Clinicopathological and Biochemical Studies on Tilipia Zilli Exposed to Climate change and Cadmium Chloride (0.25p.p.m.)

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Abstract

Heavy metals are recognized as cumulative toxic substances causing serious health hazards to man depending on their concentration. Forty fish (Tilipia Zilli) were collected from Abbassa Sharkia government and fed commercial fish diet. Thirty fish were exposed to cadmium chloride (0.25p.p.m.) and 30°C temp. For 21 days. Ten fish were kept without treatment (control). Haematological analysis of the exposed group demonstrated a marked elevation in serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum glucose, urea, creatinine, sodium, potassium and phosphorus, while serum calcium, haemoglobin and PCV were reduced. Histopathological examination of the fish exposed to cadmium chloride revealed necrobiotic changes of hepatocytes and epithelial lining of renal tubules spleen showed depletion of melanomacrophage centre. Necrosis of the gill filaments was also noticed. It could be concluded that cadmium chloride at 0.25 p.p.m induced deleterious effects in fish such as damage of liver, kidney, spleen and gills, which were reflected on the biochemical and hematological parameters. Heavy metals induced cumulative effect; therefore equivalent lesions of fish may occur in humans. Moreover, immune suppression could play an important role in predisposing for further infections conditions. [Report and Opinion 2009;1(2):80-89]. (ISSN: 1553-9873).

Key words: Pollution – cadmium, fish, immunity.

INTRODUCTION

Heavy metals are persistent contaminants in the environment that come to the forefront of dangerous substances such as cadmium, lead, mercury, copper and zinc causing serious health hazard in humans and animals [1-10]. The agricultural and industrial wastes partially treated or without treatment are being discharged into surface water [11-16]. Such metals are absorbed from polluted water through gills, skin and digestive tract of fish by bio-concentration and bio-magnification. Chronic cadmium toxicity or "itai-itai" disease was recorded [17-20].

Cadmium toxicity was interfered with calcium/phosphorus ratio [21, 22] Suppression of cell mediated and humoral response of mammals exposed to sublethal dose of cadmium has been reported [23-26].

Histopathological examination of fish exposed to cadmium showed edema of secondary gill lamellae, degeneration of hepatocytes and epithelial lining of renal tubules. Degeneration and necrosis in the gill lamellae of fish exposed to cadmium were noticed [13-19].

Heavy metals are recognized as cumulative toxic substances causing serious health hazards to man depending on their concentration.

MATERIALS AND METHODS

Experimental design: Total of forty fish 100-200 gm body weight of each was acclimatized a tized to laboratory conditions for two weeks before use. They were divided into control group (10 fish) and experimental group (30 fish) that was exposed to cadmium chloride at a concentration of 0.25 p.p.m. and 30°C temp. for 21 days.

Blood samples were collected from the
caudal vein after 7 and 21 days of exposure. Serum for biochemical analysis and heparinized blood for hematological investigations were obtained from each sample.

**Biochemical analysis:** Test kits of Bio Merieux (France) were used for evaluation of serum glutamic pyruvic transaminase and glutamic oxaloacetic transaminase [20]. Serum glucose was assessed according to Trinder [23]. Serum urea and creatinine were determined using kits of Bio Merieux (France). The concentration of cadmium, sodium, potassium and calcium were detected by using atomic spectrophotometry according to Forstner[13]. **Hematological examination:** Blood hemoglobin (Rb) was assessed by Drabkin [12]. Hematocrit value was carried out by using microhaematocrit capillary tubes, centrifuged at 1200 r.p.m. for 5 min.

**Bacteriological examination**

Bacterial isolation was done from skin, liver and kidney of fish on blood tryptose agar, MacConkey agar and tryptic soy agar plates. The plates were incubated aerobically and anaerobically. The bacterial isolates were identified morphologically and biochemically, according to Nomiyama [18].

The serum IgM was measured according to Fuda et. al. [15]. Antisera for fish were prepared by immunizing rabbits as previously described by Fuda et.al [15]. The procedure for labeling antibody fragment with enzyme was performed.

Elisa assay procedure: Assay was carried out in 96-well polystyrene ELISA microtiter plates (Titertex, Horsham, P A). The microtiter plates were coated with rabbit antigrey mullet IgM and were fractionated by DE-52 at a concentration of 40 μg/ml in 0.01 MPBS. A volume of 150 μl was dispensed into each well and incubated for 4 hrs at 4°C.

