RESPIRATORY RESPONSE OF ASIAN CATFISH, *CLARIAS BATRACHUS*, TO FLUORIDE

Anand Kumar, Sandeep Bajpai, Nalini Tripathi, Madhu Tripathi[a]
Lucknow (U.P.), India

SUMMARY: The respiratory response of Asian catfish, *Clarias batrachus*, Linn., to sub-lethal concentrations of fluoride (F) (35 and 70 mg F ion/L from NaF) was investigated. Changes in gill tissue and in blood were examined after 30 and 60 days of exposure to F. In the gill filaments, cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial swelling, lifting, and clubbing at the tip of secondary lamellae were observed. Significant decreases (<0.01 to <0.001) occurred in red blood corpuscles (RBCs) and hemoglobin content (Hb). Notable decreases also occurred in packed cell volume (PCV) and oxygen carrying capacity of blood, together with an increase in erythrocyte sedimentation rate (ESR) after exposure to the two sub-lethal concentrations of F in the tank water. These changes were greater with the higher concentration of F and the longer duration of exposure.

Keywords: Blood parameters; Catfish (*Clarias batrachus*); Fluoride toxicity to catfish; Gill histology; Oxygen carrying capacity.

INTRODUCTION

Fluoride (F) toxicity is a global issue now becoming more widely recognized. In many countries, groundwater is the major source of F,[1-2] but it also occurs in commerce and industry.[3-4] It is sufficiently soluble in water to be taken up by plants and absorbed by animals.[5-6] Recently, we reported that F can cause genotoxic alterations in fish.[7] In India, more than 66 million people including 6 million children under 14 years of age are estimated to be affected with skeletal, dental, or visceral fluorosis by consuming F contaminated water.[8] A great deal of research work has been conducted on skeletal and non-skeletal fluorosis in humans and mammalian experimental models. However, studies on F toxicity to fish are relatively limited.[9-12] In the present investigation, the extent of damage to gills and alterations in the haematological parameters of fresh water Asian ("walking") catfish, *Clarias batrachus*, Linn, were examined after sub-lethal exposure of F for 30 and 60 days to 35 and 70 mg F ion/L.

MATERIALS AND METHODS

Healthy specimens of *Clarias batrachus* (weight 50±6.5 g, length 16±0.5 cm) were procured from local resources in Lucknow. They were brought to the laboratory in large plastic containers and were kept for 15 days under standard laboratory conditions. They were fed with minced goat liver once a day, and the water of the aquaria was renewed on alternate days.[12] The physico-chemical characteristics of the water during acclimation were determined by standard APHA[13] methods: pH 7.1±0.04, dissolved O_2 6.60±2.32 mg/L, hardness 120±3.31 mg/L as $CaCO_3$, temperature 25±1.5ºC, and electrical conductivity 440.24±3.50 µohms/cm. The source of F was NaF (ER grade) obtained from Qualigens Fine Chemicals Limited, Mumbai, India. For the study, the fish were divided into three

[a]For correspondence: Aquatic Toxicology Research Laboratory, Department of Zoology, University of Lucknow, Lucknow - 226 007 (U.P.) India. E-mail: drmtripathi@gmail.com

groups of 15 fish. Two experimental groups were exposed to 35 and 70 mg F ion/L (1/10th and 1/5th of the 96-hr LC_{50} value), and one group without F added to the water served as control. At the end of 30 and 60 days, 6 fish from each group were anesthetized with MS-222 (tricaine methanesulfonate).The blood was collected by severing off the caudal peduncle into vials containing heparin (45 units/mL blood) as anticoagulant and used for determination of red blood corpuscle (RBC) count, hemoglobin (Hb%), erythrocyte sedimentation rate (ESR) and packed cell volume (PCV). The RBC count was conducted in a modified Neubaur's chamber after saline dilution of the blood, and the hemoglobin content was determined by the cyanomethemoglobin method.[14] The erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) were measured by Wintrobe's method.[14] The mean corpuscular haemoglobin concentration (MCHC) and O_2 carrying capacity of blood were calculated by using the standard formula.[14]

Gills of control and F-exposed fish were dissected out and subjected to routine histological techniques.[15] Paraffin sections were cut at 4-6 μm thickness and were stained with haematoxylin and eosin (HE). Histopathological changes were observed under a light microscope, and selected sections were microphotographed.

