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Accumulation of Cadmium Chloride and its Effect on Biochemical Parameters of Liver in Freshwater Fish, *Channa punctatus* (Snakeheaded)

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ABSTRACT

Cadmium chloride is a toxic heavy metal occurring in the environment as a pollutant that is derived from agricultural and industrial wastes. In the present study *Channa punctatus* (Bloch) was exposed to different sub-lethal concentrations of cadmium chloride to know the cadmium chloride accumulation in liver and effect on biomolecules. The results indicated that the accumulation of cadmium chloride in the liver increased with respect to increase in concentration and time of exposure. Among the bio chemicals there was an elevation in the level of blood glucose and depletion in level of protein and glycogen content with respect to exposure time and concentration of cadmium chloride.The present study conveys that the presence of heavy metal ions in water bodies induces stress to the fishes and alter the physiological process leading to toxic effects on fishes and leading to imbalance in the aquatic community.

Keywords Cadmium chloride, *Channa punctatus,* Biomolecules, Accumulation, Liver.

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INTRODUCTION

Channa punctatus (Bloch) is a freshwater common snake-headed murrel belonging to the family Channidae of the order Perciformes. Channa punctatus, have wide geographical distribution, they are bottom-dwelling habits, having ability to respond to environmental pollutants and serve as an importance as an economic food source (Nagpure *et al.* 2012). Cadmium chloride is widely used in industries along with the other heavy metals (Novelli *et al.* 2000) which imparts a wide range of physiological effects on fish and aquatic organisms (Iger *et al.* 1994). The metal accumulation in fish has led to damage to the organ structure (Giar *et al.* 2007). Chronic sub-lethal exposure to cadmium chloride leads to accumulation in the fish kidneys, liver and gills (Hollis *et al.* 1999, McGeer *et al*. 2000)which are different from one tissue type to other (Lange *et al*. 2002) and these tis-

sues have been identified as the main storage sites for Cadmium (Kim *et al.* 2004). Biochemical parameters are also sensitive for metal accumulations detecting the potential adverse effects which affects the activities of various enzymes which are considered to be sensitive biochemical indicators before hazardous effects of toxicants (Gul *et al*. 2001). There are studies which coveys that in fish blood glucose level shows significant alterations with response to the change in environmental factors (Chen *et al.* 2004, Jarup and Akesson 2009) and even be affected under toxic stress, which reflects on variations in carbohydrate metabolism (Joseph 2009). Ashrafizadeh *et al*. (2018) Reports decrease in liver glycogen and an increase in blood glucose levels in *Cyprinus carpio* on exposure to lead nitrate. In the present investigation an effort was made to estimate the amount of cadmium chloride accumulation in liver of *Channa punctatus* (Bloch) and to know the effect of sub-lethal exposure of cadmium chloride to and bio chemicals Blood glucose (BG), Glycogen (GLY), Lactate (LAC) and Pyruvate (PYR).

MATERIALS AND METHODS

Experimental fish

Channa punctatus (Bloch) were collected from the river streams and dams of Thippagondanahalli and Manchanbele comes under Arkavathi River with the help of local fisher men Bengaluru, south taluk, Bengaluru District. Fish were acclimatized to laboratory conditions for two weeks prior to experiments. Seven twenty fishes, weighing 36.45 ± 1.12 g, were divided into twelve groups (n=10). For the present study, technical-grade Cadmium chloride, monohydrate, AR ($CdCl_2$; 98.0% EC, maximum limits of impurities, iron 0.0005% and sulfate 0.005%) manufactured by Hi Media Laboratories Pvt Ltd Mumbai, India, was procured and used for the study. 1.8274 g/l was dissolved in double distilled water to obtain 1000 ppm stock solution and further this Stock solution was diluted to 5, 10, 15 ppm concentrations and were exposed for 24, 48, 72 h. The 10 to 12, unexposed, group served as the control. Glass aquaria with a capacity of 80 L (100 × 50 × 40 cm) were used, for each concentration. The water was changed once a week to maintain the environmental conditions and $CdCl_2$ concentrations (Proenca and Bittencourt 1994). Dissolved oxygen was added with diffused air at the top of the biological filter. Fish were fed twice daily with a 32% crude protein diet at a rate of 2% of body weight.