Blocking was achieved after one washing with 200 μl of 0.01 MPBS + 0.1 % 20 μl per well and two washings with 200 μl of PBS +1% PBS. 0.01% thiomerosol was added to each well and incubated for 2 hrs at room temperature.

Incubation of samples and standards after washing was carried out as described above. 100 μl of sample and standard were placed into the appropriate wells in the microtiter plates and incubated at room temperature.

Incubation with peroxidase labeled antibody after washing was done as described above, each well received 150 μl of peroxidase labeled antibody 1:1600 in PBSBSA, followed by incubation for 12 hrs at room temperature.

**Histopathological examination:**

Specimens from gills, liver, Kidney and spleen were collected from both control and exposed groups at the end of experiment. The samples were fixed in 10% neutral buffered formalin. Five-micron thick paraffin sections were prepared and stained with H&E for microscopic examination [27]

Statistical analysis: The obtained data were subjected to the student T test.

**RESULTS**

**Serum biochemical analysis:** Fish exposed to cadmium chloride (0.25 p.p.m) showed a significant increase of SGPT and SGOT activity with pronounced elevation of urea and creatinine by 1st, 2nd, 3rd week of exposure. High level of sodium and potassium in serum of exposure fish was noticed (Table 1).

Hyperglycaemia and hypocalcemia were noticed along the experimental period with marked elevation of serum cadmium (Table 2).

**Haematological profile:** Reduction of Hb concentration and P.C.V value were observed (Table 2).

**Bacteriological examination:** Pure culture of Streptococcus spp., Staphylococcus spp. Agrobacterium spp., Flavobacterium spp. and Lactobacillus spp. were isolated from the internal and external organs of exposed fish (Table 3).

**Determination of fish IgM:** There was a significant decrease in total protein and IgM level from the first week of exposure until the end of last week (Table 4).

**Pathological findings**

Macroscopic lesions of exposed fish revealed congestion in all internal organs after 21 days. Liver appeared friable and dark red. Peritoneal haemorrhages around the operculum, and abdominal cavity were observed. Congestion and edema of gill lamellae were seen (Fig.1).

Histopathological examination revealed necrobiotic changes in hepatocytes and disorganization of hepatic cord (Fig.2). Kidney...
showed shrinkage of glomerular tufts and degeneration of proximal tubular epithelium (Fig. 3). The anterior Kidney showed depletion of melanomacrophage center (Fig. 4). Sloughing of epithelial lining of secondary gill lamellae with lymphocytes, eosinophils, polymorph infiltration were observed (Fig. 5). Necrosis of both primary and secondary gill lamellae was sometimes seen (Fig. 6). Hyperplasia of primary and secondary lamellar epithelium associated with shortening and fusion of gill lamellae with obliteration of interlamellar space were noticed (Fig. 7). Rupture of pillar cells and capillaries associated with lamellar telangiectasis were also observed (Fig. 8).

Table 1: Effect of cadmium chloride .25p.p.m. on kidney and liver function of Tilipia Zilli

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>SGOT U/L</th>
<th>SGPT U/L</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
<th>Sodium Meg</th>
<th>Potassium Meg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week (control)</td>
<td>90.0 ± 0.17</td>
<td>20.7 ± 1.7-5</td>
<td>3.29 ± 0.27</td>
<td>0.76 ± 0.72</td>
<td>124 ± 0.57</td>
<td>4.19 ± 0.07</td>
</tr>
<tr>
<td>1st week of exposure</td>
<td>91.00 ± 2.40</td>
<td>25.5 ± 0.73</td>
<td>3.90 ± 0.34</td>
<td>0.81 ± 0.01</td>
<td>131 ± 0.76</td>
<td>4.60 ± 0.02</td>
</tr>
<tr>
<td>2nd week control)</td>
<td>90.0 ± 0.10</td>
<td>22.1 ± 1.48</td>
<td>3.30 ± 0.28</td>
<td>0.75 ± 30</td>
<td>120.3 ± 4.8*</td>
<td>4.33 ± 0.58</td>
</tr>
<tr>
<td>2nd week of exposure</td>
<td>125 ± 0.45*</td>
<td>29 ± 2.1*</td>
<td>3.91 ± 0.13**</td>
<td>0.90 ± 0.19**</td>
<td>138 ± 0.70**</td>
<td>5.9 ± 0.08</td>
</tr>
<tr>
<td>3rd week (control)</td>
<td>90 ± 2.2</td>
<td>20.00 ± 0.05</td>
<td>3.20 ± 0.27</td>
<td>0.72 ± 0.27</td>
<td>126 ± 4.2</td>
<td>4.1 ± 0.09</td>
</tr>
<tr>
<td>3rd week of exposure</td>
<td>136 ± 2.46*</td>
<td>35 ± 1.56*</td>
<td>4.6 ± 24*</td>
<td>0.99 ± 0.18**</td>
<td>148 ± 7.6</td>
<td>6.25 ± 0.13</td>
</tr>
</tbody>
</table>

