Volume : 3 | Issue : 12 | December 2014 • ISSN No 2277 - 8179

Research Paper

Acute And Chronic Toxicity of Cadmium to Male *Clarias Batrachus* Linn. With Special Reference to Their Haematological Changes

Biology

KEYWORDS : *Clarias batrachus*, cadmium, LC50, ethology, haematology.

Mr. Kishore Dhara	Deputy Director of Fisheries, Gour Banga Zone, Govt. of West Bengal, Meen Bhavan, Mangal Bari, Malda-732142, India.
Mr. Dip Mukherjee	Part Time Lecturer, Post Graduate Department of Zoology, Maulana Azad College, 8, Rafi Ahmed Kidwai Road, Dharmatala, Kolkata – 700013, India.
Dr. Nimai Chandra Saha	Additional Director of Public Instruction, Govt. of West Bengal, Education Directorate, Bikash Bhavan, Salt Lake, Kolkata-700091, India.

ABSTRACT

The present study was done to evaluate the acute and chronic toxicity of cadmium to Clarias batrachus along with its effects on ethological and haematological changes of fish. 96h median lethal concentrations (LC50) of cadmium chloride along with its 95% confidence limits was 82.66 (75.09-90.54) mg/l. Excessive mucous secretion in the body surface, signs of stress like erratic fin movement, hyperactivity, difficulty in breathing, suffocation and surface attachment of fish followed by death were recorded in the higher concentrations (82.5, 92.5 and 95.0 mg/l) during 72 and 96h of exposure. During chronic toxicity tests, total erythrocyte count (TEC), haemoglobin (Hb) content, haematocrit (Hct) percent, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), blood triglyceride, cholesterol, serum albumin, serum globulin and serum albumin-globulin ratio of Clarias batrachus varied significantly with increasing exposure time (60 and 90 days) at all sublethal concentrations of cadmium (4.14 and 8.27 mg/l).

Introduction

Cadmium occurs naturally in the environment in insignificant amounts but its release to the environment is steadily increasing due to anthropogenic activities causing pollution of soil and aquatic ecosystems (Gad, 2005).

The concentration of cadmium in unpolluted fresh waters is generally less than 0.001 mg/l (Fleischer et al., 1974; Friberg et al., 1974; Hiatt & Huff, 1975); the concentration of cadmium in seawater averages about 0.00015 mg/l (Fleischer et al., 1974; Hiatt & Huff, 1975). Unfortunately, still higher concentrations of cadmium than the permissible limit have been reported by some workers in different water bodies. Blood physiology is a vital index to monitor health status of a number of fish species; scanty reports are available on the haematological changes of fish following exposure to cadmium (Kori-Siakpere et al., 2006). The use of haematological parameters as indicators of stress to toxic substances can provide information on the physiological response of fish to the changing surroundings (Kori-Siakpere & Ikomi, 2011). The cadmium entering the aquatic ecosystem may not directly impart toxicity to the organisms being in low concentrations; however it can be accumulated in aquatic organisms through bio-concentration, bioaccumulation and the food chain process (Mauskar, 2007; Jayakumar & Paul, 2006; Omer et al., 2012). The transport of cadmium in the blood is mediated by the erythrocytes and albumin. Finally, it is stored mainly in the liver and kidneys as metallothionein (Gad, 2005).

In the present investigation, attempts were made to determine 96h median lethal concentrations (LC_{50}) of cadmium to freshwater fish, *Clarias batrachus* along with its behavioural responses. The study was also conducted to determine the chronic effects of cadmium at sublethal doses to fish for developing a more mechanistic understanding through haematological changes. This study will also help to design environmental monitoring strategies and ecosystem conservation measures.

Materials and methods

Test organisms used in the bioassay comprised of adult male *Clarias batrachus* (mean length 17.11 ± 0.96 cm, mean weight 59.90 ± 7.01 g). The fish was acclimatized to the test condition for ten days before their use. Analytical grade cadmium chloride, $CdCl_2.H_2O$ (purity 98%, molecular weight 201.32 g/mol; E. Merck, made in Germany) was used as the test chemical.

