EFFECT OF FLUORIDE ON GROWTH BIOINDICATORS IN STINGING CATFISH, HETEROPNEUSTES FOSSILIS (BLOCH)

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SUMMARY: The toxicity of fluoride (F) on protein and lipids, as growth bioindicators, was evaluated in “stinging” catfish, Heteropneustes fossilis, after their exposure to a sublethal concentration of NaF (77.20 mg F ion/L) for three months. Alterations in the amounts of these biomolecules in the gill, liver, kidney, and muscle tissues of the F-exposed fish were recorded after 45 and 90 days and compared to controls. Significant depletion of protein and lipids in these tissues occurred after both periods. The depletion was duration dependent, which suggests that F interferes with the availability of biomolecules required for proper growth and development of fish after chronic exposure to F.

Keywords: Biomolecules; Fluoride toxicity; Lipid; Protein; Soft-tissues; Stinging catfish.

INTRODUCTION

Natural waters are often contaminated by either untreated or partially treated wastes of industrial and agricultural origin containing various chemical pollutants. Owing to their bio-accumulative and non-biodegradable properties, these chemicals constitute a core group of persistent aquatic contaminants. Among them, fluoride (F) has emerged as one of the major pollutants in ecotoxicological studies. It is a persistent bioaccumulator that accumulates in visceral organs of aquatic animals including fish being continuously exposed to the contaminated medium. The accumulation of F disturbs the metabolic machinery that is interlinked with the structural integrity of cells and tissues and alters the biochemical profile of the exposed organisms.

Recently, we reported that F can induce genotoxicity and can cause respiratory dysfunctions in Asian catfish. Deleterious effects of F on biochemical contents in different tissues of fish has also been observed in our laboratory and reported earlier. The present study is a continuation to our previous studies on Asian catfish. Here the fish were exposed only to a higher concentration of F to observe changes in the level of growth biomolecules after different durations of F exposure. Obviously, it is of concern that alteration in the level of these biomolecules may result in decline of fish growth and population as well as lower nutritive value of the harvested product, which is not conducive for profitable aquaculture.

MATERIALS AND METHODS

Healthy specimens of Heteropneustes fossilis (mean weight 12.04±0.20 g and length 13.09±0.20 cm) were procured from the local fresh water resources in Lucknow. They were transported to the laboratory in large plastic containers filled with fresh water to minimize stress. Fish were checked for disease as well as injury and rinsed in 0.1% KMnO₄ solution to avoid infection and were acclimated for about 20 days in dechlorinated tap water contained in a large steel tank with proper aeration.

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For the experiment, healthy fish were sorted out and transferred into aquaria measuring 60×40×45 cm holding 40 L of water. They were divided into two groups of 15 fish in each group. Group I served as the control (without F added to the water) and group II was exposed to a sublethal concentration of F; 77.20 mg F ion/L (one fifth of 96-hr LC₅₀ value). The source of F was NaF (ER grade) obtained from Qualigens Fine Chemicals Limited, Mumbai, India. A stock solution of 10 mg F/mL was prepared by dissolving a weighed amount of NaF in 500 mL of double distilled water, which was further diluted to the desired concentration with chlorine-free tap water. The physico-chemical properties of the holding water was determined according to APHA¹¹ methods: temperature 27±1.5°C, pH 7.3±0.2, dissolved oxygen 7.8±1.5 mg/L, alkalinity as CaCO₃ 225–230 mg/L, hardness as CaCO₃ 250–290 mg/L, and F 0.1 mg/L. Fish were fed with pieces of dried prawn, and the aquaria water was renewed on alternate days and supplemented with a fresh dose of NaF.

At the end of 45 and 90 days, 6 fish from the control and experimental groups were removed and sacrificed for sampling. Gill, liver, kidney, and muscle tissue were carefully dissected out and subjected to biochemical determinations. Total protein was estimated by the method of Lowry et al.¹² and lipid estimation followed the methods of Folch et al.¹³ The determinations were replicated three times, and the data were analyzed by the Student t test.

**RESULTS**

Changes in the levels of protein and lipid (mg/g) in different tissues in the control and experimental fish after 45 and 90 days of exposure to F are shown in Tables 1 and 2. The results reveal significant reductions in both protein and lipid content of tissues in decreasing order: gill, kidney, liver, and muscle.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Exposure Duration (Days)</th>
<th>Control (mg/g)</th>
<th>Experimental (77.20 mg F/L)</th>
<th>% Decrease (↓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>45</td>
<td>10.18±0.44</td>
<td>7.76±0.60†</td>
<td>23.77</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>13.03±0.20</td>
<td>6.26±0.12†</td>
<td>51.95</td>
</tr>
<tr>
<td>Liver</td>
<td>45</td>
<td>46.30±0.12</td>
<td>40.00±0.11†</td>
<td>13.58</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>51.36±0.14</td>
<td>45.50±0.10†</td>
<td>11.40</td>
</tr>
<tr>
<td>Kidney</td>
<td>45</td>
<td>23.25±0.60</td>
<td>18.11±0.12†</td>
<td>22.10</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>25.51±0.34</td>
<td>16.20±0.37†</td>
<td>36.49</td>
</tr>
<tr>
<td>Muscle</td>
<td>45</td>
<td>100.80±1.35</td>
<td>96.21±1.22*</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>116.12±1.75</td>
<td>105.56±0.31†</td>
<td>9.09</td>
</tr>
</tbody>
</table>