* Segnificant P< 0.05  ** highly significant P < 0.01

Table2: Some hematological and biochemical changes in Tilipia Zilli exposed to cadmium chloride.

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>P.C.V %</th>
<th>Hemoglobin gm/dl</th>
<th>Glucose mg%</th>
<th>Cadmium p.p.m</th>
<th>Calcium mg/dl</th>
<th>Phosphonis mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week (control)</td>
<td>21.00 ± 0.06</td>
<td>8.7 ± 0.3</td>
<td>62 ± 1.20</td>
<td>0.05 ± 0.01</td>
<td>5.6 ± 0.32</td>
<td>4.2 ± 0.07</td>
</tr>
<tr>
<td>1st week of exposure</td>
<td>17.9 ± 0.05</td>
<td>8.1 ± 0.01</td>
<td>68 ± 0.46</td>
<td>0.08 ± 0.016</td>
<td>4.00 ± 0.87</td>
<td>6.1 ± 0.21</td>
</tr>
<tr>
<td>2nd week control)</td>
<td>22 ± 0.29</td>
<td>8.1 ± 0.36</td>
<td>60 ± 0.05</td>
<td>0.054 ± 0.068</td>
<td>5.4 ± 0.91</td>
<td>4.1 ± 0.66</td>
</tr>
<tr>
<td>2nd week of exposure</td>
<td>17 ± 1.97</td>
<td>7.00 ± 0.98**</td>
<td>70 ± 1.93**</td>
<td>0.12 ± 1.03*</td>
<td>4.2 ± 0.73*</td>
<td>6.4 ± 0.12*</td>
</tr>
<tr>
<td>3rd week (control)</td>
<td>20.0 ± 1.32</td>
<td>8.1 ± 0.07</td>
<td>62.2 ± 0.70</td>
<td>0.04 ± 0.01*</td>
<td>4.4 ± 0.73</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>3rd week of exposure</td>
<td>16.9 ± 0.8</td>
<td>6.91 ± 0.23*</td>
<td>84 ± 0.02*</td>
<td>0.16 ± 082*</td>
<td>3.5 ± 0.88*</td>
<td>6.1 ± 0.6*</td>
</tr>
</tbody>
</table>

* Segnificant P< 0.01  ** highly significant P < 0.05
Table 3: Bacteriological recovered in Tilapia Zilli exposed to cadmium chloride (0.25 p.p.m).

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>External surface</th>
<th>Internal organs</th>
<th>Internal organs liver</th>
<th>Gills</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrobacterium spp.</td>
<td>4.1x10^7</td>
<td>3.8x10^6</td>
<td>3x10^4</td>
<td>5x10^7</td>
</tr>
<tr>
<td>Flavobacterium spp.</td>
<td>7x10^7</td>
<td>6.2x10^6</td>
<td>6x10^3</td>
<td>7.3x10^8</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>6x10^5</td>
<td>5x10^4</td>
<td>6.2x10^3</td>
<td>4.2x10^6</td>
</tr>
<tr>
<td>Streptococcus SPP.</td>
<td>5x10^7</td>
<td>9x10^5</td>
<td>7x10^6</td>
<td>3x10^7</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>3.3x10^3</td>
<td>4.4x10^6</td>
<td>2x10^3</td>
<td>2x10^6</td>
</tr>
</tbody>
</table>

Table 4: Influence of cadmium chloride 0.25 p.p.m on IgM and protein level.

