INFLUENCE OF CYANIDE ON SOME ANTIOXIDANT ENZYMES OF FRESHWATER FISH, *CIRRHINUS MRIGALA* (HAMILTON)

Shwetha Alavandi and Basaling B. Hosetti*

Toxicology Laboratory, Department of Studies and Research in Applied Zoology, Kuvempu University, Jnana Sahyadri, Shankaraghatta – 577451, Karnataka, India

**Abstract:** Sublethal toxicity of cyanide to freshwater fish, *Cirrhinus mrigala* was evaluated to determine its effect on the activities of some antioxidative enzymes. Changes in succinate dehydrogenase (SDH), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6-PDH) were observed in tissues of the tested animal. Cyanide significantly decreased the activity of SDH, and G6-PDH in the liver, gill and brain tissues of the fish. The elevation of LDH indicated metabolic disorders and a clear response against energy depletion. Changes in the dehydrogenase activity in cyanide treated fish tissues may be due to fluctuations in oxidative metabolism. It is concluded that the cyanide intoxication exerted a profound impact on the enzymatic activity of the fish, as observed in the hitherto study.

**Key words:** cyanide, enzymes, sublethal toxicity, *Cirrhinus mrigala*.

**Introduction**

Cyanide is extremely destructive chemical which can kill both target and non-target organisms when expelled in the environment (Dube and Hosetti, 2011). Industrial wastewaters, especially those from electroplating baths, manufactures of paints and synthetic fabrics, aluminium works, plastics, metal finishers, coal gasification processes, petroleum refineries and pesticidal agents are some important sources of cyanide contamination in aquatic environment (Kim et al., 2008). The U.S. Environmental Protection Agency (1980) considered cyanide as a priority toxicant and proposed acute and chronic level tests for cyanide in freshwater. The most significant effects of cyanide in fish include hypoxia (Dube and Hosetti, 2011), susceptibility to predation (Eisler, 1991), growth inhibition (Dixon and Leduc, 1981) and behavioural alterations (Shwetha and Hosetti, 2009). Other documented manifestations in fish include biochemical alterations, especially antioxidant activity thereby causing oxidative damages at cellular and molecular levels (David et al., 2008; Al-Ghanim and Mahboob, 2012).

*Corresponding author: e-mail: hosetti57@gmail.com*
Many organisms have evolved protective mechanisms to minimize the damages caused by the toxic chemicals and normal oxidative products of cellular metabolism (Jokanović, 2001). Oxidative stress caused by oxygen free radicals is generally considered a serious mechanism in the pathogenesis of many infirmities (Livingstone, 2001). Oxidative stress occurs when the critical balance between oxidants and antioxidants is imbalanced due to the depletion of antioxidants or excessive accumulation of the reactive oxygen species, or both, leading to damage (Scandalios, 2005). Antioxidants, which absorb the reactive oxygen species prior to their interaction with cellular components, are the first line of defence against exogenous oxidative stress (Besaratinia et al., 2001). Under normal conditions antioxidants protect the cells and tissues from oxidative damage (Ahmad et al., 2000).

Like many pesticides, cyanide is capable of producing oxidative stress in aquatic species and exerts its toxicity (Bhattacharya et al., 2009). Oxidative stress causes a wide range of genetic, metabolic, and cellular responses (Valavanidis et al., 2006). Studies on toxicant induced effects on various oxidative enzymes in fish and other aquatic organisms can provide the information about the eco-toxicological consequences of toxicant misuse (Ercal et al., 2001). The aim of this study, therefore, is to assess the impact of sub-chronic exposure to cyanide on enzyme contents in fish, C. mrigala. The hitherto study may provide useful information for ecological risk assessments.

Material and Methods

A stock of juvenile fish (C. mrigala) was procured from State Fisheries Department, Bhadra Reservoir Project, Shimoga District, Karnataka State, India. They were acclimatized to the experimental conditions for a week before use and fed with commercial fish food pellets. Only active specimens (5±0.5 g, 7±0.5 cm) with no signs of stress and injury were used in the study. Prior to the start of the bioassay, the fish were treated with 0.1% KMnO4 to remove any dermal adherents and bacterial contamination. Dechlorinated tap water (temperature 27±1°C, pH 7.2±0.2, dissolved oxygen 6.3±0.4 mg/l, hardness 23.2±3.4 mg/l as CaCO3, phosphate 0.39±0.002 µg/l, salinity 0.01 ppt, specific gravity 0.001 and conductivity less than 10 µS/cm and a light period of 12 h/day) was used throughout the experimental period and water was renewed every 24 h. Sodium cyanide (97% purity, Loba Chemical, Mumbai) was used as toxicant, prepared by dissolving it in double distilled water. The toxicant solution in the test chambers was replaced with a fresh solution of the same concentration every 24 h. The fishes were subjected to sublethal levels of cyanide (40% of 96 h LC50) for 7 and 14 days. The experiment was run in triplicates.
Influence of cyanide on some antioxidant enzymes of *Cirrhinus mrigala*

