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ORIGINAL ARTICLE

EFFECT OF ARSENIC ON LIPID PEROXIDATION AND ANTIOXIDANTS SYSTEM IN FRESH WATER FISH, *LABEO ROHITA*

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ABSTRACT

The present study is investigated to analyze the impact of arsenic on brain and gill tissues of fresh water fish, *Labeo rohita*. In the present study, the lipid peroxidation (LPO) and antioxidant system such as glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT) were observed in both control and arsenic exposed fish, *Labeo rohita*. The present study showed that the lipid peroxidation (LPO) was increased and antioxidant system such as glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT) were decreased in arsenic exposed fish, *Labeo rohita*. The present study concleded that the arsenic compounds could reduce antioxidants system in the fresh water fish, *Labeo rohita*.

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Key words: Arsenic LPO Antioxidants Brain Gill

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1.INTRODUCTION

Heavy metals enter into aquatic habitats by a number of routes and cause hazardues effect on their morphology and physiology. Heavy metal pollution of water is a major environmental problem facing the modern world. Heavy metals have a unique property of accumulation over a period of time, along a food chain and a very high level can be accumulated in an organism from very low level concentration in water and sediments (Shrivastava and Sathyanesan, 1987).Metals are introduced into the environment by a wide range of natural and anthropogenic sources (Wepener *et al*., 2001) and with anthropogenic being either domestic or industrial (Seymore., 1994). Heavy metals are often present at elevated concentrations in aquatic ecosystems due to the rapid growth in population (Seymore, 1994), the increase in industrialization (Pelgrom *et al*., 1994), the increase of urbanization and socio-economic activities, exploration and exploitation of natural resources, extension of irrigation and other modern agricultural practices and the lack of environmental regulations (Seymore., 1994).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 (Wang and Huang, 1994). 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).

Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish(Tulasi *et al*.,1992). The heavy metal in the tissue of fishes may cause various physiological defects and mortality. The fishes which are largely being used foe the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuits *et al*.,2004).

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*., 1996]. Fish gills, which serve as the primary uptake site in fish for trace metals, represent the most important targets when exposed to elevated levels of ambient metals (Newman and Jagoe, 1994). The gills are in direct contact with the contaminate medium (water) and have the thinnest epithelium of all the organs and

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metals can penetrate through the thin epithelia cells (Bebianno *et al*.,2004).

Lipid per oxidation is alchemical mechanisms capable of disrupting the structure and function of the membranes, that occurs as a Lipids. Heavy metal causes cell membrane damage like Lipid per oxidation which leads to the imbalance between synthesis and degradation of enzyme protein (Padi and Chopra,2002).Antioxidants are known to reduce oxidative radical inducer receptions. They inhibits per oxidation of membrane lipid by scavenging Lipid peroxidyl radicals. Antioxidants systems include antioxidant enzymes (e.g., superoxidase dismutuse, Catalase, Gluathione peroxidase and glutathione) that remove ROS, thereby protecting organisms from oxidative stress.Glutathione is a commonly occurring tripeptide that scavenges oxidizing agents by reacting with them. Glutathione is a tripe tide metabolic regular and as putative indicator of health. Its main function is detoxification of endogens metabolic peroxides through glutathione per oxidize pathway and also exogenesis substances such as heavy metal.

Glutathione peroxidase (GPx) is the well-known antioxidant enzyme against oxidative stess, which in turn requires glutathione as co-factor. It catalyzes the oxidation of GSH to GSSG at the expense of H_2O_2. It contains selenium molecules at the active sites and transtens reducing equivalents from glutathione to H_2O_2 and producing water and GSSG. GPx is antiperoxidative enzyme present in the cell and mitochondrial matrix. Catalase (CAT) is an enzyme , which is present in most cell, and catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a heme containing protein, and is an efficient inhibitor when H_2O_2 accumulates in the tissue containing ferrous ions. It is mainly found in the peroxisomes, and removes H_2O_2 produced oxidation.

2.MATERIALS AND METHODS

Labeo rohita was collected from the fish farm located in Pinnalur, 20 km away from the overnment 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 static renewal toxicity test were conducted according to the methods recommended by American Public Health Association (APHA) (1989). In the present investigation the toxicity of Arsenic trioxide the median lethal concentration (LC50) of Arsenic f analyzed. The LC50 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 LC50 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, 2 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. 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 LC50 values were one tenth of the 96 hr LC50 sublethal concentration respectively. 96 hr LC50 value for arsenic was found at 1.89 ppm. Hence the one tenth of 96hr LC50 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.