RESULTS

Haematology: The F concentration and duration dependent decrease in RBC count, Hb%, PCV, and O_2 carrying capacity of blood for the fish are recorded in Tables 1 and 2. The decrease in RBC count and Hb% was significant ($p<0.01$, $p<0.001$) at both F concentrations and exposure periods, while a significant ($p<0.01$) decline in O_2 carrying capacity of blood was recorded after 60 days at the lower F concentration and was highly significant ($p<0.001$) at the higher F concentration after 30 and 60 days of exposure. The decrease in PCV was significant after 60 days at the lower F concentration ($p<0.05$) and more significant at the higher concentration ($p<0.01$) in both exposure periods. The concentration and duration dependent increase in ESR was found significant after 60 days at the lower F concentration ($p<0.05$) and both exposure periods at the higher concentration ($p<0.05$, $p<0.001$). The change in MCHC was found insignificant throughout the experiment (Tables 1 and 2).

Histopathology: Gills of control fish showed normal appearance of primary and secondary lamellar epithelial cells, chloride cells, and pillar cells. Gills of fish exposed to sub-lethal concentrations of F for 30 days and 60 days showed several pathological changes. The magnitude of these changes increased with increasing time of exposure. Gills of fish exposed to F for 30 days showed swelling in primary and secondary lamellar epithelium and clubbing on the tip of secondary lamellae at the lower F concentration, while lifting in lamellar epithelium, fusion and degeneration of secondary lamellae, and vacuolation in epithelial cells were observed at the higher F concentration.

However, after 60 days of exposure to F, thickening and rupturing of primary and secondary lamellar epithelium, pre-necrotic and degenerative changes in

chloride cells and pillar cells were observed at the lower F concentration, and thinning, lifting in primary and secondary lamellae, necrosis, vacuolation, and hyperplasia in chloride cells and pillar cells were observed at the higher F concentration.

Table 1. Effect of F on blood parameters of *C. batrachus* after 30 days exposure. (Values are Mean±SEM)

Parameters	Group I (Control)	Group II (35 mg F ion/L)	Group III (70mg F ion/L)
RBCs (10^6/mm^3)	3.74±0.180	2.72±0.120†	2.36±0.190†
Haemoglobin (g%)	15.02±0.20	13.97±0.22†	12.65±0.25‡
O_2 carrying capacity (mL O_2/g Hb)	18.77±0.31	17.46±0.23	15.81±0.25‡
ESR (mm/hr)	0.58±0.02	0.64±0.02	0.70±0.03*
PCV (%)	34.16±0.62	33.92±0.62	29.75±0.58†
MCHC (%)	43.25±1.47	45.12±1.35	45.02±1.37

Compared with the control *p<0.05, †p<0.01, ‡p<0.001

Table 2. Effect of F on blood parameters of *C. batrachus* after 60 days exposure. (Values are Mean±SEM)

Parameters	Group I (Control)	Group II (35 mg F ion/L)	Group III (70mg F ion/L)
RBCs (10^6/mm^3)	3.62±0.050	2.42±0.680†	1.99±0.220†
Haemoglobin (g%)	14.40±0.26	12.74±0.34†	9.34±0.35†
O_2 carrying capacity (mL O_2/g Hb)	18.00±0.32	15.92±0.22†	12.95±0.32‡
ESR (mm/hr)	0.60±0.04	0.84±0.06*	1.02±0.05‡
PCV (%)	33.74±0.37	30.25±1.05*	27.80±1.07†
MCHC (%)	42.73±1.37	43.51±1.37	43.27±1.53

