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RESEARCH ARTICLE

BIOCHEMICAL CHANGES OF ANTIOXIDANTS ENZYMES IN CAT FISH (*HETEROPNEUSTIS FOSSILIS*) BLOCH AFTER FLUORIDE EXPOSURE

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ABSTRACT

The aim of the study was to evaluate the assessment of oxidative stress induced by fluoride which causes adverse effect in aquatic ecosystems like other pollutants. Study has been carried out on certain biomarkers in brain of cat fish (*Heteropneustis fossilis*). Biomarkers selected for stress monitoring were Lipid peroxidation (LPO) and antioxidant defense system enzymes, mainly Catalase (CAT), Superoxide dismutase (SOD), Reduced glutathione (GSH), Glutathione peroxidise (GPx) and Glutathione S-Transferase (GST) activities in brain of fish exposed to 35 mg F/L and 70 mg F/L of water for 45 days. Oxidative stress due to exposure to fluoride was observed in brain of cat fish and it was evident by the increased in LPO, GST level and decreased level of CAT, GSH, SOD and GPx levels in the brain tissues. Oxidative and antioxidant profiles indicate that chronic exposure to the fluoride is capable of inducing oxidative stress in fish.

INTRODUCTION

Fluoride is an essential trace element for humans and animals. In human beings chronic fluoride toxicity by the intake of a moderate or high dose of fluoride. Fluoride pollution in the aquatic ecosystems is mainly due to industrial activities (such as mining and processing of phosphorus rock and the manufacturing of aluminium) and agricultural activities (the applications of fluoride containing fertilizers and pesticides) **(Camargo, 2003).** The first major natural source of inorganic fluoride is the weathering of fluoride minerals. Volcanoes are the second major natural source through the release of gases with hydrogen fluoride (HF) into the atmosphere (CEPA, 1994). Higher concentration of fluoride in water and its consumption leads to destruction of enamel of teeth and a number of ill effects which are collectively referred as 'Fluorosis'. Fluorosis is a disease arising due to excess intake of fluoride. It has been clearly mentioned that increased fluoride consumption can cripple man **(Susheela and Ghosh 1990, Tripathi, 2007).** Fluoride causes its potential adverse health effects. Efforts to reduce fluoride related health problems should more seriously since concentrations of fluoride is indicating high in the water resources Endemic fluorosis is threatening the health of humans. The problems associated with excess of fluoride exposure amplifies the biochemical stress in the body by generating imbalance between reactive oxygen species (ROS) and antioxidants there by inducing oxidative stress **(Mahaboob and Sujitha, 2012).** At high concentration, it causes adverse changes in the soft tissues like brain, kidney, liver and ovary leading to adverse effect in neurological, behavioural and physiological functions. There are strong evidences suggesting the involvement of oxidative stress in the mechanism of fluoride toxicity. A number of antioxidants exist to balance the cellular production of ROS, maintaining the intracellular redox balance by preventing the cellular damage caused by ROS.

MATERIALS AND METHODS

Experimental animals and chemicals

The fresh water cat fish *H. fossilis* (mean weight 27.04±0.19 g and length 16.7±0.20 cm) were purchased from the local market in Lucknow and used for the chronic toxicity. They were acclimated to laboratory conditions for one month prior to the experiments. The specimens were given prophylactic treatment by bathing them twice in 0.05% KMnO4 solution for 2 minutes to avoid any dermal infections. The NaF (AR grade) obtained from Qualigens Fine Chemicals Limited, Mumbai, India.

Biochemical Assays

Preparation of Homogenate

The brain was homogenized in10% (w / v) ice cold chilled sodium phosphate buffer (0.1 M, pH 7.4) using a Potter-Elvehjem

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homogenizer. A part of this homogenate was used for biochemical estimations and the other part was centrifuged at 9,000 rpm for 30 minutes at 4°C to using a Sigma refrigerated centrifuge to obtain the supernatant which was further used for SOD, CAT, GPx, GST and protein estimations.

