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Research Article

ALTERATION IN PIGMENTATION AFTER FLUORIDE EXPOSURE IN STINGING CATFISH, *HETEROPNEUSTES FOSSILIS* (BLOCH)

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ABSTRACT
The present study was undertaken to investigate the toxicological impact of fluoride (F^-) on chromatophores in skin of fresh water fish, *Heteropneustes fossilis* after chronic exposure. The fish was exposed to two sublethal concentrations i.e. 38.60 mg F/L (low concentration) and 77.20 mg F/L (high concentration) for three months and the observations were recorded after 45 and 90 days. Marked change in colouration was observed in both the experimental groups as compared to control. There were distinguished melanophores in the pigment aggregation state or pigment dispersion state. On the basis of structure, melanophores were identified as reticulate, stellate and punctuate type. After chronic exposure to F^-, several alterations were observed in chromatophores in comparison to control such as breaking of chromatophores as well as dendritic processes, change in structure and morphology, transformation of one type into another, increased distance between adjacent chromatophores, increase or decrease in number and finally complete loss of cellular entity. The observations were found to be concentration dependent, i.e. the changes were more severe in higher concentration in comparison to lower concentration exposed group.

Key Words: *Fluoride, Pigmentation, Heteropneustes Fossilis, Chromatophores*

INTRODUCTION
Chromatophores are pigment containing and light reflecting cells present in the skin of various vertebrates like fishes, amphibians, reptiles and mammals (Zarnescu, 2007). Among them fish, amphibian and reptiles possess rapid colour change with response to the changing environment. The brilliant colouration in fishes is generated as a result of absorption of light rays by pigmentary substances contained in dendritic melanophores, xanthophores and by scattering and reflection of light by iridophores (Hawkes, 1974). Lightening and darkening of fish skin occurs due to aggregation and dispersal of pigment. The aggregation of pigment granules, melanosomes to the centre of the cell results in skin lightening whereas dispersal of pigment throughout the cells results in darkening.

Colour change in fish performs various functions such as protection from intense illumination, aggressive colouration to escape from predators and colour display for courtship, mating and reproduction (Fujii, 2000). The change in chromatophore pattern is under the control of nervous and endocrine systems (Hoar, 1987; Nagabhusnam and Sarojini, 1989 and Tripathi *et al.,* 2005). Environmental information is processed in the central nervous system and transmitted to the melanophores, where both the hormonal and neuronal regulations result in appropriate chromatic reactions (Pradeep *et al.,* 2007). A part from this, several environmental factors such as light, water quality, temperature, salinity and chemicals/pollutants are also known to affect colour change (Fujii, 1969; Watanabe *et al.,* 1965; Tripathi *et al.,* 2005 and Pradeep *et al.,* 2007).

There are various reports indicating adverse effect of pollutants in fishes but scanty information is present regarding their effect on pigmentation. Since fluoride has been reported to affect nervous as well as endocrine systems in animals (Guan *et al.,* 1999; Gao *et al.,* 2009; Sharma *et al.,* 2007 and Wang *et al.,* 2009). it might also be having adverse effects on pigmentation in fishes after chronic exposure. *Heteropneustes fossilis* is a common edible fish in India whose skin is vulnerable to contaminated/polluted water due to lack of scales. Integumentary melanophores respond immediately to

pollutants (Singh and Duttamunshi, 1992) due to prolonged and direct contact to the contaminated media. Keeping these facts into consideration the study was designed to investigate the effect of sub-lethal concentrations of fluoride on pigmentation in fish.

MATERIALS AND METHODS

Healthy fishes of either sex (16.0±0.5 cm long and weighing 30.0±2.0 gms) were procured from local fish market and transported to laboratory in large plastic containers. They were sorted for disease and injury, rinsed in 0.1% $KMnO_4$ solution and acclimated to laboratory conditions for 20 days in large steel tanks. They were fed with dry prawn pieces, once every morning and the water was renewed on alternate days.

