Mercury Induced Biochemical Alterations as Oxidative Stress in *Mugil cephalus* in Short Term Toxicity Test

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ABSTRACT

Mugil cephalus juveniles of size 2.5 ±0.6cm were exposed to mercury in short term chronic toxicity test through static renewal bioassay to detect the possible biochemical agent as biomarkers in aquatic pollution and in estuarine contamination as specific. Lipid peroxidation levels, glutathione *S*-transferase, catalase, reduced glutathione and acetylcholinesterase were studied as biochemical parameters.Increased thio-barbituric acid reactive substances levels were observed under exposure to mercury, leading to the oxidative damage resulting in lipid peroxidation. Decreased activities of antioxidants, catalase and increased glutathione-S-transferase under short term chronic exposures to mercury were more prominent in metal stress suggesting activation of physiological mechanism to scavenge the ROS produced. Decreased values of reduced glutathione on prolonged exposures to mercury indicate utilization of this antioxidant, either to scavenge oxy-radical or act in combination with other enzymes. The acetylcholinesterase activity was found to be decreased during mercury exposure. The results also suggest that mercury alters the active oxygen metabolism by modulating antioxidant enzyme activities, which can be used as biomarker to detect sub-lethal effects in aquatic pollution.

Key words: Acetylcholinesterase; antioxidants; catalase; oxidative stress, reactive oxygen species, Mercury, lipid peroxidation.

INTRODUCTION

Estuarine pollution is an ongoing activity started long back however intensified during the last few decades, and currently the circumstances has become alarming, especially in India[1]. Metals are natural components; however become contaminants of the aquatic environment, due to anthropogenic activities[2].Bioavailability and indestructible nature are the most fundamental property of heavy metal exerting toxic effects on living organisms when they exceed a certain concentration limit[3]. Heavy metals in metal accumulating organisms are linked to their ability to bind incoming metals, thereby controlling their intracellular availability leading to tolerance ability of test organisms. Oxidative stress induced by metals could be the best indicator and often interpreted as a failure of detoxification mechanisms in metal active sites such as mitochondria[4]. Cellular measurements and its responses to chemical contaminants like heavy metals in test organisms are used as bio-indicators from aquatic environment allowing early detection of biological effects as well as assessment of the extent of contamination of pollutants[5,6].

Depletion of glutathione and sulfhydryl groupsof protein due to heavy metals results in increased Reactive oxygen species (ROS) production such as, hydrogen peroxide,superoxide anion and hydroxyl radicals[7]. Superoxide anion and hydrogen peroxide is generated from sequential reduction of oxygen[8]. Another reactive species peroxynitrite is produced when superoxide anion rapidly reacts with nitric oxide and has the potential to trigger cellular death[9]. ROS are measured as crucial intermediaries for the metal-triggered tissue injuries and apoptosis[7].There must be effective anti-oxidation systems in the organisms to prevent

oxidation induced damage. Some components of anti-oxidation systems involve reduced glutathione (GSH) and antioxidant enzymes including free radical scavenging enzymes, such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidases (GPX) and Glutathione reductase (GR). Other related enzymes are Glyoxalase I (GI), Glyoxalase II (GII) and Glutathione *S*-Transferase (GST). GSH reduces ROS under oxidative stress, with the concomitant formation of theoxidized glutathione (GSSG) [10]. Particularly in the aquatic environment, oxidative stress is one of the ecological significance, providing a sink for many pollutants that are capable of causing oxidative stress[11]. Alterations in the activity of enzymes and related biomarkers are the potential tools for aquatic toxicological research[12].

Fish being a source in nutrient cycling and maintaining community balances in aquatic ecosystem play an important role in energy flow and are regarded as high protein to man[13]. Hence convenience of fish for assessing environmental conditions in aquatic ecosystem as test organisms has gained eminence in recent years[14]. Fish are considered as suitable bio-monitors for environmental pollution and they are exposed to the heavy metals *in vitro* and to study the effectsof heavy metals in aquatic ecosystems[15]. The study related to antioxidant defense system is being increasingly reported due to its potential ability to provide biochemical biomarkers that can beused in environmental monitoring system such as aquatic pollution and estuarine contamination in specific[11]. Tools involving biomarkers in environmental monitoring confer significant advantages over traditional chemical measurements because measured biological effects can be meaningfully linked to environmental consequences so that environmental concernscan be directly addressed[16]. Hence, in the present study the biochemical parameters such as lipid peroxidation levels, Glutathione *S*-transferase, catalase, reduced glutathione and acetylcholinesterase were measured by exposing juveniles of *Mugil cephalus* to mercury under short-term toxicity tests (static renewal).