Static replacement bioassays were used for both 96h acute toxicity tests and 90 days chronic toxicity tests following the methods outlined in American Public Health Association (2012). The physicochemical properties of water used during the experiment were: temperature 27.5 ± 1.36 °C, pH 7.4 ± 0.76, free CO_2 12.0 ± 2.34 mg/l, DO 5.2 ± 1.12 mg/l, alkalinity 167 ± 4.54 mg/l as $CaCO_3$ and hardness 127 ± 5.63 mg/l as $CaCO_3$.

Acute toxicity tests for fish were conducted in 15l glass aquaria holding 10l of water in the laboratory. Each concentration was accompanied by four replicates. Ten organisms were used in each replicate. The fishes were not fed 24h before and during the bioassays. The number of dead fishes was counted every 24h and removed immediately from the test medium to avoid any organic decomposition and oxygen depletion. Mortality rate at different concentrations and at different times of exposure was analyzed using the computer software R version 2.14.0 (US EPA, 1999) and probit analysis (Finney, 1971) for determining 96h median lethal concentrations (LC_{50}) with 95% confidence limits of cadmium chloride to fish.

The ethological changes like movement pattern, mucous secretion and sudden death syndrome (erratic fin movement, hyperactivity, difficulty in breathing, suffocation, surface attachment and convulsion followed by death) of the fish exposed to different doses used during acute toxicity tests (0, 70.0, 75.0, 82.5, 92.5 and 95.0 mg/l of cadmium) were observed following the method of Rand (1985).

Chronic toxicity tests were conducted in 300l cement vats in the outdoor for 90 days using two different sublethal concentrations (4.14 and 8.27 mg/l) of cadmium and a control. The vats were arranged in 3 blocks each with 4 vats as per Randomized Block Design (Gomez & Gomez, 1984) thereby giving four replicates for each of the two sublethal concentrations and control. Each vat was stocked with ten adult *Clarias batrachus* for haematological study. In addition to natural food, the stocked fish were fed a mixture of rice bran, mustard oil cake and fish meal (1:1:1) 6 days a week @ 5% of their body weight. The blood samples of fish were analysed at every 30 days interval with a total exposure period of 90 days. Blood samples were taken by puncturing the caudal vessels using EDTA (Ethylene diamine tetra acetic acid) as anticoagulant. Haemoglobin (Hb), Haematocrit (Hct), Total erythrocyte count (TEC), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and

Mean Corpuscular Volume (MCV) were estimated following the methods of Tvedten (1989), Campbell and Murru (1990) and Dacie and Lewis (1977). Serum albumin, Serum globulin, cholesterol and triglyceride levels were measured using standardized kits. Serum albumin/globulin (A/G) was obtained by the ratio of the values of serum albumin and serum globulin.

The values of haematological parameters of fish were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for determining significant differences among the means (Gomez & Gomez, 1984).

Results and Discussion

The 96h median lethal concentration (LC_{50}) with 95% confidence limits of cadmium chloride to fish is given in Table 1. No mortality was observed in the control group during the experiment. In the present study, 96h LC_{50} value of cadmium to *Clarias batrachus* (82.66 mg/l) almost corresponds with the LC_{50} values of fathead minnow, *Pimephales promelas* (72.6 mg/l) and green sunfish, *Lepomis cyanellus* (66 mg/l) (Pickering & Henderson, 1966). In *C. batrachus*, significant relationship between mortality rate and exposure times were not observed ($p>0.05$). The relationship between mortality rate and exposure concentrations was significant at different exposure times for *C. batrachus* ($p<0.01$).