Compared with the control *p<0.05, †p<0.01, ‡p<0.001

DISCUSSION

Blood is an overall reflector of body health, and its haematology is useful in the detection and diagnosis of metabolic disturbances and disease processes. F is now well known to be haemotoxic for lower and higher vertebrates.[17-19] It has also been reported to induce genotoxicity, which may be one of the reasons for disturbed cellular activities in fishes.[7]

In the present study, the decrease in RBC count by F may be responsible for reduction in Hb content, possibly from the destructive action of F on the

erythrocyte (RBC) membrane resulting in lower viability of the affected cells. The damaging effect of any toxicant on erythrocytes may be secondary while primary action may be on erythropoietic tissues, causing either a decrease in RBCs production or an increased rate of erythrocyte destruction.[20] In this connection, a major concern is the effect on the ability of haemoglobin molecules to bind oxygen loosely and reversibly for their capacity to transport O_2. The observed decrease in O_2 carrying capacity in F-exposed fish may be due to the reduction of RBCs and Hb%. This finding is supported by the observations of Chatterjee and Ganguli[21] and Sampath et al.,[16] who have also reported decreased O_2 carrying capacity of fish after exposure to Cu, Zn, and mahua oil cake. Another possibility for the decrease in O_2 carrying capacity in the present study may be damage of gills under F stress leading to loss of respiratory area. This is clearly evident from histological examination of the gills.

The decline in PCV (packed cell volume) observed in the present investigation may be the result of the reduction in RBC count and Hb content in *C. batrachus*. The findings are supported by the observations of Gupta et al.[22] and Saxena et al.,[9] who have reported similar changes in *Channa punctatus* after exposure to F. The gradual increase in erythrocyte sedimentation rate (ESR) values from 30 to 60 days in F exposed groups can be attributed to the accumulation of F in RBC. Similar findings were reported by Gupta et al.[22] and Saxena et al.[9] The slight variation in MCHC values may be due to changes in haemopoietic activity caused by F.

Gills of *C. batrachus* exposed to either 35 or 70 mg F ion/L showed swelling in primary lamellar epithelium, shortening, and fusion of secondary lamellae, hyperplasia and hypertrophy in chloride cells. All these changes were similar to those reported by Gupta[10] in *Channa punctatus* and Kumar and our group[11] in *C. batrachus* following exposure to F. Hyperplasia of epithelium results in an increase of the diffusion distance affecting the exchange of gases. Fusion of lamellae may be considered to cause a decrease in the total respiratory area of the gills, thus resulting in a decreased O_2 uptake capacity of the gills. In this condition fish fail to get adequate oxygen for total metabolic activities, and they therefore visit the surface more frequently, as found in the present study. Earlier workers have also reported that increased thickness of the epithelial layers results from hyperplasia following exposure to the pesticide endosulfan.[23]

The increased visits to the surface of the water by fish exposed to F indicate impairment of respiration and the respiratory mechanism by reduction of O_2 uptake through the gills and blood. Possible reasons for the observed decrease of RBC and Hb content include blocking *de novo* synthesis of Hb at some stage and/ or by promoting increased destruction of erythrocytes or delay in their maturation in a way that interferes with normal haematopoiesis. F can also hamper the absorption of iron at the intestinal mucosa or impair enzymes at the corpuscular level or cause increased destruction of erythrocytes by interfering with the cell-plasma relationship. One or more of these factors might be operative in producing anemia in fish, thus causing respiratory dysfunctions.

ACKNOWLEDGEMENT

The authors are grateful to Professor Minakshi Shrivastava, Head, Department of Zoology, University of Lucknow, for providing necessary laboratory facilities.