Tissue preparation

At the end of day 7 and day 14 of exposure, fish were sacrificed and liver, brain and gill tissues were collected immediately, washed with distilled water, blotted and weighed before homogenization. Tissues were homogenized using a glass homogenizer with respective homogenizer solution and centrifuged. Five percent of tissue homogenates (w/v) were prepared in 0.25 M ice cold sucrose solution using a glass homogenizer, centrifuged at 2,500 rpm for 15 minutes and the supernatant was taken as the source of enzyme. The enzyme activity is expressed as $\mu$M formozan formed/mg protein/h.

Enzyme estimation

Succinate dehydrogenase (SDH) activity in liver, brain and gill tissue was estimated by the method of Nachlas et al. (1960). Lactate dehydrogenase (LDH) activity was estimated by the method of Govindappa and Swamy (1965). Glucose 6-phosphate dehydrogenase (G6PDH) activity was determined by the method of Bergmeyer and Bernt (1965).

Statistical analysis

Each assay was replicated three times, and values thus obtained were converted to the percentage of the respective control and analysis of variance (ANOVA) was employed using SPSS 13.0 for windows (SPSS Inc., Chicago, IL, USA). The analysis was made between the means of the control and cyanide treated fishes using Duncan’s new multiple range test (Daniel, 1987).

**Results and Discussion**

No mortality was observed during the acclimation period or throughout the experimental period. Cyanide, the noxious chemical, has been found to bring about changes in the activity of all the three enzymes studied. In all the cases, differences between the means of the control and cyanide treated fishes were considered statistically significant at $p<0.05$.

SDH is a vital enzyme of citric acid cycle that catalyzes the reversible oxidation of succinate to fumarate. In the present investigation it can be visualized that there was a rapid depletion of SDH activity in all tissues of fish treated with sublethal concentration of cyanide compared to the control (Table 1). The maximum decrease was observed in brain (-18.19% and -31.24%), followed by liver (-15.54% and -26.76 %) and gill (-11.55% and -18.45%) on day 7 and day 14 of exposure. The enzyme activity was more pronounced on day 14.
Decreased SDH activity clearly indicates depletion in the oxidative metabolism at the level of mitochondria leading to depression of TCA cycle. A similar decrement in the SDH activity was observed by Jacob Doss et al. (2007) in freshwater fish exposed to cypermethrin. The same trend was also reported in different organisms exposed to different chemicals (Sudharsan et al., 2000; Al-Ghanim and Mahboob, 2012). A decrease in SDH activity under toxic stress was associated with the inhibition of mitochondrial respiratory mechanism, or derangement in ultra structure, architectural integrity and permeability of mitochondria (Archanakumta and Gaikwad, 1998). Decreased SDH activity of all tissues in the present study clearly indicates depletion in the oxidative metabolism at the mitochondrial level leading to despair of TCA cycle under the cyanide intoxication.

Table 1. Changes in the activity levels of SDH in different tissues of cyanide treated Cirrhinus mrigala.

<table>
<thead>
<tr>
<th>Tissues/Duration in days</th>
<th>Liver Mean ± SD Change (%)</th>
<th>Brain Mean ± SD Change (%)</th>
<th>Gill Mean ± SD Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.24 ± 0.025</td>
<td>0.39 ± 0.021</td>
<td>0.86 ± 0.041</td>
</tr>
<tr>
<td>7</td>
<td>1.04 ± 0.061</td>
<td>-15.54</td>
<td>0.32 ± 0.091</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-18.19</td>
<td>0.76 ± 0.016</td>
</tr>
<tr>
<td>14</td>
<td>0.91 ± 0.092</td>
<td>-26.76</td>
<td>0.27 ± 0.039</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-31.24</td>
<td>0.70 ± 0.066</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 5). The enzyme activity is expressed as µM formozan formed/mg protein/h. Means for organs are significantly different (p < 0.05) from each other.  

LDH is hydrogen transferring enzyme that catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD⁺ as hydrogen acceptor. LDH, an indicator of anaerobic metabolism, was expected to exhibit increased activity at lower oxygen levels. This pattern was noticed in brain tissue in which LDH activity was significantly higher on day 14 (58.04%) of treatment compared to day 7 (35.60%). Similar trends were also evidenced in liver (39.85% and 25.26%) and gill (19.13% and 8.09%) tissue of the fish exposed to cyanide (Table 2). Increased activity of LDH is a characteristic feature of a shift from aerobic to anaerobic metabolism leading to elevated rate of pyruvate conversion into lactate, resulting in lactic acidosis (Abdel-Hameid, 2009). Data from the present study demonstrated a significant increase in LDH activity under hypoxia. Increased LDH activity in liver of Cyprinus carpio was reported by Kamalaveni et al. (2003). Similarly, increased LDH activity in freshwater fish by arsenic treatment was reported by Shobha Rani et al. (2000).
Table 2. Changes in the activity levels of LDH in different tissues of cyanide treated *Cirrhinus mrigala*.

