J. Ecophysiol. Occup. Hlth. 16(3&4), 2016, 66–71

DOI : 10.15512/joeoh/2016/v16i3&4/15695

Alteration in Antioxidant Biomolecules after the Exposure to Fluoride in Fresh Water Fish *Heteropneustes fossilis*

Neelam Singh*, and Madhu Tripathi

Department of Zoology, Aquatic Toxicology Research Laboratory, University of Lucknow, Lucknow UP – 226007, India; nealneelam120@gmail.com

Abstract: *Fluoride contamination in aquatic ecosystem has been recognized as one of the major problems worldwide and it is imposing a serious threat to aquatic organisms. It induces adverse physiological and bio-chemical effect in animals. The present study has been planned to observe toxic effect of Sodium fluoride on antioxidative biomarkers like LPO, SOD, and GSH in a fresh water catfish Heteropneustes fossilis. Fish were exposed to two sub lethal concentrations of Sodium fluoride for 30 days. After exposure, fish were sacrificed for collection of tissues for biochemical assay. It was found that SOD, GSH decreased significantly while LPO increased significantly in treated fish as compared to control. Results indicate concentration dependent induction of oxidative stress and subsequent alternations in the activities of non enzymatic and enzymatic antioxidants like LPO, GSH and SOD.*

Keywords: Antioxidants, Fluoride, GSH, LPO, SOD

Introduction

Aquatic ecosystem receives all the pollutants and toxicants present in atmosphere. Fluoride is also one among such toxicant which comes in the environment as a result of natural geochemical activities and anthropogenic activities. Its level is increasing in the environment gradually. In aquatic bodies also it is increasing day by day. In unpolluted surface waters, fluoride concentration is usually 0.01–0.3 mg/L, although higher concentrations have been reported in waters of volcanic areas Mahvi *et al*., (2006), Dobaradaran *et al*., (2009). From different parts of India fluoride level has been reported to range from 1-29 ppm Chinoy *et al*., (1991). Fluoride is a chemically active ionized element. It can affect oxygen metabolism and induce the production of O_2^- free radicals Inkielewicz and Krechniak, (2004). In aquatic environments, a high level of fluoride has acute and chronic toxic effects e.g., altered biomolecule level, growth reduction, impaired reproduction and even death of organisms Tripathi *et al*., (2005,2009), Kumar *et al*., (2007) Ochoa *et al*., (2009) Bajpai *et al*., (2009), (2010), (2012), Shamsollahi *et al*.,(2015). Previous studies have revealed that fluoride induces excessive production of oxygen free radicals and causes a decrease in biological activities of some antioxidant enzymes like Super oxide dismutase (SOD), Catalase and Glutathione peroxidase (GPx) Wang *et al*.,2003; Shanthakumari *et al*., (2004). Antioxidants are part of defensive mechanism and play important role in health and disease of animals. Free radicals are produced in the animals as by-products of normal metabolism and as a result of exposure to radiation and some environmental pollutants. These are normally neutralized by a system in the body that include the antioxidant enzymes (SOD, Catalase, and Glutathione peroxidase) and the

***Email: nealneelam120@gmail.com**

nutrient-derived antioxidant small molecules (vitamin E, vitamin C, carotenes, flavonoids, glutathione (GSH), uric acid, and taurine). Barbier *et al*., (2010) has reported that fluoride causes disruption of enzyme activity, inhibition of proteins synthesis, alternation of gene expression. Tripathi et al., (2009) has found chromosomal aberration induced by fluoride in kidney of *Clarias batrachus*. Aquatic animals living in fluoride contaminated water are continuosly exposed to high concentration of fluoride and they enter the food chain. Due to bioaccumulation phenomenon, fluoride level is further enhanced. Fish are extremely sensitive to many waterborne toxicants, because pollutants come in the contact of gills directly. Present work is aimed to evaluate the effect of fluoride on the antioxidants activity like SOD, LPO, GSH in different organs of fresh water fish *Heteropneustes fossilis* (Singhi).