Tissue analysis

After the treatments the fishes were sacrificed and dissected, liver was collected and freeze-dried. Later the liver was washed in fresh water three times and rinsed in double distilled water and was blotted with dry filter paper. Tissues were dried overnight at 80 C, approx. 0.3 g dry tissue was weighed and digested. The digested solutions were diluted with double distilled water and subjected to atomic absorption spectrophotometry. The tissue digestion was conducted according to the method described by Allen (1989) Cadmium chloride concentration was measured using an Atomic Absorption spectrometer (Varian – Spectra, 220 FS).

Glycogen

Glycogen content in the liver was estimated using the anthrone reagent method as described by Caroll *et al.* (1956). The organs were digested with 3 ml of hot 30% potassium hydroxide. The digested solution was cooled and 3.75 ml of absolute ethanol was added to it. The entire mixture was left overnight in a refrigerator. Then the mixture was centrifuged for 15 min at 2500 rpm. Supernatant was decanted and 10 ml of warm distilled water was added to the residue to dissolve the precipitated glycogen. To 0.2 ml of this 1.8 ml of distilled water and 0.5 ml of 2 % anthrone reagent dissolved in 72 % concentrated sulfuric acid were added and heated in a boiling water bath exactly for 10 minutes. The mixture was cooled and the optical density of the color developed was measured in a spectrophotometer at a wavelength of 620 nm. A blank and glucose standard were also run similarly. The glycogen content is expressed as mg of glycogen/g wet wt of the organ.

Blood glucose

Glucose in the samples was determined by colorimetric method as described by Nelson and Som-

ogyi(1952) of blood was collected and 3.9 ml of deproteinizing solution (5% zinc sulfate and 0.3 N sodium hydroxide in 1:1 ratio) was added and the mixture was centrifuged at 3000 rpm for 10 min. To 1 ml of the supernatant from each of these mixtures 1 ml of alkaline copper reagent was added, shaken vigorously and heated in a boiling water bath exactly for 20 min. Then it was cooled and 1 ml of arseno molybdate color reagent was added. Entire solution was made to 10 ml with distilled water and the absorbance was measured in a spectrophotometer at a wavelength of 540 nm. A blank and glucose standards were also run simultaneously. Glucose content was expressed as mg of glucose/100 ml of blood.

Protein

About 500 mg of liver sample was homogenized in 10 ml of 0.85%, chilled KCI in a homogenizer, 1ml of this homogenized mixture was added to1.0ml of 0.1N NaOH. The solution was digested for 10 minutes in a boiling water bath and was cooled the mixture was centrifuged for 10 min at 4000 rpm. The supernatant was diluted to 25 ml with 1.0 N NaOH. From this test solution 0.2, 0.5, 1.0 ml aliquots were taken as test sample. The volume in all the test tube was made up to 1ml with 0.1 N NaOH. Then 5 ml of reagent C (alkaline copper sulfate) was added to all the test tube followed by through mixing and left for 10 minutes. This was followed by rapid addition of 0.5 ml Folin's reagent followed by immediate mixing. The optical density was measured at 660 nm black was also prepared by adding all the reagents to 1ml of distilled water of the test sample simultaneously color development in standard protein solution (0.2mg/ml) for the calibration curve was carried out. The observations were plotted on a graph. Tissue protein values were expressed as mg/ml (Lowry *et al.* 1951).

Statistical analysis

The data obtained were statistically analyzed. One-way analysis of variance (ANOVA) was applied to compare the means obtained for different parameters among different tissues, concentrations and durations. A p-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Cadmium chloride accumulation in the liver

The highest amount of Cadmium chloride residue was found in the liver exposed to 15 ppm for 72 hours (µg/g dry weight). Significant differences ($p < 0.05$) between the treatments and control were recorded. In case of cadmium chloride exposed to 24 h at 5, 10, 15 ppm, 0.4±0.7, 0.6±0.6, 0.9±0.7 µg/g dry weight was recorded respectively. In case of 48 h of exposure an accumulation of 0.7±1.1, 1.1±1.2, 1.7±1.4 µg/g dry weight was recorded in 5, 10, 15 ppm concentration respectively. Whereas, in case of 72-h exposure 1.6±1.1, µg/g dry weight 3.2±2.1 µg/g dry weight, 4.1±1.3 µg/g dry weight was recorded (Table 1). Many studies have investigated Cadmium salts accumulation and distribution among organs. The distribution of accumulated Cadmium salts in organs differs between these studies, however the inconsistencies in these studies may be due to the differences in the Cadmium concentrations and exposure times used (Wu *et al.* 1999). Many studies on fish have demonstrated that the distribution of Cd is tissue specific and depends on the exposure route, as reported by Guinot *et al*. (2012), Nasser *et al*. (2015). The results in the present study are in accordance with data obtained by Filipovic *et al.* (2006) who argued that after long term exposure metals are transferred to storage organs such as the liver or kidney.