Biochemical Studies

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

Estimation of lipid peroxidation in the tissue

The lipid peroxidation was estimated by the method of Nichans and Samuelson (1968). In this method thiobarbituric acid reactive substances (TBARS) were measured by their reaction with thiobarbituric acid (TBA) in acidic condition to generate a pink colouredchromophore, which was read at 535 nm in an UV spectrophotometer. All the tissues were isolated and tissue homogenate was prepared in Tris-Hcl buffer (pH 7.5). 1.0 ml of the tissue homogenate was taken in a clean test tube and 2.0 ml of TBA-TCA-HCl reagent was added and then mixed thoroughly. The mixture was kept in a boiling water bath (60°C) for 15 minutes. After cooling the mixture the pink colouredchromophore was read at 535 nm against the reagent blank in UV spectrophotometer. 1,1,3,3-tetramethoxy propane was used to construct the standard graph. The values are expressed as n moles/mg wet wt. of tissue.

The tissues were homogenized in phosphate buffer and centrifuged at 2500 rpm for 5 minutes. 0.2 ml of the supernatant was taken in a clean test tube and 1.8 ml of EDTA solution was added. To this 3.0 ml of precipitating reagent was added and mixed thoroughly and kept for 5 minutes before centrifugation at 3000 rpm for 10 minutes. After centrifugation the aliquot was filtered. 2.0 ml of the filterate was taken in a clean test tube and then 4.0 ml of 0.3M disodium hydrogen phosphate solutions and 1.0 ml of DTNB reagent were added. The appearance of yellow colour was read at 412 nm in UV spectrophotometer. Reduced glutathione was used to construct the standard graph. The values are expressed as µmole/mg wet wt.of tissues

Estimation of Glutathione peroxidase (GPX) in tissues

The level of glutathione peroxidase activity was determined by the method of Rotruck *et al*. (1973). The tissues were homogenized in Phosphate buffer and centrifuged at 2,500 rpm for 5 minutes. 0.2 ml of clear supernatant was taken in a clean test tube and then the following enzyme mixture was added. The enzyme mixture contained 0.2 ml of phosphate buffer, 0.2 ml 0.4nM of EDTA, and 0.1 ml of sodium azide. In this reaction, the reaction mixture was mixed well and kept for two minutes at 37°C in an incubator. After the incubation period, 0.2 ml of reduced glutathione and 0.1 ml of H_2O_2 were again added to the above mixture and incubated at 37°C exactly for 10 minutes. The reaction was arrested by the addition of 0.5 ml of 10% TCA. The colour was developed and then read at 412 nm. Reduced glutathione was used to construct the standard graph. The values are expressed as µmoles of GSH utilized/min/mg protein

Estimation of catalase (CAT) in tissues

The activity of catalase was determined by the method of Sinha (1972). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the pressure of hydrogen peroxide. The chromic acetate formed was measured at 620 nm.The catalase preparation was allowed to split hydrogen peroxide for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate/acetic acid mixture and the remaining hydrogen peroxide as chromic acetic acid is determined calorimetrically. The tissues were homogenized in phosphate buffer solution and centrifuged for 10 minutes at 2000 rpm. In a clean dry test tube 0.9 ml of phosphate buffer, 0.1 ml sample (tissue homogenate) and 0.4 ml of hydrogen peroxide were added. After 30 to 60 seconds 2 ml of dichromate acetic acid mixture was added. The tubes were kept in boiling water bath at 37°C for 10 minutes and then allowed to cool in the room temperature. The colour developed was read at 620 nm in UV spectrophotometer. Hydrogen peroxide was used to construct the standard graph.The values are expressed as µ moles H_2O_2 consumed/min/mg protein.

Statistical analysis

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

3.RESULTS

In the brain tissue of normal fish, the level of lipid peroxidation was 2.01±1.21 nmole/g wet wt. of tissue. During the sublethal concentration of arsenic, the level of lipid peroxidation was increased upto 3.19±1.27 nmole/g wet wt. of tissue when compared to control. The level of lipid peroxidation was 1.91±0.92 nmole/g wet wt. of tissue in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the increased trend of lipid peroxidation(2.26±1.82 nmole/g wet wt. of tissue)(Fig. 1).

