Enzymatic changes in the kidney and brain of freshwater murrel, *Channa striatus* (Bloch) on short term exposure to sub-lethal concentration of lead nitrate

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ABSTRACT

The effect of short term exposure to sub-lethal concentration (20 ppm) of lead nitrate [Pb(NO₃)₂] on the activities of alkaline phosphatase, acid phosphatase, succinate dehydrogenase and superoxide dismutase in the kidney and brain of the freshwater murrel, *Channa striatus* was studied at 24 and 72 h of exposure. The alkaline phosphatase activity was increased, probably due to the adverse effect of lead nitrate on the glucose re-absorption and trans-phosphorylation. The decline in acid phosphatase activity was associated with the tissue damage caused by lead nitrate toxicity. The inhibition of succinate dehydrogenase activity indicated impairment of oxidative metabolic cycle and dependence on the anaerobic glycolytic pathway to meet the energy demands. The reduction in the superoxide dismutase activity revealed an increased oxidative stress on the nervous tissue leading to the lipid peroxidation and damage of brain.

Keywords: Acid phosphatase, Lead nitrate, Metal toxicity, Succinate dehydrogenase

Introduction

Environmental stressors and the associated risks have always been an inherent part of society (Adeyemo et al., 2008). The emission of anthropogenic pollutants has resulted in long-term ecotoxicological effects in different parts of the world (Ramesh et al., 2009). Aquatic ecosystems are especially sensitive to exposure to toxic contaminants. Pollutants, either individually or in combination may have sub-lethal effects at the cellular, organ and individual level (Adeyemo et al., 2008). Environmental pollutants such as metals pose serious risks to many aquatic organisms by affecting genetic, physiological, biochemical and behavioural parameters (Scott and Sloman, 2004). Among the aquatic habitants, fish is most susceptible to these elemental contaminants and more vulnerable to metal contamination than any other aquatic organism (Alinnor, 2005).

Toxicants in fish are additive as well as synergistic (Depledge, 1978). Anthropogenic contamination of aquatic environment by cadmium and lead has increased substantially in the last several decades with consequent elevation in the tissues of aquatic organisms at all trophic levels (Abdel-Moati and Farag, 1991). This has negative potential alterations in the behaviour, haematology and histology of the organism (Tawari-Fufeyin et al., 2008). Lead is a naturally occurring metal present in the earth’s crust, rock, soil, and water. Most waterborne lead is derived from human activities such as mining and smelting, coal burning, cement manufacturing, and use in gasoline, batteries, and paint (Ramesh et al., 2009). Toxicity of lead in the lung-breathing animals is generally manifested through the contaminated air. In fish, the toxicity of lead is however induced via the gills, their main respiratory organs. Lead as a pollutant induces lipid peroxidation in tissues and causes an irreversible damage to the respiratory organs of fish. The toxic effect of lead is primarily the inactivation of enzymes and proteins via the binding to sulfhydryl groups. It also impairs Ca²⁺ uptake causing ion regulatory damage. These metabolic activities that occur in fish are reflected in changes of the biochemical parameters of various organs and tissues (Lebedeva et al., 1988; 1993). However, very little information is available on changes in enzyme activities caused by lead nitrate. The aim of the present study was to determine the toxicity of lead nitrate [Pb(NO₃)₂] on the activity of the marker enzymes alkaline phosphatase, acid phosphatase, succinate dehydrogenase and superoxide dismutase (ALPase, ACPase, SDH and SOD) in the kidney and the brain of the freshwater murrel, *Channa striatus* and to measure the degree of tissue damage in these organs.

Materials and methods

Healthy specimens of *Channa striatus* (80 ± 4 g body weight, 20 ± 2 cm length) were collected from local paddy
fields, transported to the laboratory and maintained in glass tanks of 100 l capacity containing chlorine free tap water. The fishes were acclimatized in the laboratory condition for a period of two weeks. They were fed with natural food viz., tilapia fingerlings, insects, earthworm etc. The water was renewed after every 24 h, leaving no fecal matter, unconsumed food or dead fish, if any. Feeding was ceased 24 h prior to the commencement of the experiment with the starvation regime continuing throughout the period of the experiment. The physicochemical characteristics of the tap water used in this study were as follows: temperature – 22 ± 2°C; pH-7, dissolved O₂ - 4.48 ± 1.6 ml l⁻¹; alkalinity - 35 ± 2 ppm and CO₂ - 8 ± 2 ppm.

Analytical grade lead (II) nitrate [(Pb(NO₃)₂ (Merck) was used to prepare stock solution (1 mg ml⁻¹) by dissolving in double distilled water. Test concentrations were prepared by diluting appropriate aliquots of the stock solution. LC₅₀ of lead nitrate was determined as per the method of Krouwer and Monti (1995) and confirmed using regression analysis. For this, a preliminary range finding bioassay was conducted, in which increasing concentrations of lead nitrate (i.e., 0, 25, 50, 75 and 100 ppm) were selected and 24 h LC₅₀ value was used to prepare stock solution. LC₅₀ in double distilled water. Test concentrations were prepared by diluting appropriate aliquots of the stock solution.