Blood glucose (BG)

From the studies it was noted that exposure of the Cadmium chloride elevated the levels of blood glucose. The data presented in Table 2 and it revealed compared to control, blood glucose level increased in all exposure (5,10,15 ppm). Maximum increase in blood glucose was observed in 15 ppm concentration

Table 1. Cadmium chloride (µg/g dry weight) in liver of *Channa punctatus* (Bloch). Mean ± SD: Values are compared with control at $p \leq 0.05$.

Concentration	5 ppm	10 ppm	15 ppm	Control
24 h	0.4±0.7a	0.6±0.6a	0.9±0.7a	0.11±0.8
48 h	0.7±1.1a	1.1±1.2a	1.7±1.4a	0.43±1.2
72 h	1.6±1.1a	3.2±2.1a	4.1±1.3a	0.92±1.6

Table 2. Biochemical parameters in the liver tissues of fish, *Channa punctatus* (Bloch) on exposure to the Cadmium Chloride at different concentrations and time of duration. *1. Significant when compared with control at p ≤0.005. 2. Values with different alphabet (lowercase) superscripts differ significantly between exposure durations. 3. Values with different numeric superscripts differ significantly between concentrations.

Tissue	Concentration	Cadmium chloride											
		5 ppm			10 ppm			15ppm			Control		
	Hours of exposure	24	48	72	24	48	72	24	48	72	24	48	72
Liver	Glucose (mg/100 ml)	75.61 ± 1.51*^{a1}	76.23 ± 1.26*^{a2}	77.14 ± 2.15*^{a3}	78.91 ± 0.73*^{b1}	79.32 ± 1.29*^{b2}	80.81 ± 2.32*^{b3}	81.23 ± 0.83*^{c1}	82.12 ± 1.24*^{c2}	83.91 ± 1.46*^{c3}	70.50 ± 0.73	71.42 ± 1.87	71.87 ± 0.52
	Protein mg/ml	51.43 ± 0.91*^{a1}	52.31 ± 1.28*^{a2}	54.25 ± 1.12*^{a3}	47.26 ± 2.11*^{b1}	46.88 ± 0.78*^{b2}	45.54 ± 1.31*^{b3}	37.13 ± 0.85*^{c1}	36.36 ± 1.43*^{c2}	35.97 ± 2.11*^{c3}	57.65 ± 0.56	58.36 ± 0.78	58.71 ± 0.52
	Glycogen (mg/g wet wt)	1.92 ± 0.56*^{a1}	1.87 ± 0.95*^{a2}	1.81 ± 1.23*^{a3}	1.79 ± 1.78*^{b1}	1.75 ± 1.34*^{b2}	1.72 ± 1.68*^{b3}	1.69 ± 1.56*^{c1}	1.62 ± 0.87*^{c2}	1.59 ± 0.76*^{c3}	1.87 ± 2.17	1.93 ± 1.54	1.98 ± 0.67

exposed for 48 and 72 h. In case of exposure for 24, 48,72 h at 5 ppm the blood glucose level ranged from 75.61 to 77.14 mg/100 ml in 10 ppm exposure 78.91, 80.81 and 82.12 mg/100ml was recorded at 24, 48, 72 h of exposure respectively. Similar trends were also followed in 15 ppm exposure 81.23, 82.12, 83.91 mg/100ml was recorded respectively. The elevation in blood glucose level in observed in the studies may be due to toxic metallic and this hyperglycemic condition may be due to stepping up of glycogenolysis or gluconeogenesis (Buha *et al.* 2019). The results were in accordance with the studies conducted by Souid *et al.* (2013) which showed hyperglycemic condition and decrease in the glycogen content of liver and Kidney tissue in *Channa punctatus* under monocrotophos toxicity.