Material and Methods

The freshwater catfish *Heteropneustes fossilis (*weight 35 - 50 g and length 17 -25 cm) were procured from the local market of Lucknow and used for the experiment. Physicochemical properties of water were determined by method of APHA *et al*., (2005). Fish were divided into three groups as mentioned in Table 1. Group1 as control and 2, 3 as experimental groups which were treated with 1/5 & 1/10 of LC_{50} dose of fluoride. Experiment was carried out for one month. During experimentation, water of all the aquaria and fluoride as per requirement of protocol was added and changed on alternate days. The NaF (AR grade) was obtained from Qualigens Fine Chemicals Limited, Mumbai, India.

Biochemical Analysis

Both control and treated fish were dissected and their gills, liver and kidney were collected. They were homogenized in a glass homogenizer in cold saline solution (0.89% NaCl). Homogenized tissues were centrifuged in a refrigerated cold centrifuge to get clear supernatant and used for the biochemical determination. Lipid Peroxidation (LPO) assay was done by Colado *et al*., (1997), GSH assay by method of Ellman (1959) and SOD estimation was done by Kakkar *et al*., (1984).

Statistical Analysis

The observed MDA, SOD and GSH values as means ± SE were statistically analysed with one way ANOVA using the Graph Pad Prism version 5.01 software programs. Dunnett's tests were employed for multiple comparisons against Control. P ***<0.0001, P** <0.001 and P* <0.05 were considered significant.

Result and Discussion

The end products of lipid peroxidation are malondialdehyde (MDA), bioactive marker of lipid peroxidation. After exposure a significant changes in lipid peroxidation (LPO) were found. LPO significantly increased in all organs of treated group as compared to control. In kidney LPO increase was less significant in LD exposure group compared to control (P**< 0.001) and in HD exposure group increase was more significant (P<0.0001). In gills LPO was increased more significantly (P***<0.0001) in both exposure groups. In liver LPO was increased less significantly (P*< 0.05) in both

Table 1. Experimental Plan for Study

Groups	Body weight	Treatment (Dose)	Stocking density	Duration of Exposure
1. Control	35 – 50g	Untreated	6	30 Days
2. Low Dose	40 – 55 g	35mg F/L	6	30 Days
3. High Dose	35 – 50g	70 mg F/L	6	30 Days

exposure groups as compared to control (Table 2., Figure 1).

Superoxide dismutase (SOD) is enzymatic antioxidant. After exposure, it was found decreased significantly in both treated groups as compared to control. In kidney and gills decrease was more significant (P***< 0.0001) whereas in liver decrease was less significant (P**< 0.001). But in all organs decrease was significant in comparison to control (Table 3 and Figure 2).

Reduced glutathione (GSH) was decreased significantly in kidney and gills of both treated groups as compared to control (P***< 0.0001), whereas in liver decrease was there but not significant in comparison to control (Table 4 and Figure 3).

The present study reveals that the SOD, GSH activities were decreased whereas MDA (Lipid peroxidation) was increased in kidney, gills and liver of Fluoride treated fish as compared to control. These findings are in agreement with the earlier reports of many workers Dierickx *et al.*,(1983), Patel *et al.*, (1988), Hai *et al.*, (1997), Sun *et al.*, (1998), Oruc E. (2011), Samanta *et al.*, (2014) Yadav *et al.*, (2015) Diamond *et al.*, (2016).

Table 2. Effect of Fluoride on the Lipid Peroxidation in Liver, Gills and Kidney of ***H.fossilis*** After One Month of Fluoride Exposure.

Organ/ Group	Control	LD	HD
Liver	1.823 ± 0.41	6.546 ± 0.89	6.461 ± 1.06
Gills	7.063 ± 0.06	29.45 ± 1.23	34.966 ± 2.25
Kidney	3.043 ± 0.04	4.61 ± 0.04	8.816 ± 0.35

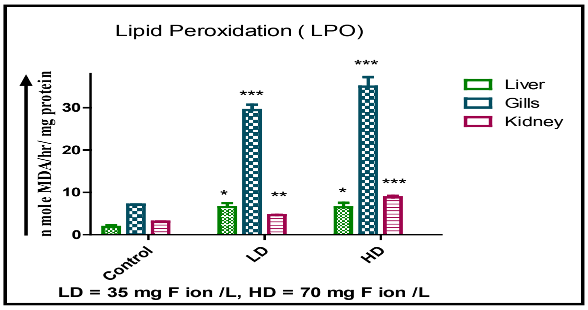

Fig. 1 Effect of fluoride on the lipid peroxidation in Kidney, gills and liver after one month of fluoride exposure. Value mean ± standard error mean, *n* = 6, P ***<0.0001, P** <0.001 and P* <0.05.

