FLUORIDE-INDUCED BIOCHEMICAL CHANGES IN FRESH WATER CATFISH (CLARIAS BATRACHUS, LINN.)
Anand Kumar, Nalini Tripathi, Madhu Tripathi
Lucknow, India

SUMMARY: The toxicity of fluoride (F) to 16-cm long fresh water male catfish (Clarias batrachus, Linn.) was evaluated after their exposure to two sub-lethal concentrations of NaF (35 mg F ion/L and 70 mg F ion/L) for 90 days. Changes in biochemical parameters in muscle, liver, and testis tissues were recorded. Significant depletion of total protein and lipids in these tissues occurred at both the lower and higher F concentrations. A significant reduction of glycogen content was found in muscle and testis at the lower concentration, but it increased in all three tissues at the higher concentration. Moreover, an increase in the level of cholesterol in muscle, liver, and testis occurred at both concentrations, but it was significantly higher (P<0.05) only at the higher concentration.

Keywords: Biochemical parameters; Catfish, Clarias batrachus; Fluoride and catfish; Fresh water catfish; Soft-tissue effects.

INTRODUCTION

Most rivers, streams, and ponds in India are severely polluted or serve as “open sewers” because domestic sewage and industrial wastes, either untreated or partially treated are discharged into them. They contain many toxic chemicals that adversely affect aquatic organisms. Owing to expanded industrial emissions and commercial uses of F compounds, the concentration of F is increasing in both ground water and surface water.

The toxic effects of elevated fluoride on various aquatic species are well documented,1-3 as are its harmful impacts on humans, livestock, and plants.3,4 Several studies on aquatic invertebrates like Daphnia, Artema, Penaeus, and Hydropsyche reveal that F affects survival, growth, behaviour, and reproduction. Likewise, F also affects vertebrates in their haematological parameters,5 morphological and behavioral parameters,6 and cellular architecture.7 F has also been shown to cause many biochemical changes and metabolic disturbances in mammals including rats, rabbits, goats, and human beings,8,9 but very few reports are available for fishes. Chitra et al.10 found that F alters enzyme activity in muscle and liver of Channa punctatus, while Gupta7 has observed that F decreased glucose and protein levels in blood and in muscles of these fish. However, these reports are not conclusive regarding the effect of F on fundamental biomolecules in fishes such as protein, lipid, cholesterol, and glycogen, all of which play important roles for survival, growth, and reproduction. Alterations in the levels of these biomolecules for to any reason may result in decline of fish population.

The present investigation was undertaken to evaluate the toxic effects of F on certain biomolecules in different tissues of fresh water catfish, Clarias batrachus, Linn.

aFor correspondence: Department of Zoology, University of Lucknow, Lucknow 226 007, India; Email: DrMTripathi@gmail.com
MATERIAL AND METHODS

Healthy living specimens of catfish, Clarias batrachus, were collected from local fresh water resources and maintained under standard laboratory conditions for 15 days. Male specimens weighing 50±5g and measuring 16±5 cm were selected for experiments conducted in aquaria measuring 60 × 40 × 45 cm. The fish were divided into three groups with 15 fish per group. Group I served as control while groups II and III served as experimental groups. The latter were treated with two sub-lethal concentrations of F: 35 mg F ion/L of water (low concentration) and 70 mg F ion/L of water (high concentration), prepared from a stock solution of NaF (Merck, Mumbai Ltd., India) containing 10.0 g NaF/L prepared by dissolving 22.11 g of NaF/L of distilled water.

The experiment was conducted for 90 days, during which the fish were fed goat liver once each day. The aquarium water was changed on alternate days, and a fresh dose of F was supplemented after feeding by addition of either 210 mL or 420 mL of stock solution of NaF to the 60 L of water in each of the two experimental aquaria. After 90 days, all the fish were sacrificed for sampling. The muscle, liver, and testis tissues in each group were dissected out and pooled for biochemical estimations.

Total protein, lipid, cholesterol, and glycogen were estimated by standard methods given by Lowry et al., Folch et al., Rosenthal et al., and Montgomery, respectively.

RESULTS

Total protein: After 90 days of exposure of Clarias batrachus to the sub-lethal concentrations of fluoride, the total protein contents in muscles, liver, and testis were decreased significantly (P<0.001) in both groups of exposed fish in comparison to the control group (Table 1).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group I (Control)</th>
<th>Group II (35 mg F ion/L)</th>
<th>Group III (70 mg F ion/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>183.20 ± 2.12</td>
<td>153.42 ± 1.78**</td>
<td>125.00 ± 1.82**</td>
</tr>
<tr>
<td>Liver</td>
<td>95.81 ± 1.03</td>
<td>60.82 ± 1.10**</td>
<td>40.72 ± 1.05**</td>
</tr>
<tr>
<td>Testis</td>
<td>60.10 ± 0.87</td>
<td>42.78 ± 1.03**</td>
<td>33.20 ± 1.10**</td>
</tr>
</tbody>
</table>

(Values are Mean ± SE, n =6); **P<0.001; n= number of observations for each value.

