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IMPACT OF CADMIUM TOXICITY ON BEHAVIOURAL AND HAEMATOLOGICAL BIOMARKERS OF FRESHWATER FISH, *CATLA CATLA*

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Abstract: The present work is aimed to evaluate LC_{50} values of Cadmium chloride for freshwater and common people edible fish, *Catla catla*, *as* well as to observe behavioural alterations treated with Cadmium. The LC_{50} values for 24h, 48h, 72h and 96h were found 14.59, 10.76, 8.45 and 5.36 mg/l, respectively. The major behavioural alterations observed during the experiments were erratic swimming, S-jerk movements and burst swimming, restlessness, muscle spasm, fin flickering, profuse mucous secretion, discolouration of the integument in exposed animals. Overall increase in opercular beats in exposed fishes was also recorded throughout the experiments. The above modulations are considered as good bio-markers to access the health status of these valuable and cherished fishes as well as worsening status of aquatic bodies in relation to metallic contaminants, particular Cadmium. Haematological indices are considered to be pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of animals exposed to pollutants. Exposure of fish to sublethal concentration of cadmium showed a significant decrease in the haemoglobin (Hb) content, total red blood cells (TRBC) and packed cell volume (PCV/Hct) at the end of 30th day as compared to control, this may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production and indicates the worsening of an organism state and developing anaemia. Whereas the total white blood cells (TWBC) increased significantly, with increase in exposure periods, may be attributed to immunological response of the fish to heavy metal. To safeguard these, it is suggested, that the cadmium levels should be monitored and be restricted to release in to the aquatic environment either in any way with heavy metals monitoring devices.

Keywords: *Catla catla*, cadmium chloride, behavioural changes, haematlogical Biomarkers, Immune response.

INTRODUCTION

Heavy metals are commonly found in natural waters and some are essential to living organisms, yet they may become highly toxic when present in high concentrations. These metals also gain access into ecosystem through anthropogenic source and get distributed in the water body, suspended solids and sediments during the course of their mobility [1]. Heavy metals when reach the aquatic bodies deteriorate the life sustaining quality of water and cause damages to both flora and fauna [2,3]. The concentration of heavy metals in the environment and in animals increases many folds due to their long half-life period and properties of non-biodegradability, bioaccumulation and biomagnifications [4].

Among them cadmium, a non-essential and non-biodegradable heavy metal has been listed in the "Black list" of European community. It has also been classified as b-class (soft) metal [5]. It enters into aquatic bodies through sewage sludge and with the run off from agricultural lands as it is one of the major components of the phosphate fertilizers [6], where it produces deleterious effects on aquatic flora and fauna by affecting various physiological, biochemical and cellular processes [7].

Cadmium is a biotoxic environmental pollutant which accumulates in the body tissues such as the lungs, liver, kidneys, bones, reproductive organs and the immune system [8,7]. The toxic effects of cadmium on organisms include nephrotoxicity, carcinoegenicity, teratogenicity and endocrine disruption [9]. Cadmium may also cause the deterioration of cell membranes by binding to metallothionein (MT) or glutathione and consequently interfer with the ability of these proteins to avoid oxidative stress. Cadmium can also replace essential metals such as copper and zinc in several metalloproteins, altering the protein conformation and affecting their activity because this element interacts ubiquitously with sulphydryl groups of amino acids, proteins and enzymes [10]. Cadmium can cause oxidative stress through several mechanisms; the fenton reaction depletion of cellular glutathione, alterations of mitochondrial electron transfer chain and inhibition of antioxidant enzymes [11].

With the discovery of new uses of cadmium, its presence in the environment has increased. Cadmium is used in the production of television picture tube phosphorus, nickel cadmium batteries, motor oils, curing agents for rubber, fungicides, phosphate fertilizers, stearate stabilizers for plastics (polyvinyl

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chloride) and shields for nuclear reactors. Cadmium is used primarily for electroplating other metals or alloys to protect them against corrosion and in the manufacture of low melting point alloys or solders.