Table 3. Effect of Fluoride on the SOD Activity in Liver, Gills and Kidney of *H.Fossilis* After One Month of Fluoride Exposure.

Organ/ Group	Control	LD	HD
Liver	1.077 ± 0.08	0.402 ± 0.11	0.389 ± 0.01
Gills	1.396 ± 0.05	0.587 ± 0.01	0.541 ± 0.05
Kidney	1.464 ± 0.03	0.654 ± 0.05	0.344 ± 0.11

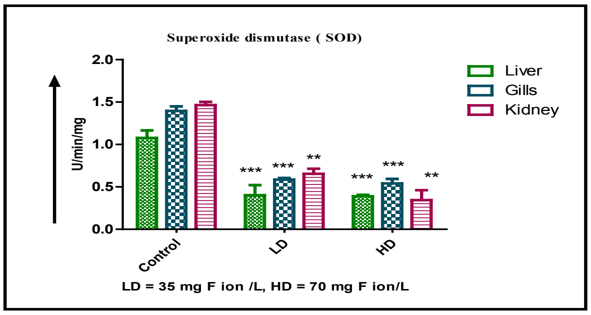

Fig. 2 Effect of fluoride on the SOD activity in liver, gills and Kidney of *H.fossilis* after one month of fluoride exposure. Value mean ± standard error mean, *n* = 6, P ***<0.0001, P** <0.001 and P* <0.05.

Increased MDA is an indicator of enhanced lipid peroxidation (LPO) of cell membrane Buyukokuroglu et al., (2002). These observation corroborate with the findings of many workers who have observed increased level of MDA in different tissues and cells of fluoride-intoxicated animal Shivarajashankara *et al.*, (2002) Mittal *et al.*, (2006), Błaszczyk *et al.*, (2008), Inkielewicz *et al.*, (2010), Basha *et al.*, (2011), Nabavi *et al.*, (2012) and (2013), Yadav *et al.*,2015). Fluoride induced increase in LPO and disturbance in the integrity of the cell membranes leading to

Table 4. Effect of fluoride on the GSH level in liver, gills and Kidney of *H.fossilis* after one month of fluoride exposure.

Organ/ Group	Control	LD	HD
Liver	0.023 ± 0.016	0.013 ± 0.0008	0.02 ± 0.015
Gills	0.03 ± 0.004	0.005 ± 0.0007	0.0015 ± 0.0004
Kidney	0.061 ± 0.007	0.006 ± 0.001	0.0055 ± 0.0020

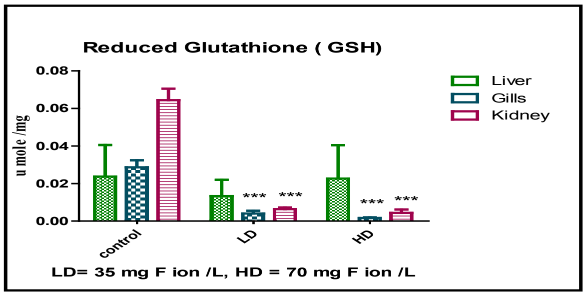

Fig. 3 Effect of fluoride on the GSH level in liver, gills and Kidney of *H.fossilis* after one month of fluoride exposure. Value mean ± standard error mean, *n* = 6, P ***<0.0001, P** <0.001 and P* <0.05.

inhibition of the membrane bound enzymes has already been reported by Basha *et al*., (2012) and Yadav *et al*., (2015).

Glutathione is a tripeptide and exists in reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) states. With higher level of oxidative stress, intracellular GSSG accumulates and the GSH/GSSG ratio decreasees and that can be used as an appropriate biomarker of oxidative stress which has been suggested by Van *et al*., (2003). The decrease in the ratio of GSH/GSSG in fish may be due to either direct scavenging of radicals or increased peroxidase activity. The increase in GSH level can benefits fish to stay alive in contaminated and polluted environment. At the same time reduction in glutathione is usually associated with enhancement of peroxidation processes in the cell membrane and leads to stress in animals. This prominently contributes in toxicity of toxicants Viarengo *et al*., (2007).