Glycogen (GLY)

Glycogen level in all the three exposure (24, 48, 72 h) at 5,10,15 ppm showed continuous decrease of the glycogen level when compared to control. Lowest glycogen level was observed in 15 ppm exposure for 72 and 48 h (1.62 and 1.59 mg/g wet weight) however the highest quantity of glycogen (1.98 mg/g wet wt) was observed in un exposed liver (control). From the data presented in Table 2 the decrease in the level of glycogen was observed with increase in concentration and exposure time. In the present study the decrement of the glycogen was observed by utilization of anaerobic glycolysis, to meet the energy warranted by the toxic environment (Tinkov *et al.* 2018). In support of this, Suryakant *et al.* (2021) reported a decrease in glycogen content in the tissues of fish, *Tilapia mossambica* exposed to methyl parathion. Bharti and Rasool (2021) also observed the decrement of glycogen with corresponding increase in blood glucose level in the fish, *Labeo rohita* under cypermethrin stress.

Protein

The level of protein after the exposure of the Cadmium chloride the level of protein was decreased when compared to control. The data presented in Table 2 and it revealed compared to control, protein content decreased in all exposure (5,10,15 ppm). Maximum decrease was observed in 15 ppm concentration exposed for 48 and 72 h. In case of exposure for 24, 48,72 h at 5 ppm the protein level ranged from 51.43 to 54.25 mg/ml. In 10 ppm exposure 47.26, 46.88, 45.54 mg/ml was recorded at 24, 48, 72 h of exposure respectively. Similar trends were also followed in 15 ppm exposure 37.13, 36.36, 35.97 mg/ml was recorded respectively. In the present study Alterations in the muscle protein content was observed in fishes exposed to the metal toxicants. Depletion in tissue protein found in the present studies may be due to increased energy loss of homeostasis, tissue repair and detoxification during stress (Tinkoy *et al.* 2018)

and plasma dissolution and renal damage caused due to a decrease in liver protein synthesis. These present findings are in fairly good agreement with the previous reports of decreased level of soluble protein and RNA content in the liver (Souid *et al.* 2013).

CONCLUSION

Experimental study indicate that cadmium chloride is widely used heavy metal ion in industries the exposure of this metal ion induces various physiological abnormalities in freshwater fish. In the present studies the exposure of different concentrations of Cadmium chloride led to accumulation of Cadmium chloride in the liver and the accumulation increased with respect to increase in concentration and time of exposure. Among the bio chemicals there was an elevation in the level of blood glucose and depletion in level of protein and glycogen content with respect to exposure time and concentration of Cadmium chloride.

REFERENCES

Ashrafizadeh M, Rafiei H, Ahmadi Z (2018) Histological changes in the liver and biochemical parameters of chickens treated with lead acetate II. *Iran J Toxicol* 12(6):1-5.

Allen SE (1989) Chemical Analysis of Ecological Materials. 2nd edn.Blackwell Scientific Publications, Oxford.

Bharti S, Rasool F (2021) Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on *Channa punctatus* (Bloch) fish. *Toxicol Reports* 8:443-455.

Buha A, Jugdaohsingh R, Matovic V, Bulat Z, Antonijevic B, Kerns JG, Goodship A, Hart A, Powell JJ (2019) Bone mineral health is sensitively related to environmental cadmium exposure-experimental and human data. *Environ Res* 176 : 108-539.

Caroll NV, Longley RW, Row JH (1956) Glycogen determination in liver and muscle by use of anthrone reagent. *J Biol Chem* 22:583-594.

Chen C, Wooster GA, Bowser PR (2004) Comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin or copper sulfate. *Aquaculture* 239 : 421–443.

Choudhury C, Mazumder R, Biswas R, Sengupta M (2021) Cadmium exposure induces inflammation through the canonical NF-κB pathway in monocytes/macrophages of *Channa punctatus* Bloch. *Fish Shellfish Immunol* 1 (2) : 110-116.

Filipovicmarijic V, Raspor B (2006) Age-and tissue dependent metallothionein and cytosolic metal distribution in a native Mediterranean fish, *Mullus barbatus,* from the Eastern Adriatic Sea. *Comp Biochem Physiol* 143: 382–387.