MATERIALS AND METHODS

Fingerlings of *Mugil cephalus* of mean 2.5 ±0.6cm in length and 0.13 ±0.02g in weight were selected for the study. Collected juveniles were immediately transported to the laboratory in air filled plastic bags and acclimatized in 200 L Fiberglass reinforced plastics (FRP) tanks with aerated natural filtered seawater. Stock solutions of mercury were freshly prepared by dissolving mercury (II) chloride in de-ionized (double distilled) water. Fresh stock solutions were prepared daily. These solutions were serially diluted to get the experimental concentration for the toxicity test. The experimental method includes static renewal (24hour renewal) test[17]. Five concentrations in a geometric series including control were prepared for the test for 14 days in short-term chronic toxicity test [18]. Toxicant and seawater were replaced on daily basis. Test animals were fed three times during the test. Maximum-allowable control mortality was 20 per cent for short-term chronic toxicity test[18]. At the final stages of the toxicity test, the tissue samples of survived test animals were pooled and made in duplicates. For the analysis of lipid peroxidation marker and antioxidant enzyme activities, 1g tissue was homogenized in chilled pestle and mortar with 5ml homogenization buffer (0.25M sucrose, 10 mM Tris, 1 mM EDTA, and pH 7.4) and centrifuged at 5,000 rpm for 15 minutes at 4°C. The resulting supernatant was the homogenate which was used for the estimation of various biochemical assays.

Lipid peroxidation (LPO)

Lipid peroxidation level was assayed by measuring Malondialdehyde (MDA), a decomposed product of polyunsaturated fatty acids. Hydro peroxides were determined by the thio-barbituric acid reaction and was measured at 532 nm in the UV-Spectrophotometer[19]. The amount of Thio-barbituric acid reactive substance (TBARS) was calculated by using an extinction coefficient of 1.56×10^5/M/cm and expressed as nmol TBARS formed /mg protein.

Glutathione *s*-transferase (GST)

Activity of Glutathione *S*-transferase (GST) was assayed at 340 nm by measuring the increase in absorbance using 1-chloro-2, 4-dinitro benzene (CDNB) as the substrate[20]. The results were

expressed as nM of GSH and CDNB conjugate formed /min/mg protein.

Catalase (CAT)

Catalase (CAT) activity was measured at 240 nm by determining the decay of hydrogen peroxide levels and was expressed as µmol of hydrogen peroxide consumed /min/mg/protein[21].

Reduced glutathione (GSH)

The reduced glutathione (GSH) was measured at 412 nm using 5, 5-dithiobis-(2-nitro benzoic acid) (DTNB) reagent[22]. The values were expressed as µmol of GSH oxidized/mg protein.

Acetylcholinesterase activity (AChE)

Acetylcholinesterase activity (AChE) activity was determined using Ellman's reagent, DTNB (5, 5'-dithio-bis (2- nitro benzoic acid); 0.5mM) and acetylthiocholine iodide (ACTI) as substrate[23, 24, 25]. The rate of change of absorbance at 412nm was recorded over 1.5 minutes at 25°C. The protein concentration of each of the sample extract was determined measured at 750 nm in UV-Spectrophotometer[26]. One-way ANOVA (Dunnetts

procedure) was used to compare the results with control using graphpad prism version 5.

RESULTS AND DISCUSSION

Scavenging enzymes at lower concentration in juvenile fish makes them vulnerable to oxidative damage when attacked by ROS[27]. *M. cephalus* exposed to exposure concentrations experienced rigorous Oxidative stress (OS) characterized by significant alterations in biomarkers,were also been observed in brain samples of the mullet[28]. Removal of H_2O_2 is an important strategy of marine organisms against oxidative stress[29]. Increased activities of CAT have been reported in several fish and invertebrate species[30, 31]. Concentration of LPO was significantly higher ($P< 0.001$) in higher concentrations of mercury due to increased levels of exposure indicating the importance of antioxidant[32].The level of total protein to mercury exposure significantly ($P<0.001$) decreased in 10 µg/l. Glutathione-*S*-transferase (GST) exhibited a significant ($P<0.001$) increase in the activity at 8 and 10 µg/l concentration of mercury. Reduced glutathione (GSH) level