The ethological changes observed in *C. batrachus* exposed to cadmium are shown in Table 2. Excessive mucous secretion in the body surface of fish was probably due to its avoidance reactions from the toxicant. The treated fish became hyperactive initially in comparison to the control. With the progress of time and increasing concentration fish also showed the signs of stress like erratic fin movement, hyperactivity, difficulty in breathing, suffocation and surface attachment mainly at the higher concentrations (82.5, 92.5 and 95.0 mg/l) during 72 and 96h exposure. Similar behavioural changes were also observed by various workers in fishes like guppy, *Poecilia reticulata* and the freshwater teleost, *Channa punctatus* to cadmium chloride (Yılmaz & Karaköse, 2004; Tiwari et al., 2011). The death of fish at all the exposures were characterized by gasping, flaring of the operculae, convulsions, loss of equilibrium and buoyancy, extreme rigor and cessation of ventilation within a very short time. Hyperactivity, erratic fin movement and convulsion of exposed fish were probably due to the effects of cadmium on central nervous system. These symptoms were much more prominent in the fish at the higher doses (82.5, 92.5 and 95.0 mg/l) of 72 and 96h exposures. In addition, white patches in the gills and red blotches at the base of tail, genital papilla and vent were observed in fish.

The mean values of blood triglyceride and cholesterol of fish increased significantly ($p<0.05$) at all the sublethal exposures as given in Table 3. Serum globulin, Hb content, TEC and Hct (%) of fish at all the exposures showed a significant reduction in their mean values from the control ($p<0.05$). Significant variation ($p<0.05$) was also observed for serum albumin and Hct (%) at all the treatments in 60d and 90d but no significant variation was recorded in these two means at 30d of exposure ($p>0.05$). MCV did not show any significant variation in any treatment at any day. MCH and MCHC (%) showed significant decrease in all sublethal concentrations at 90d only ($p< 0.05$). A significant decrease was observed in the mean values of serum albumin at all the concentrations of 60d and 90d exposures. Serum A/G ratio significantly increased at 4.14 mg/l but significantly decreased at 8.27 mg/l throughout the experiment. Similar changes in haematological values in teleosts were also recorded due to physiological stress, disease and toxic environmental conditions (Kori-Siakpere et al., 2006). Haemoglobin is the oxygen-carrying component in the blood of fish and its significant decrease at all sublethal concentrations at 60 and 90 days in the present experiment can be used as a good indicator of anaemia. The significant reduction of TEC, haemoglobin and Hct (%) in the present study during 60 and 90 days in all exposures was probably due to internal bleeding and haemolysis from the damaged tissues of different vital organs like kidney, gills and liver preceded by bio-concentration and bioaccumulation of cadmium through the food chain during sublethal exposure (Mauskar, 2007; Kori-Siakpere et al., 2006; Jayakumar & Paul, 2006; Omer et al., 2012). The reduction in MCH and MCHC values was attributed probably as a defence mechanism against the toxic effect of cadmium through the stimulation of erythropoiesis. The reduction in these values of fish may also be related to the decrease in RBCs, Hb and Hct due to exaggerated disturbances that occurred in both metabolic and haemopoietic activities of fish exposed to the pollutant (Kori-Siakpere & Ikomi, 2011). The elevated triglyceride and cholesterol levels for all exposed fish blood in the present study were also corroborated in the blood, muscle and liver tissues of various other fishes exposed to different toxicants (Al-Attar, 2005a, 2005b; Mohamed & Gad, 2008; Desai et al., 2002). The elevated level of triglyceride in blood of fish induced by cadmium may be attributed to thyroid and liver dysfunction leading to enhanced cholesterol and triglyceride synthesis and/or reduced cholesterol and triglyceride catabolism (Tietz, 1987).

The present finding highlights the toxicity of cadmium to fish during their acute and chronic exposure. Acute toxicity studies are among the first steps in determining the water quality requirements of fish. These studies reveal the toxicant concentrations (viz. LC_{50}) that cause fish mortality even at short time exposure. The LC_{50} values of the present study may provide useful data to set up national and local water quality criteria (WQC) for cadmium. The ethological responses of fish are extremely sensitive to toxicant; therefore the ethological responses are the most sensitive parameters for measuring the neurotoxicity (Doving, 1992). The present haematological study shows that cadmium poses a serious threat to the biological functions of fish during their chronic exposure. On the basis of haematological studies, it would be possible to forecast the physiological state of fish in natural water bodies as well as the possible mechanism of action of a toxicant to the fish body.

