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ORIGINAL ARTICLE

EFFECT OF MERCURIC CHLORIDE ON LIPID PEROXIDATION AND GLUTATHIONE IN THE FRESH WATER FISH, *CATLA CATLA*

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ABSTRACT

The present study is aimed to investigate the effect of mercury in the lipid peroxidation (LPO) and glutathione (GSH) in mercury exposed fish, *Catla catla*. In the present study, the level of lipid peroxidation and glutathione were observed in the mercury exposed fish for 14 days. The present study showed that the level of lipid peroxidation increased and glutathione decreased in the mercury exposed fish.

Keywords: Mercuric Chloride, LPO, GSH, *Catla catla*

1.INTRODUCTION

Heavy metals enter into aquatic habitats by a number of routes and cause hazardous effect on their morphology and physiology. Heavy metal pollution of water is a major environmental problem facing the modern world [Shrivastava and Sathyanesan, 1987]. 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; Bose *et al.*, 1994).

Mercury is one of the heavy metal found in nature. The release of mercury from amalgam dominates exposure to inorganic mercury and may have an acceptable risk among the general population.The most frequent chemical forms to which humans are exposed are mercury vapory, HgO, $Hgcl_2$, methyl mercury and amalgam. The inorganic mercury poisoning leads to functional and structural alter rations in many or gains [Radi and Farghaly,2000] mercury posses a inorganic mercury is present in the surface water, it is often trans former into methyl mercury by micro organisms. It this from, it can be accumulated in the form, it can be cause human intoxication [USEPA,1987] human activities play a major role in polluting environment by toxic and carcinogenic metal compounds. Hence industrial pollution of the environment with metal compounds is be corning a serious problem [Chogule et al, 2005].

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2.MATERIALS AND METHODS

Procurement of experimental animal

The fresh water fish,*Catla catla* 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.

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 mercuric chloride (0.013ppm : 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. After experiment, the fish each from the respective experimental as well as control groups were sacrificed. The gills, liver and kidney 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 coloured chromophore, 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 coloured chromophore 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.

Estimation of reduced glutathione (GSH) in the tissues

The level of reduced glutathione was determined by the method of Beutler and Kellay, (1963).This method was based on the development of yellow colour when dithio-dinitro bis benzoic acid (DTNB) was added to compounds containing sulfhydryl groups**.** 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

Statistical analysis

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

3.RESULTS

Level of lipid peroxidation (LPO) in gill tissue

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 mercury, the gill tissue showed the increased trend of lipid peroxidation(2.26±1.82 nmole/g wet wt. of tissue). The percent change over the control was 36.12(Table 1).

Level of lipid peroxidation in liver tissue.

In the normal liver tissue, the level of lipid peroxidation content was 1.87±0.92 nmole/g wet wt. of tissue when the fish exposed to mercury, the level of lipid peroxidation content was increased upto 2.26±1.82 nmole/g wet wt. of tissue. The percent change over control was20.85(Table 1).

Level of lipid peroxidation (LPO) in kidney tissue

The level of lipid peroxidation present in the kidney tissue of normal fish was 2.27±0.19 nmole/g wet wt. of tissue. The level of lipid peroxidation was increased upto 2.85±1.92 nmole/g wet wt. of tissue when the fish exposed with sub lethal concentration of mercury. The percent change over control was 25.55(Table 1).

Table 1.Level of lipid peroxidation (LPO) in the selected tissue of fresh water fish *Catla catla* exposed with sub-lethal concentration of mercuric chloride

Tissues	Control	14 days	% COC
Gill	1.91±1.61	2.60±1.69	36.12
Liver	1.87±0.92	2.26±1.82	20.85
Kidney	2.27±0.19	2.85±1.92	25.55

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

Table 2. Level of glutathione (GSH) in the selected tissue of fresh water fish *Catla catla* exposed with sub-lethal concentration of mercuric chloride

Tissues	Control	14 days	% COC
Gill	17.40±1.79	11.20±1.63	-35.63
Liver	22.70±1.63	17.12±1.92	-24.58
Kidney	19.95±1.72	12.39±1.62	-37.89

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

Level of glutathione (GSH) in gill tissue

The level of amino acid was 17.40±1.79 µ moles/g wet wt. of tissue in the control gill tissue. At sub lethal concentration of mercury, the gill tissue showed the decreased trend of glutathione (11.20±1.63 µ moles/g wet wt. of tissue). The percent change over the control was –35.63(Table 2).

