



EFFECT OF SODIUM FLUORIDE ON ORGANIC RESERVES OF SOME TISSUES OF *HETEROPNEUSTES FOSSILIS*

PRINCE KUMAR SRIVASTAVA, NEETU SINGH RUHELA, SADGURU PRAKASH AND K. K. ANSARI

Department of Zoology, M.L.K. (P.G.) College, Balrampur

ABSTRACT

After 90 days exposure of *H. fossilis* to different sub-lethal concentrations of sodium fluoride (25 mg/L, 50 mg/L and 75 mg/L), glycogen, total protein and cholesterol in kidney, brain and gill tissues were significantly decreased. None of these changes was observed in the control group.

KEY WORDS: Fluoride, Biochemical parameters, *H. fossilis*.

INTRODUCTION

Fluoride is an essential trace element that helps in mineralization, development and functions of bones and teeth. Fluoride in very low levels has been found to help in the prevention of dental caries as well as to lower osteoporosis. However, excessive ingestion of fluorine and its compounds found in water cause a crippling disease known as 'fluorosis' of its profound affinity for calcified tissues. Thus, fluoride toxicity is becoming a matter of grave concern, as many countries have been declared endemic for fluorosis. This makes it imperative for scientists to focus on precise toxic effects of fluoride on skeletal and non-skeletal tissues, so that effective therapeutic agents can be developed. The permissible limits of fluorides in drinking water as suggested by Bureau of Indian Standards (BIS, 1983) vary between 0.6 to 1.2 ppm while World Health Organization (WHO, 1984) permits a maximum of 1.5 ppm of it. Hence the present study was taken to investigate the toxic effects of sodium fluoride on certain biomolecules in kidney, brain and gill tissues of freshwater Catfish *Heteropneustes fossilis*.

MATERIALS AND METHODS

Healthy, male fishes of equal size (15.0 ± 0.5 cm) and weight (38 ± 2.0 gm) were collected from local fresh water resources and maintained under standard laboratory conditions for 15 days. The fish were divided into four groups with 10 fish per group. Group 1 served as control. While group II, III and IV exposed with 25 mg/L, 50 mg/L and 75 mg/L sodium fluoride, respectively. The water of all groups was changed on alternate days. After 90 days, all the fish were sacrificed for sampling. The kidney, brain and gill tissues in each group were dissected out and homogenized. The homogenate was

centrifuged at 3500 rpm for 20 minutes. The supernatant was used for the estimation of glycogen, total protein, total lipid and cholesterol by Caroll *et al.* (1956), Lowery *et al.* (1951), Folch *et al.* (1957) and Rosenthal *et al.*, (1957) methods, respectively. The data were analysed by student's 't' test to determine the significance of the changes from control.

RESULTS AND DISCUSSIONS

After 90 days of exposure of *Heteropneustes fossilis* to sublethal concentrations of sodium fluoride, the glycogen, total protein, total lipid and cholesterol contents in kidney, brain and gills were decreased significantly in all groups of fluoride exposed fishes (Table 1).

After 90 days exposure to sodium fluoride, the glycogen content was significantly reduced in kidney, brain and gill. Glycogenolysis in kidney, brain and gill in fluoride induced fishes suggests enhanced conversion of glycogen to glucose which increases the blood glucose content to meet an increased energy requirement under stress conditions. During stress condition fish need more energy to detoxify the toxicant and to overcome stress. Storochkova and Zhavoronkov (1983) reported that fluoride act as activator many glycolytic enzymes, consequently, the decrease in glycogen content in fluoride exposed fishes. Depletion of glycogen stored in kidney, brain and gill may be inhibition of AChE activity in brain and gill of exposed fishes. Due to inhibition of AChE, concentration of acetylcholine (ACh) increases which stimulate the secretion of catecholamine that brings about glycogenolysis and hyperglycemia through raised levels of cyclic AMP (Gopal and Khanna, 1993; Natrajan, 1984; Nilsson *et al.*, 1976; Terrier and Perrier, 1975 and Begum, 2004). Sharma and Gopal (1995) have also