Acknowledgements

We thank the Head, Postgraduate Department of Zoology and the Principal, Barasat Government College for allowing us to carry out the research work.

Table 1: 96h lethal concentrations (LCs) with 95% confidence limits of cadmium to *Clarias batrachus* (Control group theoretical spontaneous response rate = 0.0000)

Point	LC values (mg/l) (95% confidence limits)	Slope ± SE	Intercept ± SE
LC 1.00	65.53 (39.81-73.10)	23.07 ± 7.92	-39.24 ± 15.20
LC 5.00	70.14 (48.74-76.56)		
LC 10.00	72.73 (54.19-78.62)		
LC 15.00	74.54 (58.12-80.16)		
LC 50.00	82.66 (75.09-90.54)		
LC 85.00	91.66 (85.31-116.31)		
LC 90.00	93.94 (87.02-124.70)		
LC 95.00	97.40 (89.40-138.59)		
LC 99.00	104.26 (93.65-169.62)		

Table 2: Impact of cadmium on the ethological responses of *Clarias batrachus* (M: movement; MS: mucous secretion; SDS: sudden death syndrome; -: none; +: mild; ++: moderate; +++: strong) at various concentrations during different hours of exposure

Dose (mg /l)	24h			48h			72h			96h		
	M	MS	SDS	M	MS	SDS	M	MS	SDS	M	MS	SDS
70.0	+	+	-	++	+	-	++	+	-	++	+	-
75.0	++	+	-	++	++	-	++	++	-	++	++	-
82.5	++	++	-	+++	++	+	+++	++	++	+	+++	++
92.5	+++	++	+	+++	+++	+	+	+++	+++	-	+++	+++
95.0	+++	+++	+	+	+++	++	+	+++	+++	-	+++	+++

Table 3: Mean values (± SD) of haematological parameters of *Clarias batrachus* exposed to control (0.00 mg/l) and different sublethal concentrations (4.14, 8.27 mg/l) of cadmium chloride in water at different days of exposure (30d, 60d, 90d) [Values are means of four replicates ± SD]