In the brain tissue of normal fish, the level of glutathione was 18.81±1.63 µmg/g wet wt. of tissues. During the sublethal

Fig.1 Level of lipid peroxidation (LPO) in the selected tissue of fresh water fish with sub-lethal concentration of arsenic

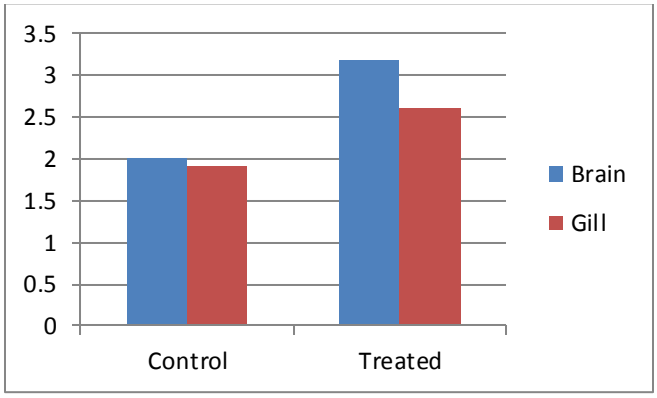


Fig. 2 Level of glutathione (GSH) in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic

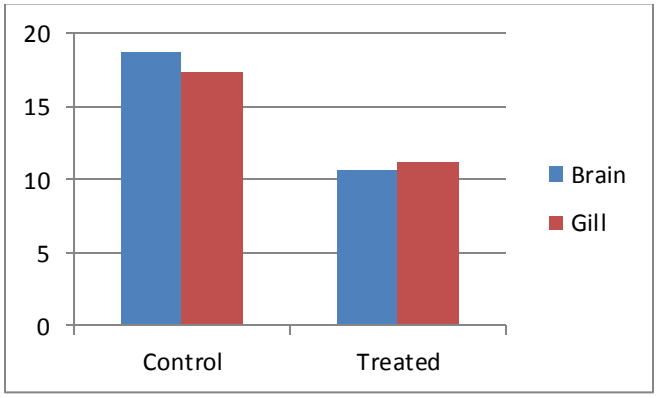
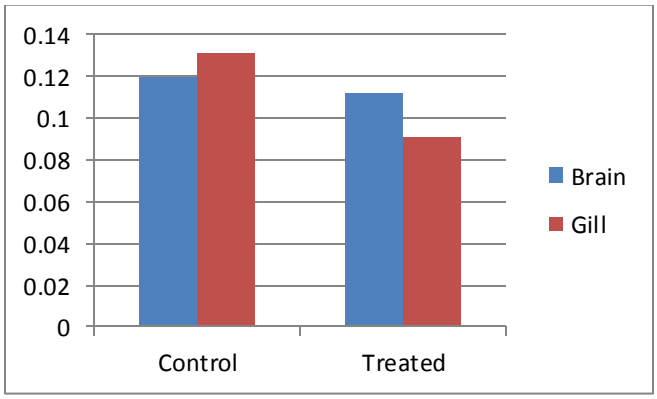


Fig. 3 Level of glutathione peroxidase(GPx) in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic

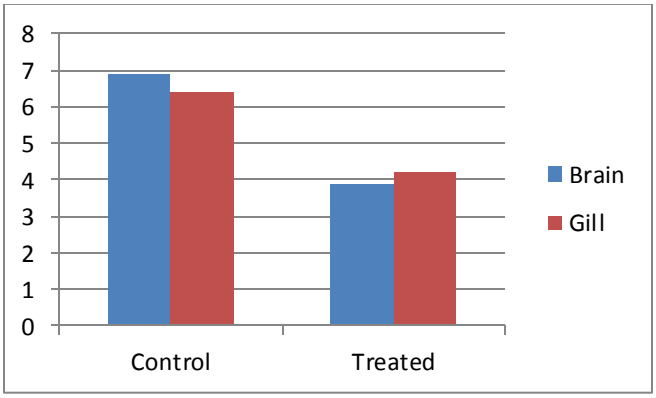


concentration of arsenic, the level of glutathione was decreased upto 10.62±1.79 µg/g wet wt. of tissues when compared to control. The level of glutathione was 17.40±1.79 µ moles/g wet wt. of tissue in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the decreased trend of glutathione (11.20±1.63 µ moles/g wet wt. of tissue).