Compared with control: *p<0.05, †p<0.01.
DISCUSSION

Duration-dependent decreases in protein and lipid contents of gill, liver, kidney, and muscle of *Heteropneustes fossilis* after sublethal exposure to F in this study indicate a deleterious effect of F on metabolism of macromolecules. The depletion may be due to blocking of the metabolism of the amino acids, thereby preventing cells from synthesizing protein. Earlier work has shown that F inhibits protein synthesis\(^{14}\) and interferes with amino acid metabolism.\(^{15}\) Another possible reason for depletion of protein may be its conversion into glucose\(^{16}\) or utilization of protein in the form of mucoprotein which is eliminated in the form of mucus by the fish to combat toxic stress.\(^9\)

Under conditions of stress, many organisms mobilize proteins as an energy source through the oxidation of amino acids. The decreased protein level observed here may also be attributed to stress-mediated mobilization of these compounds to fulfill increased demands for energy by the fish to cope with the environmental stress after exposure to toxicants.\(^{17}\) On the other hand, Saxena and Mani\(^{18}\) have suggested that decline in protein content may be related to decreased food intake, increase energy cost of homeostasis, tissue repair, and detoxification mechanism during stress. Reduced feeding intensity, after F exposure in fish, has also been observed in different studies in our laboratory.\(^{9,19-20}\)

Lipids also play an important role in metabolic activities of animals because they are a source of energy and are involved in the building of cellular components. They are stored in the form of metabolites and provide energy when an organism faces adverse conditions.\(^{21}\) Significant depletion of lipids in gill, liver, kidney, and muscle of the exposed fish in the study may be associated with the inhibition of lipid synthesis by F or comparatively more utilization of stored lipids as an immediate source of energy to withstand stress. *In vitro* inhibition of fatty acid oxidation reported by Johnson and Lardy\(^{22}\) has been shown by Zebrowski et al.\(^{23}\)

### Table 2. Effect of fluoride on total lipid content (mg/g wet tissue) in different tissues of *Heteropneustes fossilis* after exposure to F for 45 and 90 days

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Exposure Duration (Days)</th>
<th>Control (mg/g)</th>
<th>Experimental (77.20 mg F/L)</th>
<th>% Decrease (↓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>45</td>
<td>21.36±0.78</td>
<td>17.31±1.10*</td>
<td>18.96</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>23.28±0.34</td>
<td>16.35±0.32‡</td>
<td>29.76</td>
</tr>
<tr>
<td>Liver</td>
<td>45</td>
<td>49.43±0.55</td>
<td>44.41±0.11‡</td>
<td>10.15</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>52.21±0.45</td>
<td>47.42±0.67‡</td>
<td>9.17</td>
</tr>
<tr>
<td>Kidney</td>
<td>45</td>
<td>26.34±0.18</td>
<td>22.35±0.15‡</td>
<td>15.14</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>23.31±0.48</td>
<td>17.48±0.22‡</td>
<td>25.01</td>
</tr>
<tr>
<td>Muscle</td>
<td>45</td>
<td>10.50±0.67</td>
<td>8.12±0.32†</td>
<td>22.66</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>13.82±0.43</td>
<td>6.40±0.15‡</td>
<td>53.69</td>
</tr>
</tbody>
</table>

Compared with the control: *p<0.05. †p<0.01, ‡p<0.001.
to occur with F with reduction of fatty acid catabolism in rats. Similarly, Singh et al.\textsuperscript{24} have found decreased lipid content in the liver of rabbits treated with F.

F is also known to inhibit the synthesis of the enzyme acyl-CoA, which is involved in fatty acid oxidation. Thus reduction of lipid in different tissues may be due to the enzymatic inhibitory action of F. The present findings are supported by the observations of Shashi et al.,\textsuperscript{25} who have reported a decreased level of lipids in rabbits after exposure to F and suggest it might be due to the inhibition of lipase by F. Similar views have been suggested recently by Kumar et al.\textsuperscript{10} after sublethal exposure of \textit{Clarias batrachus} to F. In their view, reduction in lipids after F exposure may be due to the deactivation of hormones that regulate lipid biosynthesis or inactivation of enzymes involved in lipid metabolism.

From the present findings we conclude that F diminishes protein and lipid metabolism in the gill, liver, kidney, and muscle tissues of fish, most probably by inactivating enzymes and hormones that regulate their synthesis and by increasing their utilization in cell repair, tissue reorganization, and to meet high energy demand during stress caused by F exposure.

**ACKNOWLEDGEMENT**

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**REFERENCES**