REFERENCES

1 Tripathi P, Sultana N. Fluoride content in ground water of some rural communities of district Unnao (U.P.). Ecol Env Cons 2006;2:279-80.
2 Dobaradaran S, Mahvi AH, Dehdashti S, Dobaradaran S, Shoara R. Correlation of fluorides with some inorganic constituents in groundwater of Dashtestan, Iran. Fluoride 2009;42(1):50-3.
3 Pesticide and Environmental Toxicology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Public health goal for fluoride in drinking water. Sacramento, CA: Office of Environmental Health Hazard Assessment; 1997.
4 Shankar BS, Reddy MTM, Balasubramanya N. Incidence of fluoride contamination in groundwater of an industrial area: a case study. Our Earth 2007;4:19-23.
5 Gikunju JK. Fluoride concentration in Tilapia fish (*Oreochromis lecostictus*) from Lake Naivasha, Kenya. Fluoride 1992;25:37-43.
6 Camargo JA. Fluoride toxicity to aquatic organisms a review. Chemosphere 2003;50(3):251-61.
7 Tripathi N, Bajpai S, Tripathi M. Genotoxic alterations induced by fluoride in Asian catfish, *Clarias batrachus* (Linn.). Fluoride 2009;42(4):292-6.
8 Ayoob S, Gupta AK. Fluoride in drinking water: a review on the status and stress effects. Crit Rev Environ Sci Tech 2006;36(6):433-87.
9 Saxena R, Gupta R, Tripathi M, Gopal K. Fluoride induced haematological alterations in the freshwater fish *Channa punctatus*. J Ecophysiol Occup Hlth 2001;1:139-46.
10 Gupta R. Pathophysiological consequences to fresh water fish, *Channa punctatus* induced by fluoride [Ph.D. dissertation]. Lucknow: University of Lucknow; 2003.
11 Kumar A. Evaluation of fluoride toxicity on reproductive system of freshwater fish [Ph.D. dissertation]. Lucknow: University of Lucknow; 2005.
12 Kumar A, Tripathi N, Tripathi M. Fluoride-induced biochemical changes in fresh water catfish (*Clarias batrachus*, Linn). Fluoride 2007;40(1):37-41.
13 APHA, AWWA, WPCF. Standard methods for examination of water and waste water. 20th ed. Washington DC: American Public Health Association;1998.
14 Dacie JV, Lewis SM. Practical haematology. 7th ed. London, Melbourne, New York: J and A Churchill, Livingstone; 1991.
15 Culling CFA. Handbook of histopathology and histochemical techniques. 3rd ed. London: Butterworth; 1974.
16 Sampath K, James R, Ali Akbar KM. Effect of copper and zinc on blood parameters and prediction of their recovery in *Oreochromis mossamibicus* (Pisces: Cichlidae). Indian J Fish 1998;45:129-39.
17 Pillai KS, Mane UH. Effect of air born fluoride on some haematological parameters of chick. Bull Environ Contam Toxicol 1985;35:510-6.
18 Karram MH, Ibrahim ThA. Effect of industrial fluorosis on haemogram of camels. Fluoride 1992;25:23-36.
19 Mishra PC, Mohapatra, AK. Haematological characteristics and bone fluoride content in *Bufo melanosticus* from an aluminium industrial site. Poll Research 1998;99:421-3.
20 Karuppasamy R, Subadhra S, Puvaneweswri S. Haematological responses to exposure to sublethal concentration of Cadmium in air breathing fish, *Channa punctatus* (Bloch). J Environ Biol 2005;26 (1):123-8.
21 Chatterjee S, Ganguli S. Effect of mahua oil cake on the blood of the fish *Clarias batrachus*. Environ Eco 1993;11:888-91.
22 Gupta R, Saroj, Gopal K, Tripathi M, Sharma UD. Fluoride induced haematological and histopathological changes in fresh water fish (*Labeo rohita*). Biological Memoirs 2001;26(2):27-31.
23 Nowak B. Histological changes in gills induced by residues of endosulfan. Aquat Toxicol 1992;23(1):65-85.