Superoxide dismutases (SODs) constitute the first line of defence against free radicals by catalysing dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen Kanemastu and Asada, (1994). In present study, it was suppressed by fluoride exposure in both treated groups as compared to control. Decreased SOD levels in organs of treated fish in study indicate decreased ability of the tissues to handle O_2^- free radicals. Similar findings on SOD have been reported in the tissues of mice exposed to high fluoride intake Sharma *et al*., (1998)., Vani *et al*., (2000). According to Habig *et al*., (1974) all the changes after fluoride exposure may be due to fluoride induced oxidative stress through free radicals, H_2O_2 and impaired the production of free radical scavengers such as GSH, CAT (catalase), GSH-Px (GSH Peroxidase), SOD (Superoxide dismutase), and GST (Glutathione S-transferase). Thus, present findings suggest that fluoride disturbed the activity of enzymatic antioxidants (SOD) and levels of non enzymatic antioxidants (MDA, GSH) in fishes and these disturbed levels of antioxidants may certainly alter the physiological activities of fish.

Acknowledgement

Authors wish sincere thank to the Head of Department of Zoology, University of Lucknow for providing us the laboratory facilities & support. We acknowledge the UGC- BSR (SRF) fellowship for financial assistance. Thanks are also to UGC- SAP and DST-Purse for special assistance to central laboratory facility.

References

APHA, AWWA and WEF (2005) Standard Method for the Examination of Water and Waste

Water, 21st Ed. American Public Health Association, Washington DC.

Bajpai, S. and Tripathi, M. (2012) Alteration in Pigmentation after Fluoride Exposure in Stinging Catfish, *Heteropneustes fossilis* (Bloch). *CibtechJournal of Zoology*., ISSN: 2319-2012, **1**.

Bajpai, S. Tripathi N, Tewari S and Tripathi, M. (2010) Retardation of Growth after Fluoride Exposure in Catfish, *Heteropneustis fossilis* (Bloch). *Bioresources for Rural Livelihood*., **1**,167 -173.

Bajpai, S., Tewari, S. and Tripathi, M. (2009) Evaluation of Acute Toxicity Levels and Behavioural Responses of *Heteropneustes fossilis* (Bloch) to Sodium Fluoride. *Aquaculture,* **10**, 37-43.

Barbier, O., Mendoza, A. L and Razo, D. M. L. (2010) Molecular Mechanisms of Fluoride Toxicity. *Chemico-Biological Interactions*.,**188**, 319–333.

Basha, PM., Rai, P and Begum, S. (2011) Evaluation of Fluoride-Induced Oxidative Stress in Rat Brain: A Multigeneration Study. *Biological Trace Element Research*., **142**,623–637.

Basha, P.M. and Sujitha, N.S. (2012) Combined Influence of Intermittent Exercise and Temperature Stress on the Modulation of Fluoride Toxicity. *Biol Trace Elem Res*., **148**, 69–75.

Błaszczyk, I., Grucka-Mamczar, E., Kasperczyk, S. and Birkner, E. (2008) Influence of Fluoride on Rat Kidney Antioxidant System: Effects of Methionine and Vitamin E. *Biological Trace Element Research,* 121, 51–59.

Buyukokuroglu, M.E., Taysi, S., Polat, F. and Gocer, F. (2002) Mechanism of the Beneficial Effects of Dantrolene Sodium on Ethanol–Induced Acute Gastric Mucosal Injury in Rats. *Pharmacol Res*., **45**, 421–425.

Camargo, J.A. (2003) Fluoride Toxicity to Aquatic Organisms: A Review. *Chemosphere*., **50**, 251-264.

Chinoy, N. J., Narayana, M. V., Sequeira, E., Joshi, S. M., Barot, J. M., Purohit R. M. Parikh, D.J. and Godasar, N.B., (1992) Studies on Effects of Fluoride in 36 Villages of Mehsana District, North Gujarat. *Fluoride*., **25,**101-10.