Level of glutathione (GSH) in liver tissue.

In the normal liver tissue, the level of glutathione content was 22.70±1.63 µ moles/g wet wt. of tissue. When the fish exposed to mercury, the level of glutathione content was decreased upto 17.12±1.92 µ moles/g wet wt. of tissue. The percent change over control was –24.58(Table 2).

Level of glutathione (GSH) in kidney tissue

The level of glutathione present in the kidney tissue of normal fish was 19.95±1.72 µ moles/g wet wt. of tissue. The level of glutathione was decreased upto 12.39±1.62 µ moles/g wet wt. of tissue when the fish exposed with sub lethal concentration of mercury. The percent change over control was –37.89(Table 2).

4.DISCUSSION

Heavy metal increases the rate of formation of active oxygen species including superoxide anion radical O_2^- and hydroxyl radical (OH) through a chain reaction (Yamanaka *et al*., 1991). The mercury induced enhancement in liver microsomal cytochrome P_{450} content and oxygen radical production was paralled by an augmented lipid peroxidative index by increased TBARS in liver. The mercury induced lipid peroxidation in liver could possibly result from an enhanced microsomal oxidative capacity (Sharma *et al*., 2005). Heavy metals such have the ability to produce reactive oxygen species, resulting in lipid peroxidation, and,

depletion of sulfhydryl groups (Stohs and Bagchi, 1995).The increased lipid peroxidation caused by mercury also leads to the formation of hydroperoxides that are removed by GSH with the help of GPx. Both the reactions lead to depletion of GSH. The toxicant may inhibit the enzymes directly impairing the functional groups or indirectly rendering the supply of glutathione.

Mercury is one the most harmful toxic and transition metal it promotes the formulation of reactive oxygen species in animals. The ROS enhances the synthesis of Lipid membrane damage and destroy the cell. The generation of toxic reactive oxygen species increases the pathological condition, when the flux of ROS exceeds the capability of the antioxidant mechanisms (Mecord et al., 1984). In the present study, the level of LPO increased and GSH was decreased in gill, liver and kidney tissue of *Catla catla* exposed to mercuric chlorine for 14 days. This result could be related to the alterations in the antioxidants enzyme activities and lipid per oxidation, which may cause biochemical dysfunction in the tissues. The present study, corroborate with findings of Nilcoli et al., (1995). They reported 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 alterations in the mitochondria by blocking in the permeability pore. The present study suggests that mercury induced Lipid peroxidation in brain could possible result from the enhanced level of cytochrome P450 would lead to high rates of radical production, which of Lipid per oxidation.

Farombi *et al*.,(2007) reported that the levels of heavy metals on biomarkers of oxidative stress as surrogate bioindicators of aquatic pollution in *Clarias gariepinus*. Sies,(1993) reported that the depletion in GSH and GPx result in the involvement deleterious oxidative changes due to accumulation of heavy metal. The heavy metals which led to induction of lipid peroxidation and alteration in the antioxidant enzymes in the organs of the fish. The concentration of heavy metal was increased in the gill, liver and kidney tissues. This may be due to the fact that gills serve as the respiratory organ in fishes through which metal ions are absorbed (Bebianno *et al*., 2004).

Farombi *etal*.,(2007) indicated significant elevation of lipid peroxidation and decreased antioxidant level in all the organs. The appa2007rent 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).

Rana *et al*.,(1996) and 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).

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