Fish is generally appreciated as one of the healthiest and cheapest source of protein and it has amino acid compositions that are higher in cysteine than most other sources of protein. Heavy metals like copper, iron and zinc are essential for fish metabolism while some others such as mercury, cadmium, arsenic and lead have no known role in biological systems. Many workers have reported the manifestation of toxic effects of cadmium on various fishes [12]. However, effects of Cadmium on freshwater fishes, especially on carp fish, *Catla catla* have been documented less [13,4,7]. The present work is aimed to evaluate LC_{50} / 96h of Cadmium chloride and to observe the variations in opercular beats, behavioural modulations and haematological indices of freshwater fish, *Catla catla,* having good nutritive value and can serves as a better bioindicator of freshwater streams, lakes and ponds.

MATERIALS AND METHODS

The freshwater fish, *Catla catla* are selected as the test organisms for the present study. They were collected from the Kalyanidam Reservoir, near Tirupati, with the help of local fishermen, and immediately brought to the working laboratory and acclimatized to laboratory conditions for 15days prior to the experiments. Stock solution of Cadmium chloride ($CdCl_2.21/2\ H_2O$, M.W. = 228.35AR Grade), was prepared by dissolving weighed amount of salt in double distilled water. For toxicity tests ten aquaria of 50 liter capacity were taken having 30 liters of dechlorinated and oxygenated tap water (Physico-chemical properties of water : pH = 7.6±0.2; Temp. = 26±2^{o}C; Alkalinity = 66±4.5 mg/L; Total Hardness = 259±2.5 mg/L; D. O. = 7.6±0.2 mg/L). Series of five concentrations of Cadmium chloride *viz*. 140, 200,280, 380, 500 mg/l (Toxic range was predetermined by exploratory tests) was prepared by adding calculated amount of stock solution. One aquarium having normal water without adding Cadmium chloride, served as control, in which control animals are maintained.

Adult 10 fishes of similar size and weight (average length 16±1.5cm and weight 21.5± 2.0gm) were introduced to each test as well as control aquaria from stocking tank. The fish were acclimated to laboratory conditions in dechlorinated tap water for 2 weeks prior to the assay in 100L capacity plastic tanks. The mean values for the test water quality were as follows; temperature 22±10C; pH 5.7±0.4; dissolved oxygen 4.7±0.7ppm hardness 36±1.24ppm. The fish were fed *ad libitum* daily with pelletized formulated feed. Fish were maintained at photoperiod of 12 light, 12h dark regime. Feeding was suspended 24 hour before start and throughout the experiment to avoid dissolved Cadmium losses due to particulate adsorption. Proper aeration was maintained in test as well as control aquaria by air pumps and stone diffusers throughout the experimental period. Mortality was recorded at 24 hrs. The LC_{50} values of various intervals were calculated according to method of Finney et al. [14].

Stock solutions of $CdCl_2.H_2O$ were prepared by dissolving toxicant in distilled water to a final volume of 10ml. The stock was then serially diluted into relevant treatment concentrations. The toxicant in the test chamber was renewed completely with fresh solution of the same concentration every 24 hour. 1/5th concentration of 96 LC_{50} is taken as sub lethal concentration for haematological analysis for over a period of 3days, 7days, 15days and 30 days. Four replicates were maintained simultaneously, 10 fish were introduced in each concentration per tank. The experimental fish were also fed with formulated feed *ad libitum* as in controls. After expiry of each exposure period blood was collected from each fish by means of a hypodermic syringe at the base of caudal peduncle, and immediately transferred to EDTA containers for haematological analysis. Haemoglobin (Hb) was estimated as cyanomethemoglobin according to Ochei and Kolhakter [15]. TRBC and TWBC were determined according to the classical method using the Neuber hematocytometer. Haematocrit was determined using microhaematocrit tubes and a haematocrit centrifuge. Blood was centrifuged at 1200 x g for 5 minutes and the haematocrit value was obtained [16].

Statistical analysis:

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multi Mample Range post hoc test using SPSS 15 software. Statistical significance was considered at $p<0.05$ level of significance.