Dr. Khalid Kamal Ansari was Awarded Ph.D in 1991. M.Sc. in Zoology (Fish & Fisheries) with First Division in 1969 from Gorakhpur University. B.Sc in 1966 from Shibli National College, Azamgarh. has seven years experience of P.G Classes in M.L.K (P.G) College, Balrampur. Forty-Two years experience of U.G Classes in M.L.K (P.G) College, Balrampur. Twenty-Four years research experiences the field of Fish & Fisheries, Fish Pathology, Environmental Science & Pest Control. Thirty One research papers published. Thirteen research scholars awarded Ph.D. Four Seminars Attended. Two research scholars enrolled. Vice-Principal of M.L.K (P.G) College, Balrampur, 2009-2010. Incharge Deptt. Of Zoology M.L.K (P.G) College, Balrampur, Since 7 April, 1993. Dean Faculty of Science M.L.K. (P.G.) College, Balrampur, Member of panel for granting permanent affiliation to B.Sc classes in Degree Colleges of Dr. R.M.L Avadh University, Faizabad in 2000. Member of Board of Studies of Zoology, Dr. R.M.L. Avadh University, Faizabad, 2006-2010. Head Examiner, Co-Examiner, External Examiner in different Universities of Uttar Pradesh. He was also the Member- Faculty of Science Dr. R.M.L. Avadh University, Faizabad, 2010. Member of Board of Studies of Zoology, Dr. R.M.L. Avadh University, Faizabad, 2006-2010. Member Executive Council Dr. R.M.L. Avadh University, Faizabad, 2008 & 2010. Expert in Selection Board of Lecturers in U.P. Higher Education Commission, Allahabad, 2007. Incharge Hot and Cold of M. L.K. (P.G.) College, Balrampur, from 2004. Administrative officer of M.L.K. P.G. College, Balrampur, July, 1999 - May, 2001. Asstt. Supdt. Dr. R.M.L. Avadh University Exam, 1995-2010. Examiner of Ph.D. Thesis in Zoology of Dr. B.R. Ambedkar University, Agra, 1999. Expert in selection board of lecturers in Intermediate, U.P. Intermediate Education Commission, Allahabad, 1996. Regional Head Examiner in Intermediate Biology Practical, U.P. Board Allahabad. Teacher Representative in Governing Body of M.L.K. (P.G.) College, Balrampur, 1986-1987. Observer in Dr. R.M.L. Avadh University Exam. Secretary of M.L.K. (P.G.) College, Balrampur, Staff Quarter Maintenance Committee, 1983-1993. Games Superintendent of M. L.K. (P.G.) College, Balrampur, 1989-1991. Organizing Secretary Maharaja Sir B.P. Singh All India Hockey Tournament, 1988 and 2001-2002. Email ID: kaykayansari@gmail.com



observed a significant rise in lactic acid concentration in blood and decrease in the activity of succinic dehydrogenase (a key enzyme of TCA cycle) in *C. batrachus* chronically exposed to carbaryl. They suggested that it is an indication of the inhibition of Krebs cycle and/or a favour of anaerobic metabolism over aerobic one due to intoxication of pesticide to meet out the immediate energy demand. Thus significant decline in tissue glycogen to meet an increased energy requirement of treated fish might be due to enhanced secretion of catecholamine under the stress of effluents.

In the present study, protein content was significantly decreased in kidney, brain and gills. Kidney and gills are the main sites of degradation and detoxification of Xenobiotics (Rao *et al.*, 1983) and the biochemical effects recorded seem to be the result of greater stress on these organs. Gill *et al.*, (1988) reported that during the initial phase of exposure to toxicant loss of enzymes due to tissue necrosis and increased metabolic activity for detoxification of the toxicant might necessitate enhanced synthesis of enzyme proteins. Proteins are mainly involved in the architecture of the

cell. During chronic period of stress are also sources of energy. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. Since fishes have less amount of carbohydrate so next alternative source of energy is protein to meet increased demand of energy. Decrease in protein of gill, kidney and brain may be due to inhibition of protein synthesis (Reddy *et al.*, 1995) in the cells of these tissue and interference of amino acid metabolism. Another possible reason may be depletion of protein for its catechization in conversion to glucose (Srivastava *et al.*, 2002).

The decreases lipid content in gill, kidney and brain of fluoride in exposed fishes may be due to inhibition of lipid synthesis as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic activity. Bat Enbury and Vanden Bergh (1972) reported that fluoride act as inhibitor of various enzymes like lipases, phosphatase and esterase. It interferes with fatty acid oxidation and also inhibits the enzyme acetyl co-A synthetase involved in fatty acid oxidation. Thus decreased lipid content in various tissues may be due to the inhibition of these enzymes.