Colado, M.I., O'Shea, E., Granados, R., Misra, A., Murray, T.K. and Green, AR. (1997) A Study of the Neurotoxic Effect of MDMA ("Ectasy") on 5-HT Neurones in the Brains of Mothers and Neonates Following Administration of the Drug during Pregnancy. *Br J. Pharmacol.,* **121**: 827–833.

Diamond, SR., Sultana, T., Servos, MR. and Metcalfe, C.D. (2016) Biological Responses to Contaminants in Darters (Etheostoma Spp.) Collected from Rural and Urban Regions of the rand River, ON, Canada. *Comparative Biochemistry and Physiology B Biochemistry and Molecular Biology*., **199**,126-35.

Dierickx, P.J. and DeBeer, J.O. (1983) Interaction of Fluoroacetamide With Rat Liver Glutathione S-Transferases: Evidence for Detoxification Roles by Defluorination. *Fluoride*., **16**, 145-51.

Dobaradaran, S., Mahvi, A.H., Dehdashti, S., Dobaradaran, S.and Shoara, R. (2009) Correlation of Fluoride with Some Inorganic Constituents in Groundwater of Dashtestan, Iran. *Fluoride*, **42**, 50-3.

Ellman, G.L. (1959) Tissue Sulfhydryl Groups. *Arch Biochem Biophys*, **82**, 70-77.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974) Glutathione-S- Transferase: The First Enzymatic Step in Mercapturic Acid Formation. *J Biol Chem*, **249**, 7130-7139.

Hai, D.Q., Varga, S.I. and Matkovics, B. (1997) Organophosphate Effects of Antioxidant System of Carp (*Cyprinus Carpio*) and Catfish (*Ictalurus nebulosus*). *Comp Biochem Physiol*, **117,** 83–8.

Inkielewicz-Stepniak, I. and Czarnowski, W. (2010) Oxidative Stress Parameters in Rats Exposed to Fluoride and Caffeine. *Food and Chemical Toxicology*., **48**, 1607–1611.

Kakkar, P., Das, B. and Viswanathan, P.N. (1984) A Modified Spectrophotometric Assay of Superoxide Dismutase. *Indian J Biochem Biophys*., **21**,130-2.

Kanemautsu, S. and Asada, K. (1994) Superoxide Dismutase: In Molecular Aspects of Enzyme Catalysis (Ed T Faku and Soda) Kodansa Tokyo, 191-202.

Krechniak and Inkielewicz (2005) Correlations between Fluoride and Free Radical Parameters in Rats. *Fluoride*., **38**, 293–296.

Kumar, A., Tripathi, N. and Tripathi, M (2007) Fluoride Induced Biochemical Changes in Fresh Water Catfish (*Clarius batrachus* , Linn). *Fluoride*., **40**, 37-41.

Mahvi, A.H., Zazoli, M., Younecian, M., Nicpour, B. and Babapour, A. (2006) Survey of Fluoride Concentration in Drinking Water Sources and Prevalence of DMFT in the 12 Years Old Students in Behshar City. *J Med Sci*., **6**, 658-61.

Mittal, M., and Flora, S.J. (2006) Effects of Individual and Combined Exposure to Sodium Arsenite and Sodium Fluoride on Tissue Oxidative Stress, Arsenic and Fluoride Levels in Male Mice. *Chemico-Biological Interactions*, **162**, 128–139.

Nabavi, S.M., Habtemariam, S., Nabavi, S.F. Sureda, A., Daglia, M., Moghaddam, H. A. and Amani, A. M. (2013) Protective Effect of Gallic Acid Isolated from *Peltiphyllum Peltatum* Against Sodium Fluoride-Induced Oxidative Stress in Rat's Kidney. *Molecular and Cellular Biochemistry*., **372**, 233–239.

Nabavi, S.M., Nabavi, S.F., Eslami, S., Moghaddam, S. and Hajizadeh, A. (2012) In Vivo Protective Effects of Quercetin Against Sodium Fluoride-Induced Oxidative Stress in the Hepatic Tissue. *Food Chemistry.,* **132**, 931–935.