Results

K. Roy George et al.

The activity of alkaline phosphatase (ALP), acid phosphatase (ACP) and succinate dehydrogenase in the kidney and brain were estimated by the method of Bessey et al. (1946). The superoxide dismutase activity in the brain was estimated by the method of Kakkar et al. (1986).

Table 1. Alkaline phosphatase activity (µg p – nitrophenol liberated µg⁻¹ (30 min) in the brain and the kidney of control and exposed fish.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>24 h (20 ppm)</th>
<th>72 h (20 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>33.16 ± 1.46</td>
<td>36.62 ± 1.47</td>
<td><strong>47.68 ± 2.11</strong></td>
</tr>
<tr>
<td>Kidney</td>
<td>49.74 ± 3.94</td>
<td><strong>85.53 ± 4.8</strong></td>
<td><strong>101.5 ± 5.88</strong></td>
</tr>
</tbody>
</table>

Values are Mean ± S.E; **Highly Significant (p<0.01)

Table 2. The acid phosphatase activity (µg p – nitrophenol liberated µg⁻¹ (60 min) in the brain and the kidney of control and exposed fish.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>24 h (20 ppm)</th>
<th>72 h (20 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>45.58 ± 5.33</td>
<td>42.91 ± 4.92</td>
<td>44.29 ± 4.62</td>
</tr>
<tr>
<td>Kidney</td>
<td>69.64 ± 4.14</td>
<td>62.61 ± 4.02</td>
<td>63.37 ± 4.73</td>
</tr>
</tbody>
</table>

Values are Mean ± S.E

Table 3. The succinate dehydrogenase activity (µg formazan liberated µg⁻¹ (30 min) in the brain and the kidney of control and exposed fish.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>24 h (20 ppm)</th>
<th>72 h (20 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>533.43 ± 5.88</td>
<td><strong>523.72 ± 5.84</strong></td>
<td><strong>300 ± 6.48</strong></td>
</tr>
<tr>
<td>Kidney</td>
<td>420.70 ± 4.79</td>
<td>344.88 ± 4.35</td>
<td><strong>237.99 ± 4.63</strong></td>
</tr>
</tbody>
</table>

Values are Mean ± S.E; **Highly Significant (p<0.01); *Significant (p<0.05)

Table 4. The superoxide dismutase activity (milliunits 100 mg tissue⁻¹) in the brain of control and exposed fish.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>24 h (20 ppm)</th>
<th>72 h (20 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.71 ± 0.03</td>
<td>0.46 ±0.02</td>
<td>0.41 ±0.02</td>
</tr>
</tbody>
</table>

Values are Mean ± S.E; *Significant (p<0.05)

The alkaline phosphatase activity in the brain increased by 10.43% at 24 h and by 43.78% at 72 h of exposure to 20 ppm concentration of lead nitrate. The change was highly significant (p<0.01) at 72 h when compared to control (Table 1). When the fishes were exposed to 20 ppm of Pb(NO₃)₂ for 24 h, the acid phosphatase activity in the brain decreased by 5.85% (~5.85%) and at 72 h also, it slightly decreased (~2.83%) as compared to control (Table 2). The decrease was not statistically significant. In the kidney, the acid phosphatase activity at 24 h of exposure insignificantly decreased by 10.09% and in 72 h by 9% (~9.0%) (Table 2). The succinate dehydrogenase activity in the brain exposed to 20 ppm of Pb(NO₃)₂ for 24 h, decreased by 1.82% (~1.82%) and the change was significant (p<0.05) in comparison to control. In 72 h, the activity decreased by 43.76% (~43.76%) and the change was highly significant (p<0.01) compared to control (Table 3). The superoxide dismutase activity in the brain significantly (p<0.05) decreased by 35.2% (~35.2%) at 24 h and by 42.25% (~42.25%) at 72 h as compared to control (Table 4).

The alkaline phosphatase activity in the brain increased by 67. 93% and at 72 h it increased by 104.06% of the control. The change was highly significant(p<0.01) at 24 h as well as 72 h when compared to control (Table 1). When the fishes were exposed to 20 ppm of Pb(NO₃)₂ for 24 h, the acid phosphatase activity in the brain decreased by 5.85% (~5.85%) and at 72 h also, it slightly decreased (~2.83%) as compared to control (Table 2). The decrease was not statistically significant. In the kidney, the acid phosphatase activity at 24 h of exposure insignificantly decreased by 10.09% and in 72 h by 9% (~9.0%) (Table 2). The succinate dehydrogenase activity in the brain exposed to 20 ppm of Pb(NO₃)₂ for 24 h, decreased by 1.82% (~1.82%) and the change was significant (p<0.05) in comparison to control. In 72 h, the activity decreased by 43.76% (~43.76%) and the change was highly significant (p<0.01) compared to control (Table 3). The superoxide dismutase activity in the brain significantly (p<0.05) decreased by 35.2% (~35.2%) at 24 h and by 42.25% (~42.25%) at 72 h as compared to control (Table 4).