During experimentation, the fishes were divided into three groups having 12 fish in each. Group one served as control (without any treatment) whereas remaining two groups were exposed to 38.60 mgF/L and 77.20 mgF/L ($1/10^{th}$ and $1/5^{th}$ of 96 hour LC_{50} value) as lower and higher concentration of fluoride respectively for a period of 90 days. The observations were recorded after 45 and 90 days. Physico-chemical properties of test water during experimentation were maintained as per APHA et al. (2005) methods such as: temperature 27±1.5°C, pH 7.1±0.02, dissolved oxygen 8.5±1.6 mg/L, alkalinity 90-102 mg/L and hardness as $CaCO_3$ 120-155 mg/L. The source of fluoride was sodium fluoride (NR grade) manufactured by Qualigens Fine Chemicals, Mumbai, India. A stock solution was prepared by dissolving weighed amount of toxicant into 500 ml of double distilled water containing 10 mgF/ml, which was further diluted with chlorine free tap water to get desired concentrations.

For chromatophore number and structure, fish from each group were taken out, anaesthetized with tricaine methanesulphonate (MS-222). Samples of skin (4x8 mm) were carefully removed from the dorsal and lateral side of the fish and immediately immersed in 0.7% sodium chloride solution (fish saline). The skin pieces were fixed in alcoholic Bouin's fluid for 24 hours. After washing it with 70% alcohol to remove excess fixative, they were dehydrated in graded series of alcohol, cleared in xylene and mounted in Canada balsam. The number of chromatophores were counted from the slide, under simple compound microscope and photographed and the data obtained was subjected to student's t-test.

RESULTS

The skin of fish is composed of two main layers. The outer layer is epidermis, which is ectodermal, non-keratinised consist of stratified squamous epithelium and inner dermis, differentiated into two layers: stratum laxum (build of loose connective tissues interspersed by blood vessels, nerves and sense organs) and stratum compactum (composed of dense connective tissue having bundles of proteinaceous collagen fibres and mesenchymal cells).

In *Heteropneustes fossilis* skin, the most abundant chromatophores were melanophores. Melanophores had cytoplasmic projections, which stick out from the centre of the cell and interwined with the collagen fiber of the connective tissue (Fig-1). These cells were detected in various physiological stages i.e. in state of pigment granule dispersion and in phase of pigment aggregation. The translocation of the pigment within the melanophore influenced morphological shape of the cell. During dispersion phase, the pigment granules were spread within the cytoplasm giving rise to many dendritic processes. While during aggregation state, all the pigment granules were concentrated in the centre of the cell giving melanophore an oval or round shape. On the basis of dispersion and aggregation of pigment in melanophores, reticulate, stellate and punctuate shapes have been observed in this study.

Chronic exposure of *Heteropneustes fossilis* to sodium fluoride altered chromatophores structure, shape, size and melanin dispersion remarkably. After 45 days of exposure, in low concentration group, chromatophores number was found increased while their size was found reduced. Shape of chromatophores changed to stellate in comparison to reticulate chromatophore of controls (Table-1; Figure 1, B).