<table>
<thead>
<tr>
<th>Tissues/Duration in days</th>
<th>Liver Mean ± SD</th>
<th>Change (%)</th>
<th>Brain Mean ± SD</th>
<th>Change (%)</th>
<th>Gill Mean ± SD</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.28 ± 0.35</td>
<td>0.39 ± 0.08</td>
<td>1.31 ± 0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.35 ± 0.05</td>
<td>+25.26</td>
<td>0.53 ± 0.15</td>
<td>+35.60</td>
<td>1.42 ± 0.05</td>
<td>+8.09</td>
</tr>
<tr>
<td>14</td>
<td>0.39 ± 0.09</td>
<td>+39.81</td>
<td>0.62 ± 0.08</td>
<td>+58.04</td>
<td>1.56 ± 0.05</td>
<td>+19.13</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 5). The enzyme activity is expressed as µM formozan formed/mg protein/h. Means for organs are significantly different (p < 0.05) from each other.

G6-PDH activity in liver, brain and gill of *C. mrigala* exposed to cyanide was significantly inhibited in both exposure periods compared with the control. This was found to be more pronounced in fish sampled on day 14 of exposure. The maximum reduction in G6-PDH activity was in brain tissue (-22.57%), followed by liver (-18.62%) and gill (-12.87%) (Table 3). Cyanide-induced decrease in G6-PDH activity reflects a reduction in glucose oxidation via pentose shunt and NADPH production for reductive syntheses in cell (Chellan et al., 1999). Similar observations were also made by Tripathi and Verma (2004) in the fish exposed to endosulphan.

Table 3. Changes in the activity levels of G6-PDH in different tissues of cyanide treated *Cirrhinus mrigala*.

<table>
<thead>
<tr>
<th>Tissues/Duration in days</th>
<th>Liver Mean ± SD</th>
<th>Change (%)</th>
<th>Brain Mean ± SD</th>
<th>Change (%)</th>
<th>Gill Mean ± SD</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.81 ± 0.012</td>
<td></td>
<td>0.94 ± 0.096</td>
<td></td>
<td>0.64 ± 0.019</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.73 ± 0.010</td>
<td>-10.28</td>
<td>0.83 ± 0.013</td>
<td>-11.59</td>
<td>0.61 ± 0.016</td>
<td>-4.93</td>
</tr>
<tr>
<td>14</td>
<td>0.63 ± 0.087</td>
<td>-18.62</td>
<td>0.76 ± 0.066</td>
<td>-22.57</td>
<td>0.56 ± 0.087</td>
<td>-12.87</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 5). The enzyme activity is expressed as µM formozan formed/mg protein/h. Means for organs are significantly different (p < 0.05) from each other.

**Conclusion**

The results presented here revealed significant responses in the activities of three selected enzymes SDH, LDH and G6-PDH. These enzymes can be potential biomarkers for early warning of adverse health effects of cyanide on aquatic
animals. The response patterns suggest that these enzymes are induced when fish are exposed to low doses of cyanide, and may have adverse health effects on other aquatic species at fairly low concentrations. Proper treatment and the disposal of cyanide containing wastes are essential for the preservation of a healthy environment. Further comprehensive studies on the chronic toxicity of cyanide to other living creatures and possible effects on human beings are needed.

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References


Influence of cyanide on some antioxidant enzymes of *Cirrhinus mrigala*


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Subletalna toksičnost cijanida na vrstu slatkovodne ribe *Cirrhinus mrigala* je ocenjivana kako bi se odredio njen uticaj na aktivnost nekih antioksidativnih enzima. U tkivima testiranih životinja su uočene promene sukcinat dehidrogenaze (SDH), D-laktat dehidrogenaze (LDH) i glukoza-6-fosfat dehidrogenaze (G6-PDH). Cijanid je značajno smanjio aktivnost SDH i G6-PDH u tkivima jetre, škrga i mozga riba. Povećanje enzima LDH ukazivalo je na metaboličke poremećaje i jasnu reakciju protiv trošenja energije. Promene aktivnosti enzima dehidrogenaza u tkivima riba tretiranih cijanidom su mogle nastati usled promena u oksidativnom metabolizmu. Može se zaključiti da trovanje cijanidom ima veliki uticaj na aktivnost enzima riba, kao što je uočeno u dosadašnjim istraživanjima.

**Ključne reči:** cijanid, enzimi, subletalna toksičnost, *Cirrhinus mrigala.*

**R e z i m e**

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*Autor za kontakt: e-mail: hosetti57@gmail.com*