The cholesterol contents were significantly reduced in kidney, brain and gills of effluent treated fishes. Gluth and Hanke (1985) reported that hypocholesteremia in pesticide treated *Cyprinus carpio* was due to accumulation of water in plasma. Begum and Vijayraghavan (2001) have also observed a decline in cholesterol content accompanied with increase in free fatty acids in toxicant treated fishes. They suggest that decreased level of cholesterol in these tissues may be probably due to increased breakdown of cholesterol into free fatty acids which are being fed to TCA cycle to meet out the energy demands during effluent stress.

Thus it can be concluded that fluoride have induced an energy crisis and altered carbohydrate, protein and lipid metabolism by exerting their toxic manifestations in *Heteropneustes fossilis* that are important in their physiological activities, survival, growth and reproduction. The observed biochemical response of present study could be used as suitable biomarkers of fluoride stress to aquatic animals.

ACKNOWLEDGEMENT

The authors are thankful to principal M.L.K.(P.G.) College, Balrampur for providing laboratory facilities.

REFERENCES

- Bat Enburg, J. J. and Vande Bergh, S. G. (1972): The mechanism of inhibition by fluoride of mitochondrial fatty acid oxidation. *Biochim Biophys Acta.*, **280**: 496-505.
- Begum, G. (2004). Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (Linn.) and recovery response. *Aquat. Toxicol.*, **66(1)**: 83-92.
- Begum, G.; Vijayraghvan, S. (2001). Carbofuran toxicity on total lipid and free fatty acids in air breathing fish during exposure and cessation of exposure in vivo. *Environ. Monit. Assess.*, **70**: 133-239.

Table 1: Effect of sodium fluoride on organic reserve of *H. fossilis* after 90 days exposure.

Tissues	Control Mean \pm SD	25 mg/L Mean \pm SD	50 mg/L Mean \pm SD	75 mg/L Mean \pm SD
Glycogen (mg/gm wet tissues)				
Kidney	7.70 \pm 0.32	7.50 \pm 0.60	6.90 \pm 0.80*	6.40 \pm 0.11*
Brain	8.30 \pm 0.70	7.80 \pm 0.80	6.30 \pm 0.82*	5.80 \pm 0.60**
Gill	5.20 \pm 0.45	4.70 \pm 0.50*	3.80 \pm 0.40**	3.40 \pm 0.30**
Total protein (mg/gm wet tissues)				
Kidney	125.10 \pm 1.38	113.80 \pm 6.70	109.00 \pm 2.98	102.90 \pm 3.80*
Brain	62.00 \pm 0.72	58.90 \pm 1.32	54.18 \pm 1.34*	51.50 \pm 42.60*
Gill	57.25 \pm 2.32	51.50 \pm 1.60*	41.80 \pm 1.80*	33.60 \pm 0.50**
Total lipid (mg/gm wet tissues)				
Kidney	8.10 \pm 0.31	6.70 \pm 0.48	5.55 \pm 1.12*	4.10 \pm 0.32**
Brain	65.38 \pm 1.31	57.15 \pm 1.30	41.10 \pm 1.25**	33.12 \pm 1.15**
Gill	9.18 \pm 0.41	8.05 \pm 0.32	7.25 \pm 1.11*	6.80 \pm 1.02**
Cholesterol (mg/gm wet tissues)				
Kidney	32.30 \pm 1.32	29.18 \pm 1.32	27.32 \pm 1.48	25.18 \pm 1.38*
Brain	42.38 \pm 0.78	38.18 \pm 1.15*	35.38 \pm 3.18*	31.48 \pm 1.47**
Gill	1.48 \pm 0.18	1.28 \pm 0.04	0.98 \pm 0.08*	0.88 \pm 0.06**

* P < 0.05; ** P < 0.001

BIS (1983): Specifications for drinking water. IS. 10500: Bureau of Indian Standards, New Delhi.