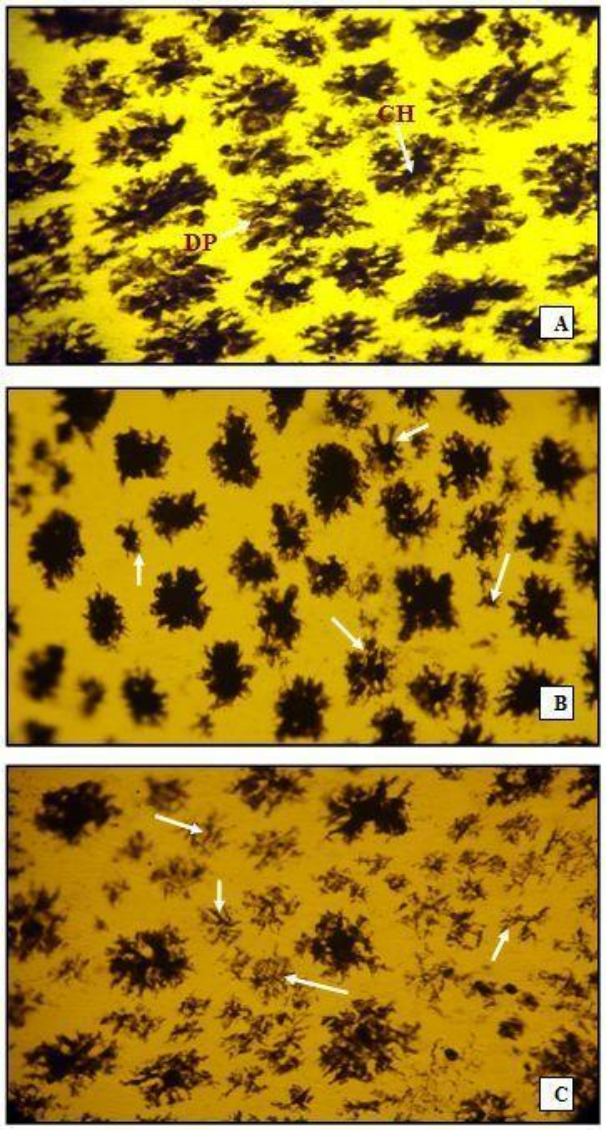

Figure 1: Photomicrographs showing effect of fluoride on chromatophores of *Heteropneustes fossilis* after 45 days exposure.

A: Showing normal chromatophore structure in control group.

B: Lower concentration fluoride exposed group showing reduced dendritic processes and increase in number of chromatophores.

C: Higher concentration fluoride exposed group showing decrease in chromatophore number, reduced dendritic processes, aggregation of melanin towards centre and breaking of chromatophore as well as dendritic processes.

CH= Chromatophore; DP= Dendritic processes

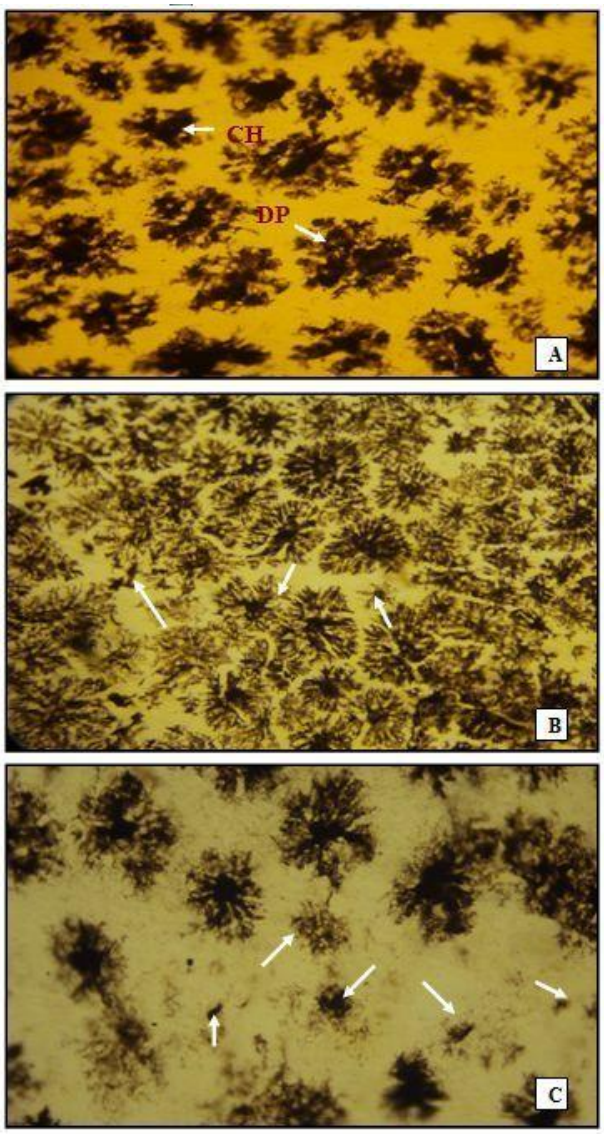

Figure 2: Photomicrographs showing effect of fluoride on chromatophores of *Heteropneustes fossilis* after 90 days exposure.

A: Showing normal chromatophore structure in control group.

B: Lower concentration fluoride exposed group showing slight increase in number of chromatophores and broken dendritic processes.

C: Higher concentration fluoride exposed group showing decrease in number of dendritic processes as well as chromatophores showing 4-5 bifid and trifid branches and marked distance between adjacent chromatophores.