<table>
<thead>
<tr>
<th>Exposure period</th>
<th>IgM/old</th>
<th>Total protein/μg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.98 ± 0.13</td>
<td>7.84 ± 0.23</td>
</tr>
<tr>
<td>1st week of exposure</td>
<td>0.80 ± 0.23**</td>
<td>7.00 ± 0.69*</td>
</tr>
<tr>
<td>2nd week of exposure</td>
<td>0.74 ± 0.84*</td>
<td>6.42 ± 0.29*</td>
</tr>
<tr>
<td>3rd week of exposure</td>
<td>0.68 ± 0.44*</td>
<td>6.2 ± 0.48*</td>
</tr>
</tbody>
</table>

* Significant P < 0.01       ** highly significant P < 0.05   ± Standard errors

Fig. 1: Congestion of all internal organs of exposed fish.
Fig. 2: Liver showing necrobiotic changes of hepatocytes (H&E X 400).

Fig. 3: Kidney showing shrinkage of glomerular tufts and degeneration of tubular epithelium (H&E X 400).
Fig. 4: Spleen showing depletion of melanomacrophage center and haemopoietic elements (H&E X 100).

Fig. 5: Sloughing of epithelial lining of secondary gill lamellae (H & E X 100).
Fig. 6: Hyperplasia and fusion of secondary gill lamellae (H & E X 200).

Fig. 7: Necrosis of both primary and secondary gill lamellae (H & E X 200).
DISCUSSION

Aforementioned data of exposed fish to cadmium chloride (0.25 p.p.m) for 3 weeks revealed an elevation of serum GPT, GOT, urea and creatinine.

These findings are in agreement with previous results. Elevation of urea and creatinine beside liver enzymes in cadmium-exposed fish may be attributed to liver and kidney injury. Reduction of calcium level in serum may have resulted from its increased excetration in urine through inhibition of calcium ATPase enzyme. On the other hand, increase of the phosphorus level in serum of exposed fish was noticed. Cadmium chloride toxicity leads to disturbance in blood electrolytes followed by skeletal changes [23, 25]. Hyperglycemia was observed in the present work which coincides with that obtained in Rainbow traut and salmogaidneri [25]. The blood glucose level was affected by the rate of carbohydrate metabolism under hypoxia and stress conditions. Hyperglycemia is attribute to stress stimuli followed by rapid secretion of both glucocorticoids and α-techoharnines from the adrenal tissue [2]. Regarding to hematological profile exposed fish, hemoglobin and P.C.V. values were decreased. These results are in agreement with previous findings [17, 18]. The erythopenia resulted from reduction of Hb concentration and P.C.V. value in Kwait Mullet due to disturbance of osmoregulatory mechanism accompanied with destruction of gill membrane and failure of gas exchange [19]. Cadmium interfered with sulpha-hydride groups of essentials enzymes [4, 25]. Heavy metals are recognized as cumulative substances leading to serious health hazards to man and animals [6-9].

In the present study, a significant decrease of IgM and total protein during the experimental period were observed. Reduction of IgM level indicated that the cadmium chloride toxicity leads to suppression of immune system of exposed fish which become susceptible to any infective agents [15, 20]. There is a significant decrease in IgM level in fish exposed to cadmium chloride if compared with control which may have resulted from high cortisol secretion that was indicated by hyperglycemia in exposed fish.

Macroscopical examination of fish exposed to cadmium chloride for 21 days revealed a congestion of all internal organs and friable bloody liver. These findings are in agreement with those mentioned by other authors [1-9].
Degeneration and necrosis of hepatocytes may be attributed to the cumulative effect of cadmium and to the increase of its concentration in the hepatic tissue during experimental period. These results agreed with Frolin et al. [14], who stated that liver has an important detoxical role of exogenous waste products as well as externally derived toxins such as heavy metals.

Necrobioitic changes of epithelial lining of renal tubules were observed especially the proximal convoluted tubules that were reflected on electrolytes reabsorption such as calcium, phosphorus, potassium and sodium. These findings come parallel to those previously reported.

It could be concluded that cadmium chloride at 0.25 p.p.m induced deleterious effect in fish such as damage of liver, kidney, spleen and gills, which were reflected on the biochemical and hematological parameters. Heavy metals induced cumulative effect; therefore equivalent lesions of fish may occur in humans. Moreover, immune suppression could play an important role in predisposing for further infections conditions.

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