Coroll, N. V.; Longley, R. W. and Rae, H. N. (1956). The determination of glycogen in liven and muscles by use of Anthrome methods. *J. Biol. Chem.*, **200**: 583-593.Folch, J.; Lees, M.; Sloane-Stanely, G. H. (1957). A simple method for isolation and purifications of total lipids from animal tissues. *J. Biol. Chem.*, **226**: 497-507.Gill, T. S.; Pant, J. C.; Pandey, J. (1988). Gil, liver and kidney lesions associated with experimental exposure to carboryl and dimethoate in fish (*Puntius conchoni*). *Bull. Environ. Contam Toxicol.*, **41**: 71-78.Gluth, G.; Hanke, W. (1985). A comparison of physiological changes in carp, *Cyprinus carpio* induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. *Ecotox. Environ. Safe.*, **9**: 179-188.Gopal, K.; Khanna, Y. P. (1993). Interaction of carbaryl with acetylcholinesterase of the teleost *Clarias batrachus*. *Toxicol. Environ. Chem.*, **39(3/4)**: 147-152.Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275.Natraja, G. M. (1984). Effect of lethal (LC50/48hr) concentration of metasytox on some selected enzyme system in the airbreathing fish, *Channa striatus* (Bleaker). *Comp. Physiol. Ecol.*, **9**: 29-33.

Nilsson, S.; Abrahamsson, R.; Grove, D. J. (1976). Sympathetic nervous control of adrenalin release from head kidney of

cod, *Gadus morhua*. *Comp. Biochem. Physiol. Ser.*, **C55**: 123-127.Rao, J. K.; Choudhary, M.; Murthy, V. S. R. (1983). Histopathology of malathion on liver and kidney of freshwater teleost, *Tilapia mossambicus* (peters). *J. Environ. Biol.*, **4**: 9-13.Reddy, A. N.; Venugopal, N. B.; Reddy, S. L. (1995). Effects of endosulfan 35 EC on some biochemical changes in the tissue and haemolymph of a freshwater field crab, *Barytelphusa guerini*. *Bull. Environ. Contam. Toxicol.*, **55(1)**: 116-121.Rosenthal, H. L.; Pfluke, M. L.; Buscaglia, S. (1957). A stable iron reagent for determination of cholesterol. *J. Lab. Clin. Med.*, **50**: 318-322.Sharma, B.; Gopal, K. (1995). Changes in lactic acid content and activity of lactate dehydrogenase and succinic dehydrogenase in *Clarias batrachus* exposed to carbaryl. *Toxicol. Environ. Chem.*, **47**: 89-95.Srivastava, N.; Kaushik, N. and Gupta, P. (2002): Zinc induced changes in the liver and muscle of fish *Channa punctatus* (Bloch.). *J. Ecophysiol Occup Hlth.*, **2**: 197-204.Storchkova, L. S. and Zhavoronkov, A. A. (1983): Fluoride as an activator of enzymatic systems. *Fluoride*, **16**: 181-186.Terrier, M.; Perrier, J. (1975). Cyclic 3',5' adenosine monophosphate levels in plasma of rainbow trout, *Salmo gairdneri* following adrenalin administration and constrained exercise. *Experientia* **31**: 196-199.

WHO (1984): Guidelines for drinking water quality Vol.1. Recommendations, World Health Organisations, Geneva, 1-30.

ADVERTISEMENT RATES

India

Inside Pages

No. of insertions:

1 Full page in B&W	:	Rs. 10000; in Colour: Rs. 20000
Half page in B&W	:	Rs. 6000; in Colour: Rs. 12000
6 Full page in B&W	:	Rs. 50000; in Colour: Rs. 100000
Half page in B&W	:	Rs. 30000; in Colour: Rs. 60000
12 Full page in B&W	:	Rs. 100000; in Colour: Rs. 200000
Half page in B&W	:	Rs. 60000; in Colour: Rs. 120000

Inside Cover Pages

1 Full page in B&W	:	Rs. 15000; in Colour: Rs. 25000
6 Full page in B&W	:	Rs. 75000; in Colour: Rs. 125000
12 Full page in B&W	:	Rs. 150000; in Colour: Rs. 250000

Back Cover Pages

1 Full page in B&W	:	Rs. 20000; in Colour: Rs. 30000
6 Full page in B&W	:	Rs. 100000; in Colour: Rs. 150000
12 Full page in B&W	:	Rs. 200000; in Colour: Rs. 300000

Other Countries

Inside Pages

1 Full page in B&W	:	\$300; in Colour: \$650
Half page in B&W	:	\$200; in Colour: \$325
6 Full page in B&W	:	\$1500; in Colour: \$3000
Half page in B&W	:	\$1000; in Colour: \$2000

Inside Cover Pages

1 Full page in B&W	:	\$450; in Colour: \$750
6 Full page in B&W	:	\$2250; in Colour: \$3500

Back Cover Pages

1 Full page in B&W	:	\$600; in Colour: \$1000
6 Full page in B&W	:	\$3000; in Colour: \$5000