In the brain tissue of normal fish, the level of glutathione peroxidase was 0.120±1.72 µmoles/mg of protein/min. During the sublethal concentration of arsenic, the level of glutathione peroxidase was decreased upto 0.112±1.62

μmoles/mg of protein/min when compared to control. The level of glutathione peroxidase was 0.131±1.68 μmoles/mg of protein/min. in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the decreased trend of glutathione peroxidase (0.091±1.79 μmoles/mg of protein/min).

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



In the brain tissue of normal fish, the level of catalase was 6.91±1.65 μmoles/mg of protein/min. During the sublethal concentration of arsenic, the level of catalase was decreased upto 3.86±0.98 μmoles/mg of protein/min when compared to control. The level of catalase was 6.40±1.02 μmoles/mg of protein/min in the control gill tissue. At sub lethal concentration of arsenic, the gill tissue showed the decreased trend of catalse(4.21±0.92 μmoles/mg of protein/min).

4.DISCUSSION

Heavy metals accumulated in the tisses of fish may catalyze reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress. Defensive mechanisms to counteract the impact of ROS are found in many mammalian species including aquatic animals such as fish. These systems include various antioxidant defense enzymes such as superoxide dismutases which catalyze the dismutation of superoxide radical to hydrogen peroxide, catalyzed action on hydrogen peroxide, glutathinone S-transferase family possessing detoxifying activities towards lipid hydroperoxides generated by heavy metals (Tjalkens *et al*.,1998).

In the present study, the level of glutathione and GPx and CAT significantly decreased but the level of LPO content increased in the brain and gill tissues of *Labeo rohita* when treated with sub-lethal concentration of arsenic for 7days. This results could be related to the alteration in the antioxidants enzyme activities and lipid peroxidation which may cause biochemical dysfunction in the tissues. The present study showed that the increased level of LPO content suggested that the excess production of ROS by mercury toxicity might be explained by its ability to produce alteration in mitochondria by blocking the permeability transition pore (Nilcoli *et al*., 1995) and alteration in mitochondrial electron transport chain. These events cause the oxidative phospharilation uncoupling and subsequent increase in ROS production (Salducci *et al*., 1999).

Farombi *et al*.,(2007) reported that the levels of heavy metals on biomarkers of oxidative stress as surrogate bioindicators of aquatic pollution in *Clariasgariepinus*. Farombi *et al*.,(2007) indicated significant elevation of lipid peroxidation and decreased antioxidant level in all the organs. The apparent increase in lipid peroxidation may be attributed to the accumulation of the heavy metals in the organs of heavy metals in the various organs. Metal catalyzed formation of ROS capable of damaging tissues such as DNA, proteins and lipids is well documented (Pandey *et al*.,2003).

Romeo *et al*.,(2000) reported that cadmium increases the formation of lipid peroxidation in rats and fishes. Several studies have been shown that there is increase in the formation of o_2 free radicals or reactive oxygen species (Stohs and Bagchi,1995). They also reported that cadmium increased the oxidative stress and attributed to the development of pathological conditions because of its long retention in the tissues. This finding is in agreement with cadmium induced oxidative stress in the tissues by increasing lipid peroxidation and altering antioxidants(Sarkar et al.,1999).

Iasmach *et al*., (2000) have reported that the study of changes in the activities of some antioxidants enzymes and the level of lipid peroxidation as an index of pollution using fish from Warri and Ethioper Rivers. Studies on antioxidant status during a free radical challenge can be used as an index of protection against the development of lipid peroxidation in experimental animals (Banerjee *et al*., 1999; Banerjee, 1999).

Sharma *et al*., (2005) reported that the enhancement of oxygen radical production may be ensured, leasing to an increased level of LPO content in rats exposed to pesticides. They also reported that the reduction of antioxidant defense system mainly responsible for generating hydroxyl radical leading to promote oxidative damage by Fenton reaction. The antioxidant enzymes SOD and GSH-Px are active scavengers free radicals, and hence are involved protecting against potential cell injury and neuropathological conditions (Hussain *et al*., 1999).

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