet wt. of tissue)	5.21±1.65	7.15±1.82
Acetylcholine (µmole/g wet wt. of tissue)	32.66±0.86	41.85±0.62
Acetylcholinesterase (µmole of acetylcholine hydrolysed/mg protein/hr)	5.12±0.62	4.06±0.27

Mean ±S.D of ten individual observations.
Significance *(p<0.05) Group I compared with group II

Carbohydrates are an important sources of energy required to various metabolic activities of the living organisms, the energy being derived as a result of oxidation. They are mainly in the form polysaccharides and disaccharides, which are hydrolyzed into monsaccharides by enzymes of digestive tract. The present study showed the level of glycogen decreased and glucose increased in the brain tissue of *catla catla* exposed to arsenic trioxide. This results indicates and extensive utilization of energy stores. In the present study, the level of glucose increased in the brain tissue to fish exposed arsenic. This result indicates that the glycogenolysis take place in the liver, where by the reserved glycogen is being slowly converted into glucose. Radha krishnaiah *et al*., (1992) reported that the level of glucose increased in the blood of *Labeo rohita exposed* to copper. The present study suggests that glycogen is being a ready source of energy, reduction in glycogen is probably due to more rapid breakdown, when releases glucose into circulatory system to meet the increased energy requirement in a stressful condition. Hinston et al.,(1973) have reported that maximum glycogen depletion corresponds to dramatic increase in glucose level in the fish *Channa punctatus* exposed to pollutants. They suggest that it might be due to some of the hepatic glycogen gaffing converted to glucose via the intermediate glucose-6 phosphate getting and entering the circulation.

Acetylcholine is the major transmitter substance in vertebrates. It is an ammonium compound. The arrival of berve impulses at the synaptic knob depolarizes presynaptic membrane, causing calcium channels to open, increasing the permeability of the membrane to calcium (Ca^{2+}) ions (Mitchell ,2004). Acetylcholinesterase is an important regulatory enzyme that controls the transmission of nerve impulses across cholinergic synapes

storage of transmitters. This might be due to alterations in cholinergic system in the tissues exposed arsenic toxicity (Sarkar et al., 1998). The present study shows the level of acetyl choline (Ach) increased and acetyl cholinesterase (AchE) decreased in brain, gill, liver and kidney tisslues or *labeo rohita* exposed to arsenic. This result suggests decrease in the cholinergic transmission and consequent accumulation of ache in the tissues. Sahib and Raman Rao,(1980a;1980b) have observed an increase in ach content consequent to decrease in the tissue. ACHE level in tilapia mossambica exposed to malathion. Coppage et al., (1975) observed the similar inhibition of ache in the brain tissue of fish exposed to malathion. Sing and Kumar, (2000) reported decrease in acetylcholinesterase activity in *Labeo rohita* exposed to malathion. Reddy *et al*., (1993) reported that inhibition of AchE with concomitant increase in ach content in the tissue of *Cyprinus carpio* exposed to fenvalerate. They also reported that this is an implication of greater inhibition in the inhibitory activity of the neural nervous system and Ach accumulated in brain and other tissues.

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