Table 1: Biochemical alterations in *M. cephalus* exposed to mercury in short-term chronic toxicity test

Concentration (µg/l)	Protein[a]	GST[b]	GSH[c]	CAT[d]	AchE[e]	MDA[f]
0	16.92 ±0.09	3.80 ±0.14	93.12 ±1.57	213.28±17.03	4.50±*0.71*	*9.94±0.41*
1	15.82 ±0.00 ns	4.55 ±0.21ns	87.45±1.70*	312.68±12.10***	6.65±0.21*	18.71±1.20*
2	14.38 ±0.53**	6.37 ±0.19***	70.76±0.67***	197.63 ±4.81 ns	2.50±0.71*	23.60±1.75**
4	14.59 ±0.59**	5.40 ±0.28**	66.93±1.36***	179.60 ±0.90 ns	2.55±0.64*	30.07±0.59***
8	13.99 ±0.00**	6.52 ±0.03***	57.39±2.45***	153.01±10.40**	1.85±0.07**	40.57±0.64***
10	12.57 ±0.71***	7.95 ±0.35***	44.69±0.76***	136.28±5.72***	1.00±0.14**	52.68±3.84***

***values are significant at P<0.001, ** values are significant at P<0.01, * values are significant at P<0.05. One way ANOVA (Dunnetts multiple comparison test (á=0.05)); Values are the mean and standard deviation. a-mg protein /g tissue, b-(Glutathione-S-transferase) GST activity nM of CDNB /min/mg protein, c- (Reduced glutathione) µmol of GSH oxidized / mg protein , d- (Catalase) µmol of H_2O_2Consumed/min/mg protein, e- (Acetylcholinesterase) nM/min/mg protein**,** f- (Lipid peroxidation) Nm of MDA/ mg protein;The concentration column (mg/l) contains '0' indicating control in the test conducted in triplicate; ns-not significant

significantly (*P*<0.001and 0.05) decreased in the 14 days of exposure compared to control in all the concentrations. CAT and LPO showed trend of significant decrease and increase in linear increase in the mercury concentration. The activity of AChE significantly (*P*<0.01 and 0.05) decreased throughout the exposure concentration. *M. cephalus* exposed in short-term chronic toxicity test showed that all the biochemical components and anti-oxidative enzymes of the oxidative stress showed significant changes in the tissues exposed to mercury Table 1.

Protein content in *M. cephalus*might be due to the proteolysis process for energy production and utilization owing to the decreased food intake of test organisms under stress[33].These data may indicate a faster rate of GSH utilization or degradation, which could be responsible for the observed lower GSH content. Moreover, increase of GSH content may be related to prevention of oxidative challenge[34]. Aquatic organisms maintain high content of GSH in tissues and increased content has the function of protection that could provide the first line of defense against the influence of toxic heavy metals[35, 36]. Esterases are considered as potential biomarkers to differentiate the levels of contaminants[37]. Maintenance of enzyme activities in relation to oxidative stress may serve as important markers of the presence of hazardous substances[38, 39]. Mullet (*Mugil* sp.) from contaminated Spanish areas revealed increased activities of antioxidant (catalase) and detoxifying GST enzymes[40,41]. Channel catfish (*Ictalurus punctatus*) exposed to effluents resulted in a significant increase in catalase activity[42].

Changes in GST activity exhibit detoxification process in fish exposed to toxic compounds[4,5]. Decrease of GST was observed in fish exposed to mercury in the present study. This induction in GST activity could indicate a defense of fish against oxidative stress damage produced by adverse conditions such as heavy metal contamination. Increased levels of lipid peroxidation(LPO) have been observed in fish under experimental conditions, upon exposure to different xenobiotics[43]. There are evidences that heavy metals like those used in the studied, produced increased LPO levels in *M. cephalus*[44]. The concurrent use of several biomarkers is important to minimize misinterpretation in cases of complex situations of pollution[45].The result indicates that fish actively generate oxidative stress and antioxidant responses which can be used as biomarkers of pollution.

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