Lipid peroxidation (LPO)

Tissue LPO was measured using the method of **Ohkawa *et al.* (1979).** Absorbance was recorded at 530 nm and the results were expressed as n moles MDA / hr / mg tissue.

Reduced glutathione (GSH)

GSH concentration was measured in brain tissue using the method of **Ellman (1959).** The absorbance of GSH-DNTB conjugate was determined at 412 nm and the concentration (nM GSH/mg protein) was calculated using standard calibration.

Superoxide dismutase (SOD)

SOD activity was analyzed using the method of **Kakkar *et al.* (1984).** Colour intensity of the chromogen was measured at 560 nm. The result was expressed as µmoles /min/mg of protein.

Catalase (CAT)

The activity of CAT was measured according to the method of **Sinha (1972).** The mixture was cooled and absorbance was read at 570 nm. The CAT activity was calculated in terms of µ moles/min/mg protein.

Glutathione peroxidase (GPx)

The GPx was measured using the procedure of **Rotruck *et al.*, (1973)**. Absorbance was read at 420 nm. The results were expressed as n moles/min/mg protein.

Glutathione S-transferase (GST)

GST was determined spectrophotometrically at 25°C by following the formation of GSH conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm using extinction coefficient of 9.6×10^3 m^{-1} cm^{-1} **(Habig *et al.* 1974).** GST activity was expressed as n mole /min/mg of protein.

Protein estimation

Protein was estimated by colorimetric method and BSA as standard **(Lowry *et al.* 1951).**

Statistical analysis

The results were expressed as mean and standard error (Mean ± SEM) and determined for all the parameters. The data was analyzed employing the analysis of variance (ANOVA) using statistical software Graph Pad In Stat Software Inc., v. 3.06, San Diego, USA. The Dunnett *test* for multiple comparisons of groups against the control was performed, to determine the significant differences among the groups.

RESULTS

Lipid peroxidation and non-enzymatic antioxidant

The LPO level was significantly increased after low dose exposure ($P < 0.05$) and increase highly significant after high dose exposure ($P<0.01$) in the brain as compared to the control group [Figure1].

The level of GSH was decreased insignificantly after low dose exposure ($P > 0.05$) and significantly after high dose exposure ($P < 0.01$), in the brain as compared to control [Figure 2].

Enzymatic antioxidants

The SOD activity decreased significantly after low dose exposure ($P < 0.05$) as well as highly significant high dose exposure ($P< 0.01$) in the brain as compared to the control [Figure 3].

The CAT activity was decreased significantly after low dose exposure ($P < 0.05$) as well as high dose exposure ($P < 0.01$) in brain as compared to control [Figure 4].

Gpx activity decreased significantly after low dose exposure ($P>0.05$) as well as highly significant high dose exposure ($P <0.05$) in the brain as compared to the control [Figure 5].

The level of GST increased significantly after low and high dose exposure ($P<0.01$) as compare to control [Figure 6].

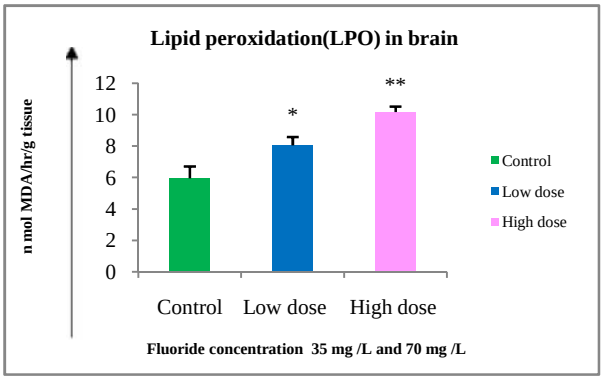

Figure 1 Effects of fluoride exposure on lipid peroxidation after 45 days. Values as mean ± SEM. Comparison with control group are significantly ($P < 0.05$) and very high significantly ($p<0.01$) from each other.