RESULTS

The LC_{50} values and their confidence limits of Cadmium chloride for *Catla catla* are summarized in Table.1. The 24, 48, 72 and 96h LC_{50} values of Cadmium chloride were 14.59, 10.76, 8.45 and 5.36 mg/l, respectively. It is observed that an inverse relationship of exposure duration and concentration was clearly evident.

Table.1: LC_{50} values of Cadmium chloride and upper and lower confidence limits in *Catla catla.*

Exposure Period (hrs.)	LC50 (mg/l)	Lower confidence limit (mg/l)	Upper confidence limit (mg/l)
24	14.59	16.03	11.85
48	10.76	13.29	8.68
72	8.45	11.28	6.56
96	5.36	8.14	3.93

The experimental fish sub lethal concentration of Cadmium chloride caused various behavioral abnormalities in fish such as - an erratic increased swimming, surfacing and hyperactivity, restlessness, abnormal swimming, and secretion of mucous which was followed by loss of balance and succumbing of fish, when they are initially exposed to cadmium chloride test solution.

Table.2: Variations in Opercular beats/minute of *Catla catla after* exposed to Cadmium chloride.

Exposure Period (hrs)	Control	Experimental
24	61 ± 2.74	84± 3.43
48	66 ± 4.78	88 ± 3.54
72	73 ± 4.26	95 ± 4.55
96	78 ± 4.55	116± 5.33

Throughout the exposure period the fishes showed various aggressive behavioral abnormalities such as nudge and nip, fin flickering, partial and S-jerk and burst swimming increased significantly ($P<0.05$) compared with control. It is also observed that the aforementioned responses are significantly and gradually increased with increasing the exposure period. The behavioral abnormalities observed in fish treated with sublethal concentration cadmium chloride are summarized in the Table.2.

Cadmium chloride induced marked effects on opercular beats/minute of *Catla catla in* the test fishes which were found significantly higher throughout the experiment (Table.3). Though a decline was noticed from 24 and 48 hrs exposure period only but the values were still significantly higher than the controls, whereas the mean values of opercular beats in 72 and 96 hours are higher than control and also other exposure periods. The differences between means of opercular beats of test animals and control animals were highly significant at 24, 48, 72 and 96 hours exposure periods. The overall fluctuations in means of opercular beats from 24 hrs to 96 hrs were found significant in test animals as well as in control animals.

The data on variations in haematological indices of *Catla catla* exposed to $1/5^{th}$ sub lethal concentration 96h LC_{50} of cadmium presented in Table.4. It is evident that the heavy metal Cadmium inflicted a drastic gradual reduction ($p<0.05$) in TRBC, Hb and Hct in fish in 30 days exposure period relative to control, whereas the total leucocyte count (TWBC) is increased with increase concentration of cadmium chloride.

Table.3: Behavioral abnormalities in *Catla catla* monitored in the present study.

S. NO	EXPOSURE PERIOD	MONITORED BEHAVIOURAL CHANGES
1	**After 24 hrs exposure**	-restlessness, rapid surfacing, peeling of skin and colour fading - slightly reduced activity - gradual increase in colour fading
2	**After 48 hrs exposure**	-gill adhesion and a thin film of mucous on gills, operculum and general body surface at this stage. - nudge and nip - fish moving towards surface water and gulping of air is increased,
3	**After 72hrs exposure**	-loss of balance and jerky movements during swimming. - the school formation, a characteristic of this fish, - weakened in test animals as compared to controls - prominent ulcerations on the base of caudal fin, pectoral fin and on trunk of fish. - hemorrhages were also identified on fins and trunk of some fish
4	**After 96hrs exposure**	- a thick mucous film was formed on whole body and gills, in all test fish - the experimental fishes lost their natural colouration and become almost reddish black / dark red in colour. - S-jerk movements and burst swimming - fin flickering

DISCUSSION

The sub lethal concentration of Cadmium chloride caused changes in various physical, behavioural and haematological indices of *Catla catla*, including the accumulation of bluish-white mucous-like substance clogged on the vital external areas of the fish skin. These deposits were suspected to be salts of cadmium. They were noticed only in the Cd treated fishes, which indicated contamination through water.