Total lipid: The total lipid content in muscle, liver, and testis was also significantly decreased (P<0.05 to P<0.001) in both groups of exposed fish in comparison to the control group (Table 2).

<table>
<thead>
<tr>
<th>Tissue</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>8.32 ± 0.35</td>
<td>5.82 ± 0.43*</td>
<td>4.10 ± 0.32**</td>
</tr>
<tr>
<td>Liver</td>
<td>72.40 ± 1.75</td>
<td>45.12 ± 1.35**</td>
<td>35.28 ± 1.22**</td>
</tr>
<tr>
<td>Testis</td>
<td>15.12 ± 0.43</td>
<td>10.02 ± 0.32**</td>
<td>9.31 ± 0.38**</td>
</tr>
</tbody>
</table>

(Values are Mean ± SE, n =6); *P<0.05; **P<0.001; n= number of observations for each value.

Cholesterol: At both F concentrations, the cholesterol content in muscle, liver, and testis was higher than in the control fish. The increase was significant (P<0.05) in muscle tissue of both groups of exposed fish, whereas it was
significant in liver and testis only in the fish exposed to the higher concentration of F (Table 3).

Glycogen: The glycogen content was significantly decreased (P<0.05) in muscle and testis at the lower F concentration, but the decrease was not significant in liver. At the higher F concentration, however, glycogen increased significantly (P<0.05) in all three tissues (Table 4).

**DISCUSSION**

The decrease caused by F in protein content of muscle, liver, and testis in *Clarias batrachus* as observed here is similar to the observations of Gupta 7 with *Channa punctatus* after exposure to F for 90 days. This decrease may be due to blocking of the metabolism of amino acids, thereby preventing cells from synthesizing protein. In fact, studies have shown that F inhibits protein synthesis 9 and interferes with amino acid metabolism. 15 Another possible reason may be depletion of protein for its utilization in conversion to glucose.16

The total lipid decrease in muscle, liver, and testis of the F-exposed catfish is in agreement with observations by Shashi et al. in rabbits.8 The decrease may be due to inhibition of lipid synthesis by F as well as increased utilization of stored lipids as a source of energy to conduct regular metabolic functions. F is well-known as an inhibitor of various enzymes like lipases, phosphatases, and esterases. It interferes with fatty acid oxidation17 and also inhibits the enzyme acyl-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 increased cholesterol content observed in muscle, liver, and testis of the F-exposed catfish is in agreement with observations by Shashi et al. in rabbits.8 The increase may be due to inhibition of lipid synthesis by F as well as increased utilization of stored lipids as a source of energy to conduct regular metabolic functions. F is well-known as an inhibitor of various enzymes like lipases, phosphatases, and esterases. It interferes with fatty acid oxidation17 and also inhibits the enzyme acyl-Co-A synthetase involved in fatty acid oxidation. Thus decreased lipid content in various tissues may be due to the inhibition of these enzymes.
well as steroidogenesis will be suppressed, and the level of cholesterol, the precursor of steroid hormones, will be increased.

It is noteworthy that the glycogen content in the fish exposed to the lower F concentration was decreased, whereas a significant increase was found at the higher F concentration. The decrease of glycogen was statistically significant in muscle and testis but not significant in liver. The decline in the liver and muscle suggests enhanced conversion of glycogen to glucose to meet an increased energy requirement under stress conditions. Increased locomotor activity of the fish during the experiment supports this suggestion. Kasthuri and Chandran have also made a similar suggestion in their study with Mystus gulio exposed to lead.23

The increased glycogen level in the fish exposed to the higher level of F may be due to disturbance of carbohydrate metabolism as it has been observed to affect enzymes involved in glycogen turnover at higher F concentration.24 Several other studies have revealed that F inhibits many glycolytic enzymes.3,10 Consequently, the decrease in glycogen content at the lower F concentration and the increase at the higher F concentration cannot be explained by same mechanism. Still, it is clear that carbohydrate metabolism is disturbed by exposure to F.

From the results obtained here, it is clear that F interferes with various metabolic activities and alters the levels of protein, lipids, glycogen, and cholesterol of Clarias batrachus that are important in their physiological activities, survival, growth, and reproduction.

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