Values are Mean ± S.E; **Highly Significant (p<0.01)
Discussion

Heavy metals produce toxic effects in the tissues of various aquatic and terrestrial animals. The toxic effects of heavy metal in fish are multidirectional and manifested by numerous changes in the physiological and biochemical processes of their body systems (Dimitrova et al., 1994). The heavy metals of principal toxicological concern are lead, mercury and cadmium (Hammond, 1973) which produce cumulative toxic effects if taken in small doses and acute toxicity at higher doses (Harrison et al., 1971). Lead is more toxic due to its long lasting effects on tissues of animals. Sub-lethal toxicity of lead in fish produces enzymatic and neurological effects (Hodson et al., 1984). In the present study, lead nitrate induced alterations in the activities of alkaline phosphatase, acid phosphatase, succinate dehydrogenase and superoxide dismutase in the kidney and brain of the freshwater murrel, Channa striatus was investigated. Kidney and brain are the most sensitive organs easily vulnerable to intoxication by lead compounds.

Alkaline phosphatase is a brush border enzyme, localized in the intestinal mucosa and kidney tubules. It is composed of several isoenzymes that are present in practically all tissues of the body, especially in cell membranes. According to Hickman and Trump (1969), it plays an active role in the re-absorption of glucose from the renal tubules. It catalyses the hydrolysis of monophosphate esters and has a wide substrate specificity. The functional activity of this enzyme was found to increase during exposure to heavy metals, as an adaptive response in mitigating metal toxicity (Vinodhini and Narayan, 2008). A similar situation was observed during the present study with increased activity of alkaline phosphatase in experimental fish exposed to sub-lethal concentration of lead nitrate as compared to the control fish group.

Increased alkaline phosphatase activity in the kidney and brain of C. striatus suggests that glucose re-absorption and trans-phosphorylation reactions catalyzed by this enzyme are adversely affected by treatment with lead (Sastry and Agarwal, 1979). Increased stimulation of alkaline phosphatase has previously been found in such pathological processes as liver impairment, kidney dysfunction and bone disease (Koop and Hetesa, 2000; Yang and Chen, 2003). In contrast with these studies, Sastry and Gupta (1978) reported that lead nitrate (6.8 mg l\(^{-1}\) for 125 days) inhibits alkaline phosphatase activities in the digestive system, especially in the liver of Channa punctatus. This indicates that chronic exposure is more toxic and lead produces cumulative effects. According to Pickering and Henderson (1964), lead toxicity is different at higher concentration and short term treatment than at low concentration and long term treatment.

Acid phosphatase is the marker enzyme of lysosome and exists in a latent form. Stimulation or inhibition of this enzyme can result in the disturbance of metabolism. Changes in the activity of acid phosphatase due to any stress, seems to be a characteristic of tissue damage. In the present study, a slight decrease in the activity of acid phosphatase in brain and kidney of C. striatus was noticed. This inhibition in enzyme activities by heavy metals like lead may be due to the direct binding of the metal with enzyme protein (Passow et al., 1961) or the toxic effects produced by them on tissues (Blackwood et al., 1961) leading to decreased synthesis of enzymes. Hirth (1964) in his in vitro studies has shown the mechanism of enzyme inhibition by heavy metals revolving around the affinity of lead and mercury to the sulfhydryl group.

Succinate dehydrogenase is an enzyme in the Kreb's cycle acting on succinate. It binds to the inner mitochondrial membrane unlike the other enzymes of the cycle which are found in the matrix. The suppression of succinate dehydrogenase activity in kidney and brain of C. striatus indicates impairment of oxidative metabolic cycle and reliance on the anaerobic glycolytic pathway to meet the energy demands (James et al., 1996). James et al. (1991; 1992) found suppression of succinate dehydrogenase activity in Oreochromis mossambicus exposed to sub-lethal doses of heavy metals like copper, zinc and cadmium, and in Heteropneustes fossilis exposed to sub-lethal levels of mercury (0.01 and 0.03 ppm). Inhibition in the activity of succinate dehydrogenase was observed in the digestive system of C. punctatus and H. fossilis, on exposure to sub-lethal concentration of lead nitrate (3.0 and 2.8 mg l\(^{-1}\)) (Sastry and Gupta, 1980).

Superoxide dismutases are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. The reduction in the superoxide dismutase activity reflects an increased oxidative stress on the tissue leading to lipid peroxidation and damage of brain (Farmand et al., 2005). From the results of the present study, it is concluded that lead nitrate is highly toxic and has profound influence on the activities of the tested enzymes in fish. These enzymes could be effectively used as potential biomarkers for metal toxicity to the freshwater fish in the field of environmental monitoring.

Acknowledgements

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Reference