The pathological effects of the deposits of $CdCl_2$ included irritation of the skin, which may be more severe in gill filaments and respiratory lamellae than the skin thus interfering with the ability of gill to carry oxygen for respiration [17]. This is in addition to the fact that the presence of metals in the water had already reduced the dissolved oxygen content of the water in which the fish lives. Of more concern is the effect on the gills than the skin because the metals only caused irritation of the latter (skin), while in the former (gill) the metals interfered with the normal ability of the gill to carry oxygen for respiration [18].

Behavioural changes of hyper-activity and jumping observed in the Cadmium poisoned fishes in all tanks were due to skin irritation, respiratory rate impairment and coughing induced by the toxicants on the fishes especially. Weak swimming and reduced equilibrium (swimming upside down) were symptoms of a dying fish. Death resulting from acute $CdSO_4$ might be due to increased heart failure, hypertension, gastric

haemorrhage, convulsion, paralysis, heart failure and suffocation [19].

Cadmium chloride poses toxic effects on the carp fish *Catla catla* which is evident by the findings of present investigations and LC_{50} values observed in present study are nearer to the reporting of other workers on different fishes [20]. Behavioral alterations have been established as sensitive indicators of chemically induced stress in aquatic organisms. Behavioural alterations like erratic swimming, restlessness and surfacing, observed in present study may be an avoiding reaction to the heavy metals as also observed by various workers [21]. The avoidance reaction may be related to narcotic effects or to change in sensitivity of chemo receptors [22]. The immediate physiological consequence of the lamellar fusion is the reduction in the surface area available for gaseous exchange, which could adversely affect respiratory physiology of the fish. A wide range of functions has been attributed to fish mucous including protection against environmental contaminants and UV radiation [23]. Many workers are of the opinion that some components of the fish mucous, probably the acidic and/or sulphated glycoprotein moieties have a metal binding and ameliorative effect against ambient toxicants [24]. Daoust *et al.* [25] suggested that the lamellar adhesion in gills might be result of contact stress, which causes erosion of mucous coating and epithelial lining leading to alterations in the chemical composition and thickness of the mucous layer due to interaction with xenobiotics. Erosion of the epithelial lining and alteration in mucous coating has also been observed in the present study also. It is well known that the presence of glycoproteins in the mucous is indicative of its metal binding capacity as cadmium specially binds –SH groups. Mucous coating on one hand prevents the further metal entry by precipitation and on other hand can also eliminate the accumulated Cadmium chloride during its sloughing.

Loss of balance of fish might be due to neurological impairment in central nervous system as it is evidenced by inhibition of e.g. AChE by Cadmium [26]. Skin ulcerations as observed in present study are similar to the report of Iger et al. [27] in fishes treated with cadmium. The overall increase in opercular beats as observed in present study is in good conformity with earlier reports on different fishes in relation to various toxicants. The increased opercular activity may be due to shock received by the fish in new toxic environment along with sensory stimulus to increase the opercular movement for proper ventilation of the gills to cope with hypoxia [28].

Cadmium induced gill necrosis in fishes has been reported by various workers [29] which may also be a cause of decrease in oxygen consumption and increase in ventilation frequency. The respiratory distress is further compounded due to the decreased hemoglobin, hematocrit and erythrocytes as observed in cadmium exposed fish [30]. Surfacing and gulping of air might be a compensatory device to cope with the oxygen deficiency as observed in *Channa punctatus* after cadmium exposure [31]. The increased gulping activity and opercular movement by the exposed fish may be the reflection of an attempt by the fish to extract more oxygen to meet the increased energy demand to with stand the cadmium toxicity. It may also be correlated to the formation of a hypoxic condition due to the interference in gaseous exchange caused by the accumulation of mucous on the gill epithelium. The present findings suggest that cadmium produces respiratory distress in fishes and opercular beats per minute can be considered as good bio-marker to access the health status of these valuable and cherished fishes as well as worsening status of aquatic bodies in relation to metallic contaminants, particular Cadmium.

Table.4: Variations in haematological indices of *Catla catla* exposed to sub lethal concentration of Cadmium chloride.