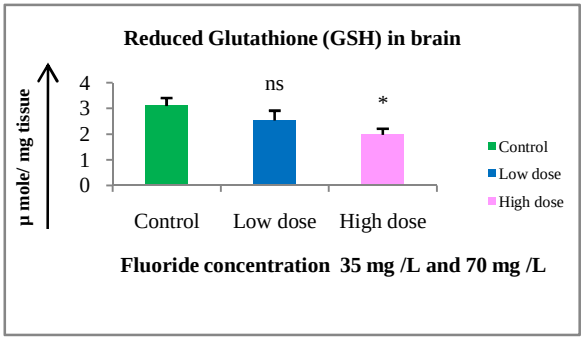

Figure 2 Effects of fluoride exposure on reduced glutathione after 45 days. Values as mean ± SEM. Comparison with control group are insignificantly ($P > 0.05$) and significantly ($P < 0.01$) from each other.

DISCUSSION

Brain tissue rich in polyunsaturated lipids is highly vulnerable to ROS mediated oxidative damage (**Siegel *et al.* 1999**). A

significant increase in the LPO level was observed after fluoride exposure in the brain. Increased level of LPO can most likely be ascribed to an excessive production of ROS, which could be related to antioxidant enzyme leakage as has been suggested by Enis *et al.* 2011 after chromium induced oxidative stress in blood and tissues.

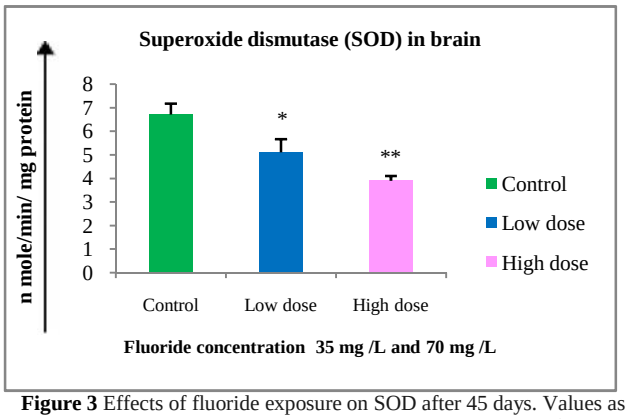

Figure 3 Effects of fluoride exposure on SOD after 45 days. Values as mean± SEM. Comparison with control group are decreases significantly ($P < 0.05$) and very high significantly ($P < 0.01$) different from each other.

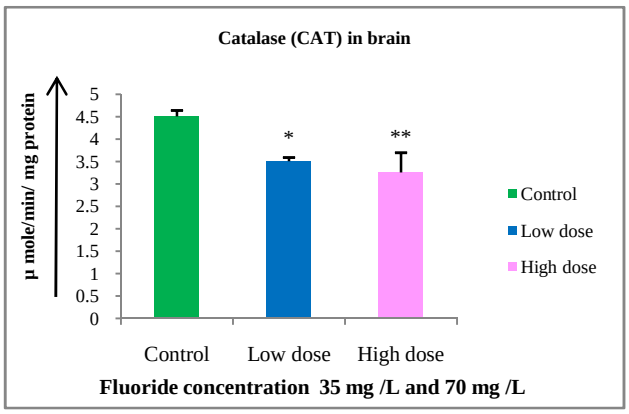

Figure 4 Effects of fluoride exposure on Catalase after 45days. Values as mean ± SEM. Comparison with control group are decreases significantly ($P < 0.05$) and very high significantly ($P < 0.01$) different from each other.

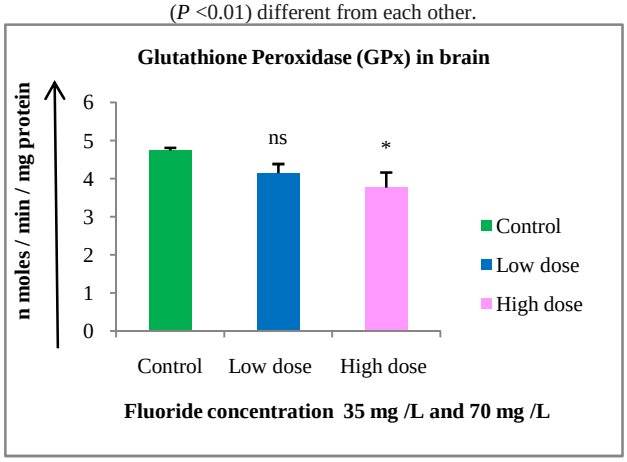

Figure 5 Effects of fluoride exposure on GPx after 45days. Values as mean ± SEM. Comparison with control group are decreases significantly ($P > 0.05$) and high significantly ($P < 0.05$) different from each other.