Giari L, Manera LM, Simoni E, Dezfuli BS (2007) Cellular alterations in different organs of European sea bass *Dicentrarchus labrax* (L.) exposed to cadmium. *Chemosphere* 67: 1171–1181.

Guinot D, Urena R, Pastor A, Varo I, Ramo J, Torreblanca A (2012) Long-term effect of temperature on bioaccumulation of dietary metals and metallothionein induction in *Sparus aurata. Chemosphere* 87: 1215–1221.

Gul S, Belge-Kurutas E, Yildiz E, Sahan A, Doran F (2004) Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake.*Turkey Environ Int* 30: 605–609.

Hollis L, McGeer JC, McDonald DG, Wood CM (1999) Cadmium accumulation, gill Cd binding, acclimation and physiological effects during long term sub-lethal Cd exposure in rainbow trout. *Aqua Toxicol* 46: 101–119.

Jarup L, Akesson A (2009) Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol* 238 (3): 201-208.

Joseph P (2009) Mechanisms of cadmium carcinogenesis. *Toxicol Appl Pharmacol* 238 (3): 272- 279.

Kim SG, Jee JH, Kang JC (2004) Cadmium accumulation and elimination in tissues of juvenile olive flounder, *Paralichthys olivaceus* after sub-chronic cadmium exposure. *Environ Pollut* 127: 117–123.

Kraal MH, Kraak MHS, DeGraat CJ, Davids C (1995) Uptake and tissue distribution of dietary and aqueous cadmium by carp (*Cyprinus carpio*). *Ecotoxicol Environ Saf* 31 (2): 179–183.

Lange A, Ausseil O, Segner H (2002) Alterations of tissue gulathione levels and metallothionein mRNA in rainbow troutduring single and combined exposure to cadmium and zinc. *Comp Biochem physiol Pharmacol Toxicol* 131: 231–243.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin-phenol reagent. *J Biol Chem*193: 265-275.

McGeer JC, Szebedinszky C, McDonald DG, Wood CM (2000) Effects of chronic sub-lethal exposure to waterborne Cu, Cd or Zn in rainbow trout. Ion-regulatory disturbance and metabolic costs. *Aqua Toxico*l 50: 231–243.

Nagpure NS, Dabas A, Kumar R, Kushwaha B, Kumar P (2012) Assessment of tissue specific effect of cadmium on antioxidant defense system and lipid peroxidation in fresh water murrel *Channa punctatus* 38:469-482.

Nasser AA, Abdel WA, Abdel W, Sayed MYH, Allam Y (2015) Haematological and biochemical parameters and tissue accumulations of cadmium in *Oreochromis niloticus exposed* to various concentrations of cadmium chloride. *Saudi J Biol Sci* 22(5):543-550.

Nelson N, Somogyi N (1952) A Photometric adaptation of Somogyi method for the determination of glucose. *J Biol Chem* 195:19-23.

Novelli Filho JLVV, Novelli ELB, Manzano MA, Lopes AM, Cataneo AC, Barbosa LL, Ribas BO (2000) Effect of tocopherol on superoxide radical and toxicity of cadmium exposure. *Int J Environ Hlth Res*10: 125–134.

Proenca CEM, Bittencourt PRL (1994)Manual de pisciculture tropical. Brasý´lia: Ibama, pp 78.

Souid G, Souayed N, Yaktiti F, Maarouf K (2013) Effect of acute cadmium exposure on metal accumulation and oxidative stress biomarkers of *Sparus aurata*. *Ecotoxicol Environ Saf* 89: 1–7.

Suryakant N, Lin M, John TM, Levine EK, Fernando RA, Kumar D (2021) Role of Autophagy in Cadmium induced hepatotoxicity and liver diseases. *J Toxicol,* pp1-14.

Tinkov AA, Gritsenko VA, Skalnaya MG, Cherkasov SV, Aaseth J, Skalny AV (2018) Gut as a target for cadmium toxicity. *Environ Pollut* 235: 429–434.

Wu SM, Weng CF, Yu MJ, Lin CC, Chen ST, Hwang JC, Hwang PP (1999) Cadmium-inducible metallothionein in tilapia *Oreochromis mossambicus. Bull Environ Contam Toxicol* 62: 758–768.