Parameter	Control	Exposure Period (Days)			
		3	7	15	30
Total Red Blood Cell Count (TRBC) RBC X10 ^{9}/l	6.47 ±3.10	5.79 ±2.44	5.03 ±2.11	4.63 ±2.15	3.16 ±1.39
% Change	____	(- 10.51)	(- 22.26)	(- 28.43)	(-51.16)
Haemoglobin (Hb) (g/ml)	122.67 ±4.16	106.54 ±2.45	85.49 ±3.6	50.67 ±4.68	38.67 ±2.16
% Change	____	(- 13.15)	(-30.31)	(- 58.69)	(-68.48)
Haematocrit (PCV) (%)	43.36 ±5.39	35.62 ±4.38	30.03 ±6.5	27.88 ±9.20	22.03 ±4.93
% Change	____	(- 7.85)	(- 30.74)	(- 35.70)	(- 49.19)
Total White Blood Cell Count (TWBC) 10^9 (g/l)	30.68 ±6.34	38.71 ±7.45	44.74 ±8.95	52.71 ±12.31	59.20 ±10.80
% Change	___	(26.17)	(45.83)	(71.80)	(92.96)

Mean values are mean ± SD of six individual observations. All values are significant at P < 0.05 to the control.
Values in parenthesis indicate percentage changes over the control.

Haematological indices are considered to be pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of animals exposed to pollutants [4]. The haematological indices such as Hct, Hb, TRBC and TWBC are used to assess the functional status of the oxygen carrying capacity and immunological condition of the bloodstream and have been used as indicators of heavy metal pollution in the aquatic environment [32]. Literature shows that changes in haematological indices of fish caused by heavy metals and their mixtures are different. They are predetermined both by the concentration of heavy metals in the water and time of exposure, and both

these factors can cause reversible and irreversible changes in the homeostatic system of fish.

The present study denotes that there were significant (P<0.05) decrease in TRBC and Hb levels after a long time exposure to sub lethal concentration of Cadmium chloride in fish, on day 30. Therefore, this may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production [33]. It may also be due to an increase in the rate at which Hb is destroyed as a result of long time exposure to Cadmium chloride.

It is well known that lead causes early mortality of mature red blood cells and inhibition of haemoglobin formation through inhibition of erythrocyte alpha-amino levulinic acid dehydratase (ALA-D). The result is anaemia at high concentration of heavy metal exposures or compensating erythropoiesis at lower exposures [31]. In the light of the present study, Hct was decreased gradually in the exposure periods of 3, 7, 15 and 30 days over the control group. A decrease in the erythrocyte count or in the percent of haematocrit indicates the worsening of an organism state and developing anaemia.

Haemoglobin concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible. A decrease in the concentration of haemoglobin in blood is usually caused by the effect of toxic metals on gills, as well as decrease in oxygen, which also suggests anaemia or confirms toxic impact of cadmium chloride in *Catla catla*.

TWBC functions majorly to fight infections, defends the body against invasion by foreign substances and to produce, transport and distribute antibodies in immune response. The increase in TWBC s compared to control noted in the result (Table 1) may be as a result of stimulation of the immune system of the affected fish to fight the toxicity of heavy metal. This finding was in accordance with the report that infections and intoxication stimulate WBC in fish. Kumar and Gopal (34) reported increase in the WBC count from different fish species to different fish species. It is also noted from the results that there was a gradual and significant (P<0.005) increase in TWBC level, in fish administered with Cadmium for 30 days compared to the control fish. The increase in leuocytes in the administered group compared to the control on day 30 may be attributed to immunological response of the fish to heavy metal.

There was decrease in Hct values in cadmium treated fish, *Catla catla* compared to controls on day 30, indicating that there was shrinking in cell sizes and it may be due to metals intoxication. With these foregoing it is obvious that if fish is exposed to this heavy metal for long period of time it may have some haematological damage which may lead to stimulation of immune deficiency diseases [35]. To safeguard these, it is suggested, that the cadmium levels should be monitored and be restricted to release in to the aquatic environment either in any way with heavy metals monitoring devices.

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