Ochoa-Herrera, V., Banihani, Q., León, G., Khatri, C., Field, J.A. and Sierra-Alvarez, R**.** (2009) Toxicity of Fluoride to Microorganisms in Biological Waste Water Treatment Systems. *Water Res*., **43**, 3177–3186.

Oruc, E. (2011) Effects of Diazinon on Antioxidant Defense System and Lipid Peroxidation in the Liver of *Cyprinus Carpio* (L.). *Environ Toxicol*., **26**,571–8.

Patel, D. and Chinoy, N.J. (1998) Influence of Fluoride on Biological Free Radical Reactions in Ovary of Mice and its Reversal. *Fluoride*, **31**, S27.

Samanta, P., Pal, S., Mukherjee, A.K. and Ghosh, A.R. (2014) Biochemical Effects of Glyphosate Based Herbicide, Excel Mera 71 on Enzyme Activities of Acetylcholinesterase(Ache), Lipid Peroxidation (LPO), Catalase (CAT), Glutathione- S-Transferase (GST) and Protein Content on Teleostean Fishes. *Ecotoxicology and Environmental Safety*., **107**, 120-125.

Shamsollahi, R. H., Zolghadr, Z., Mahvi, H. A., Hosseini, S. S. and Mossavic, N. S. (2015)

The Effect of Temperature, Water Hardness, and Exposure Time on Fluoride Toxicity in the Aquatic Environment. *Fluoride*., **48**, 338-344.

Shanthakumari, D., Srinivasalu,S. and Subramanian,S. (2004) Effect of Fluoride Intoxication on Lipid Peroxidation and Antioxidant Status in Experimental Rats. *Toxicology*, **204**, 214- 228.

Sharma, A. and Chinoy NJ (1998) Role of Free Radicals in Fluoride-Induced Toxicity in Liver and Kidney of Mice and its Reversal. *Fluoride*, **31**, S26.

Shivarajashankara, Y.M., Shivashankara, A.R.,. Bhat, Gopalakrishna. P. and Hanumanth, Rao. (2002) Brain Lipid Peroxidation and Antioxidant Systems of Young Rats in Chronic Fluoride Intoxication. *Fluoride,* **35**, 197–203.

Sun, G., Qiu, L., Ding, G., Qian, C. and Zheng, Q.(1998) Effects of Beta-Carotene and SOD on Lipid Peroxidation Induced by Fluoride: An Experimental Study. *Fluoride*, **31**, S29.

Tripathi, M. and Tripathi, A. (2005) Effect of Fluoride on Growth of Fingerlings of *Channa punctatus*. *Flora and Fauna*, **11**, 111 -114.

Tripathi, M., Tripathi, A. and Gopal, K. (2005) Impact of Fluoride on Pigmentation of a Freshwater Fish *Channa punctatus*. *J.Appl. Biosci*., **31**, 35-38.

Tripathi, N., Bajpai, S. and Tripathi M. (2009) Genotoxic Alterations Induced by Fluoride in Asian Catfish, *Clarias batrachus* (linn.) . *Fluoride*., **42**, 292–296

Van, der. Oost. R., Beyer, J. and Vermeulen, N.P.E. (2003) Fish Bioaccumulation and Biomarkers in Environmental Risk Assessment: A Review. *Environmental Toxicology and Pharmacology*, **13**, 57–149.

Vani, M.L. and Reddy, K.P. (2000) Effects of Fluoride Accumulation on Some Enzymes of Brain and Gastrocnemius Muscle of Mice. *Fluoride*., **33**, 17-26.

Viarengo, A., Lowe, D., Bolognesi, C., Fabbri, E. and Koehler, A. (2007) The use of Biomarkersin Biomonitoring: A 2-Tier Approach Assessing the Level of Pollutant-Induced Stress Syndrome in Sentinel Organisms. *Comparative Biochemistry and Physiology*., **146C**, 281–300.

Yadav, S.S., Kumar, R., Khare, P. and Tripathi, M. (2015) Oxidative Stress Biomarkers in the Freshwater Fish, *Heteropneustes fossilis* (Bloch) Exposed to Sodium Fluoride: Antioxidant Defense and Role of Ascorbic Acid. *Toxicology International*., **22,** 71.