Parameters	Days								
	30	60	90	30	60	90	30	60	90
	Dose (mg/l)								
	0.00	4.14	8.27	0.00	4.14	8.27	0.00	4.14	8.27
Triglyceride (mg/dl)	64.00^{a} ± 4.97	72.18^{b} ± 3.12	78.00^{b} ± 6.06	68.12^{a} ± 4.10	80.14^{b} ± 1.66	84.00^{b} ± 6.16	63.06^{a} ± 6.91	82.30^{b} ± 6.95	89.00^{b} ± 3.74
Cholesterol (mg/dl)	226^{a} ± 5.16	234^{a} ± 4.24	251^{b} ± 11.60	224^{a} ± 3.65	252^{b} ± 8.49	273^{c} ± 10.95	215^{a} ± 4.69	264^{b} ± 5.16	287^{c} ± 14.53
Serum Albumin (g/dl)	2.40^{a} ± 0.04	2.52^{a} ± 0.07	2.46^{a} ± 0.06	2.48^{b} ± 0.03	2.58^{c} ± 0.04	2.29^{a} ± 0.03	2.42^{c} ± 0.04	2.21^{b} ± 0.05	1.94^{a} ± 0.04
Serum Globulin (g/dl)	3.36^{b} ± 0.15	2.98^{a} ± 0.07	2.77^{a} ± 0.27	3.16^{b} ± 0.05	2.71^{a} ± 0.05	2.61^{a} ± 0.13	3.27^{b} ± 0.04	2.85^{a} ± 0.06	2.81^{a} ± 0.17
Serum A/G ratio	0.72^{a} ± 0.04	0.85^{b} ± 0.03	0.90^{b} ± 0.09	0.78^{a} ± 0.02	0.90^{b} ± 0.06	0.88^{b} ± 0.04	0.74^{b} ± 0.74	0.78^{c} ± 0.78	0.69^{a} ± 0.69
Hb (g/dl)	15.0^{a} ± 1.22	13.5^{ab} ± 0.71	12.0^{b} ± 1.78	14.9^{a} ± 1.09	10.7^{b} ± 0.34	10.0^{b} ± 1.41	15.4^{a} ± 0.32	10.4^{b} ± 0.37	9.0^{c} ± 0.74
TEC (10^{6}/mm^{3})	3.22^{a} ± 0.11	2.86^{b} ± 0.03	2.79^{b} ± 0.14	3.18^{a} ± 0.02	2.35^{b} ± 0.03	2.22^{b} ± 0.14	3.27^{a} ± 0.02	2.21^{b} ± 0.04	2.10^{b} ± 0.15
Hct (%)	38.63^{a} ± 3.83	35.14^{a} ± 2.18	34.40^{a} ± 2.88	38.17^{a} ± 2.17	29.39^{b} ± 2.06	28.37^{b} ± 1.83	39.30^{a} ± 1.19	28.78^{b} ± 3.23	27.12^{b} ± 2.04
MCV (μ^{3}m)	119.83^{a} ± 9.10	122.83^{a} ± 6.74	123.35^{a} ± 9.42	120.04^{a} ± 7.00	125.08^{a} ± 8.96	127.87^{a} ± 5.25	120.20^{a} ± 4.25	130.14^{a} ± 13.51	129.20^{a} ± 4.18
MCH (pg)	46.56^{a} ± 3.07	47.19^{a} ± 2.18	42.92^{a} ± 4.82	46.85^{a} ± 3.33	45.53^{a} ± 1.28	44.98^{a} ± 4.84	47.17^{a} ± 1.11	47.06^{a} ± 1.34	42.89^{b} ± 2.52
MCHC (%)	38.89^{a} ± 1.04	38.47^{a} ± 1.93	35.01^{a} ± 5.26	39.23^{a} ± 4.84	36.55^{a} ± 2.93	35.20^{a} ± 3.82	39.21^{a} ± 1.40	36.45^{ab} ± 3.83	33.25^{b} ± 2.77

^{a,b,c} Dissimilar superscript letters between two concentrations of a row at 30, 60 and 90 days indicate significant difference (DMRT, $p < 0.05$)

