

Clinicopathological, Biochemical and Microbiological Change on Grey Mullet Exposed to Cadmium Chloride

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Abstract: Forty fish grey mullet (*mugilcephalus*) were collected from Abbassa Sharkia government and fed commercial fish diet. Thirty fish were exposed to cadmium chloride (0.25 ppm) 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.

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 were acclimatized A sized 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 ppm 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, MacConcky 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 labelling 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 was fractionated by DE-52 at a concentration of 40 µg/ml in 0.01 MPBS. A volume of 150µl 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 labelled antibody after washing was done as described above, each well received 150 µl of peroxidase labelled antibody 1:1600 in PBSBSA, followed by incubation for 12 hrs at room temperature.

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

RESULTS

Serum Biochemical Analysis: Fish exposed to cadmium chloride (0.25 ppm) 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).

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

Exposure time	SGOT U/L	SGPT U/L	Urea mg/dl	Creatinine mg/dl	Sodium Meg	Potassium Meg
1 st week (control)	90.6±0.013	20.4±1.73	3.9±0.27	0.78±0.73	120±0.58	4.29±0.07
1 st week of exposure	93.0±1.20	24.3±0.63	3.7±0.34	0.83±0.017	130±0.76	4.50±0.03
2nd st week control)	91.00±0.15	23.2±1.43	3.0±2.29	0.75±30	120.3±4.8	4.2±0.57
2nd st week of exposure	123±0.18*	29.7±2.19**	3.90±1.14**	0.90±0.19*	138±0.70	5.97±0.08**
3 rd week (control)	93±1.2	29±0.05	3.20±0.29	0.62±0.27	123±3.3	4.00±0.09
3 rd week of exposure	139±2.39*	34±1.04*	4.8±0.24*	0.97±0.02*	149±7.9	6.05±913*

*Segnificant P<0.05 ** highly significant P <0.0

Table 2: Some hematological and biochemical changes in fish exposed to cadmium chloride

Exposure time	P.C.V %	Hemoglobin gm/dl	Glucose mg%	Cadmium p.p.m	Calcium mg/dl	Phosphonis mg/dl
1 st week (control)	21.00±0.07	8.9±0.4	65±1.21	0.060±0.12	5.8±0.33	4.37±0.07
1 st week of exposure	18.8±0.05	8.1±0.02	69±0.47	0.09±0.016	4.00±0.88	5.00±0.23
2nd st week control)	23.00±0.29	8.2±0.37	60±0.05	0.055±0.067	5.5±0.92	4.00±0.66
2nd week of exposure	18.00±1.98**	7.1±2.99**	70±1.94**	0.12±1.94*	4.2±0.84*	6.4±0.080**
3 rd week (control)	21.9±1.45	8.2±0.08	62±0.71	0.06±0.023	4.4±0.74	3.98±0.1
3 rd week of exposure	16.9±0.82*	6.9±2.5*	85±0.03*	0.17±0.85**	3.6±0.85*	6.2±0.05*

* Segnificant P< 0.05 ** highly significant P <0.01 ± Standard errors P.C.V. = Packed cell volum

Table 3: Bacteriological recovered in fish exposed to cadmium chloride (0.25 pp m)

Bacterial strain	External surface	Internal organs	Internal organs liver	Gills
<i>Agrobacterium spp.</i>	31×10 ⁷	28×10 ⁶	2×10 ⁴	4×10 ⁸
<i>Flavobacterlum spp.</i>	6×10 ⁷	6.2×10 ⁶	6×10 ³	63×10 ⁸
<i>Staphylococcus spp.</i>	6×10 ⁵	5×10 ³	6.2×10 ²	4.2×10 ⁵
<i>Streptococcus SPP.</i>	5×10 ²	9×10 ⁴	7×10 ⁵	3×10 ⁵
<i>Lactobacillus spp.</i>	3.3×10 ²	4.4×10 ⁵	2×10 ³	2×10 ⁵

Table 4: Influence of cadmium chloride 0.25 ppm on IgM and protein level

Exposure period	IgM/old	Total protein/neg/dl
Control	0.98±0.13	8.84±0.23
1 st week of exposure	0.80±0.23**	7.20±0.69
2 nd week of exposure	0.74±0.84*	6.52±0.29
3 rd week of exposure	0.68±0.44 *	6.3±0.48

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

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).

DISCUSSION

Aforementioned data of exposed fish to cadmium chloride (0.25 ppm) for 3 weeks revealed a 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 excretion 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 trout and salmogaideri [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 "-techolamines 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 erythropenia 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 sulphahydride

groups of essential 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.

Necrobiotic 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 ppm 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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