Similar observation has been reported by Velma and Tchounwou, 2010 after chromium induced oxidative stress in fish. As regards the reduction in GSH level, it may be due to the direct conjugation of GSH, with electrophiles generated after fluoride exposure. Similar decrease has already been reported after exposure to other organophosphate methyl parathion on fresh water fish **(Diana *et al.* 2006).**

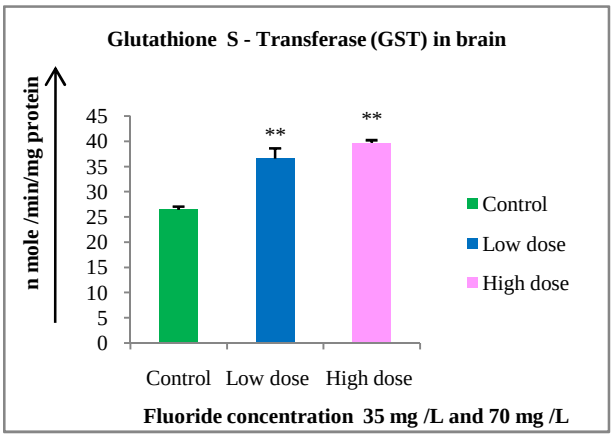

Figure 6 Effects of fluoride exposure on GST after 45days. Values as mean ± SEM. Comparison with control group are increase significantly ($P < 0.01$) and very high significantly ($P < 0.01$) different from each other.

SOD is considered to a first line of defense against deleterious effects of ROS in the cell by catalysing dismutation of superoxide radicals to hydrogen peroxide to molecular oxygen **(Kanemastu and Asada 1994).** In present study indicates that decreased SOD activity in brain as compared to control found excessive ROS may have inactivated SOD leading to further ROS accumulation and enhanced tissue damage. Similar result has been reported by Sharma *et al.* 2012 in his study with trichlorfon-induced oxidative stress.

CAT is one of the sensitive enzyme biomarkers and its activity is modulated by various factors including over production of superoxide radicals **(Kono and Fridovich, 1982; Pandey *et al.* 2001)**. In this study decreased CAT activity in the brain may be explained on the basis of direct binding of the metal to –SH groups of the enzyme molecule. Dabas *et al.* 2014 show similar result with cadmium induced genotoxicity and oxidative stress response in *Labeo rohita.* In the present study, different dose of fluoride decreased the GPx activity in brain of fish as compared to control. It may be due to reduced level of glutathione (due to over production of free radicals) which is used as substrates for GPx. **Branco *et al.* 2012** were found similar result in fish brain exposed to methyl Hg and Se.

Glutathione S-transferases are a family of multifunctional enzymes that are involved in the detoxification of both xenobiotics as well as endogenous reactive compounds of cellular metabolism. Fluoride exposure increased the level of GST in brain as compare to control. It may be due to decreased level of GSH. Meena *et al.* 2014 show similar result glutathione s-transferase and catalase activity in different tissues of marine catfish *Arius arius* on exposure to cadmium.

CONCLUSION

Our results indicate that antioxidant enzyme assays can be used as a bio indicator for chronic exposure to fluoride in the catfish of *H. fossilis.* This could be related to the alterations in antioxidant enzyme activities and other biomarkers of oxidative stress in *H.fossilis* which may cause biochemical dysfunction in this species. In addition, the results provide evidence that

enzymatic and non enzymatic biomarkers of oxidative stress can be sensitive indicators of aquatic animals.

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