In high concentration group after same duration the number of chromatophores was found decrease. Dendritic processes were found to be reduced in comparison to controls in most of the cells and melanin was found aggregated in centre, leaving almost transparent dendritic processes. Breaking of chromatophores as well as dendritic processes was also noticed at some places (Figure 1 C).

Table 1: Effect of fluoride on chromatophores after exposure to two sublethal concentrations for different durations

Chromatophore Type	Exposure Duration (Days)	Control	Low Concentration (38.60 mgF/L)	High Concentration (77.20 mgF/L)
Reticulate	45	34.83±1.51	18.52±1.94**	9.80±1.54**
	90	39.33±1.62	28.16±1.86*	13.65±1.89**
Stellate	45	16.15±1.01	43.83±1.90**	31.16±1.60**
	90	10.18±1.34	40.66±1.61**	23.80±1.15**
Punctate	45	2.50±0.42	5.16±0.60*	10.58±1.38**
	90	5.34±1.36	12.36±1.18*	20.52±1.34**

*Values are Mean±S.E.M; N= 6 (Number of observations per value); *p<0.01; **p<0.001 (Compared to control)*

After 90 days of exposure, in low concentration group there was a slight increase in chromatophore number and considerable decrease in chromatophore size. The dendritic process was found reduced and broken at some places (Figure 2 B). In high concentration exposed group after 90 days, heavy breaking of dendritic processes as well as chromatophores was noticed. The dendritic processes were found highly reduced leaving 4-5 smaller bifid and trifid branches in most of the chromatophores. Some punctate type chromatophores were also seen in comparison of reticulate type of controls. Dark coloured small granules were found released in matrix after breaking of dendritic processes. Most of the chromatophores were with loss of their cellular entity. Maximum distance was recorded between two adjacent chromatophores during this stage (Figure 2 C).

DISCUSSION

In the present study, marked changes in chromatophores of fish were noticed after chronic exposure to fluoride. The alterations included decrease in chromatophore size, increase in number and change of reticulate shape into stellate type or punctate, retracted and reduced dendritic processes, centrally aggregated melanin pigments with transparent extremities of chromatophores, broken chromatophore and increased distance between two adjacent chromatophores. The chromatophores showed alteration in the shape, density and dimension after fluoride exposure which indicates that fluoride has a definite accelerative effect on dispersion of the melanin pigments and its effect was duration and dose dependent.

The results of the study are in accordance with the findings of Tripathi *et al.,* (2005) who have reported similar alterations in chromatophores of fresh water fish *Channa punctatus* after fluoride exposure, Kesherwani (2006) after cadmium exposure in fresh water catfish *Heteropneustes fossilis* and Pradeep *et al.,* (2007) after methyl parathion exposure in *Oreochromis mossambica*. Decrease in the chromatophore size and number indicates the impairment of pigment cells (Singh and Duttamunshi, 1992). Chromatophores especially melanophores have been shown to differentiate and to die by aptoptosis under the influence of factors that regulate motile responses. These factors are likely to utilize common intracellular signaling pathways used in part to regulate both types of changes (Sugimato, 2002).

The size of chromatophore decreased with the density due to gradual cell death and apoptosis. The process of cell death includes loss of cell activity, cell fragmentation and phagocytosis of the fragments (Sugimato *et al*., 2000). The fusion of dendritic processes and further deterioration in the shape of

melanophores with increasing period of toxicant exposure is consistent to the findings of Pandey *et al.,* (1981).

Heteropneustes fossilis is a catfish, whose skin is vulnerable to polluted water due to lack of scales. Integumentary melanophores respond immediately to pollutants (Singh and Dutta Munshi, 1992). Several workers have described the physiological colour change, nervous and endocrine control on colour change and neurohormones on controlling chromathophore patterns (Fujii, 1969; Pouchet, 1872, 1876; Bagnara and Hadley, 1973; Hoar, 1987 and Nagabhusnam and Sarojini, 1989). Fluoride has been reported to affect endocrine system in animals. The colour change and alterations in chromatophores observed in the present study after fluoride exposure may be due to the endocrine mediated effects or due to direct cytotoxic effects.

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