REFERENCE

Al-Attar, A.M. (2005a). Changes in haematological parameters of the fish, Oreochromis niloticus treated with sublethal concentration of cadmium. Pak J Biol Sci, 8(3), 421-424. | Al-Attar, A.M. (2005b). Biochemical effects of short-term cadmium exposure on the freshwater fish, Oreochromis niloticus. J Biol Sci, 5(3), 260-265. | American Public Health Association (APHA) (2012). Standard methods for the examination of water and wastewater. E.W. Rice, R.B. Baird, A.D. Eaton, & L.S. Clesceri (Eds.), American Public Health Association, American Water Works Association, Water Environment Federation: Washington DC. | Campbell, T.W., & Murru, F. (1990). An introduction to fish haematology. Compendium of continuing education in Veterinary science, 12, 525-533. | Dacie, V.J.S., & Lewis, M.S. (1977). Practical Haematology. ELBS edition. | Desai, H.B., Nanda, B., & Panigrahi, J. (2002). Toxicological effects on some biochemical parameters of freshwater fish Channa punctatus (Bloch.) under the stress of nickel. J Environ Biol, 23(3), 275-277. | Doving, K.B. (1992). Assessment of animal behaviour as method to indicate environmental toxicity. Comp Biochem Physiol, 100, 247-252. | Finney, D.J. (1971). Probit analysis. Cambridge University Press: London. | Fleischer, M., Sarofim, A.F., Fassett, D.W., Hammond, P., Shacklette, H.T., Nisbet, I.C. & Epstein, S. (1974). Environmental impact of cadmium: a review by the panel on hazardous trace substances, Environ Health Perspect, 7, 253-323. | Friberg, L., Piscator, M., Nordberg, G.F., & Kjellstrom, T. (1974). Cadmium in the environment, Cleveland, OH: CRC Press. | Gad, S.C. (2005). Cadmium. In Encyclopedia of Toxicology, vol. 1-4, P. Wexler (Ed.), Academic Press, Elsevier: USA. | Gomez, K.A., & Gomez, A.A. (1984). Statistical procedures for agricultural research, John Wiley and Sons, New York, USA. | Hiatt, V., & Huff, J.E. (1975). The environmental impact of cadmium: an overview, Int J Environ Stud, 7, 277-285. | Jayakumar, P., & Paul, V.I. (2006). Patterns of cadmium accumulation in selected tissues of the catfish Clarias batrachus (Linn.) exposed to sublethal concentration of cadmium chloride. Vet arhiv, 76, 167-177. | Kori-Siakpere, O., Ake, J.E.G., & Avworo, U.M. (2006). Sublethal effects of cadmium on some selected haematological parameters of Heteroclarias (A Hybrid of Heterobranchus bidorsalis and Clarias gariepinus), Int J of Zoological Research, 2, 77-83. | Kori-Siakpere, O., & Ikomi, U. (2011). Alterations in some haematological parameters of the African Snakehead Parachanna africans exposed to cadmium, Not Sci Biol, 3(4), 29-34. | Mauskar, J.M. (2007). Cadmium an Environment Toxicant. Newsletters 61. Retrieved from http://www.cpcb.nic.in/upload/Newsletters/Newsletters_61_CADMIUMAn%20EnvironmentToxicant-March-2007.pdf | Mohamed, F.A.S., & Gad, N.S. (2008). Environmental pollution-induced biochemical changes in tissues of Tilapia zillii, Solea vulgaris and Mugil capito from Lake Qarun, Egypt. Global Veterinaria, 2(6), 327-336. | Omer, S.A., Elobeid, M.A., Fouad, D., Daghestani, M.H., Al-Olayan, E.M., Elamin, M.H., Virk, P., & El-Mahassna, A. (2012). Cadmium bioaccumulation and toxicity in Tilapia fish (Oreochromis niloticus). J of Animal and Veterinary Advances, 11(10), 1601-1606. | Pickering, Q.H., & Henderson, C. (1966). The acute toxicity of some heavy metals to different species of warmwater fishes, Int J Air water Pollut, 10, 453-466. | Rand, G.M. (1985). Behavior. In G.M. Rand, & S.R. Petrocelli, (Eds.) Fundamentals of Aquatic Toxicology Methods and Applications. 221.13. Washington: Hemisphere Publishing Corporation. | Tietz, N.W. (1987). Fundamentals of Clinical Chemistry. USA: Saunders WB Co. | Tiwari, M., Nagpure, N.S., Saksena, D.N., Kumar, R., Singh, S.P., Kushwaha, B., & Lakra, W.S. (2011). Evaluation of acute toxicity levels and ethological responses under heavy metal cadmium exposure in freshwater teleost, Channa punctata (Bloch). Int J of Aquatic Sci, 2(1), 36-47. | Tvedten, H. (1989). The complete blood count and bone marrow examination: General comments and selected techniques. In M.D. Willard, H. Tvedten & G.H. Turnwald (Eds.) Small animal clinical diagnosis by laboratory methods. Philadelphia, USA: Saunders. | US EPA. (1999). Probit Program Version 1.5. Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, (U. S. Environmental Protection Agency, Cincinnati, Ohio 45268), http://www.epa.gov/nerleerd/stat2.htm. | Yılmaz, M., Gül, A., & Karaköse, E. (2004). Investigation of acute toxicity and the effect of cadmium chloride (CdCl2.H2O) metal salt on behavior of the guppy (Poecilia reticulata). Chemosphere, 56(4), 375-380. |