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Effects of Cadmium Chloride on Hematological Profiles in Freshwater Fish *Channa punctatus* (Bloch)

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ABSTRACT

Cadmium chloride is a toxic heavy metal that can induce imbalance in various physiological, biochemical and cellular processes in aquatic organisms. Hematological parameters allow the most rapid detection of alterations in physiology exposed to the heavy metals thus, it could be considered as possible biomarkers for detecting heavy metal pollution. The present study reflects the effect of Cadmium chloride on hematological parameters with sub lethal exposure to Cadmium chloride for 48 and 96 h, the results indicated significant decrease in Hb (Hemoglobin), RBC (Red Blood Corpuscles), PCV (Packed Cell Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration) and PLT (Platelets Count) were noticed and WBC (White Blood Corpuscles) and MCV (Mean Corpuscular Volume) was significantly increased with respect to time of exposure and concentration.

Keywords *Channa punctatus*, Hematology, Cadmium chloride, Sub lethal exposure.

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INTRODUCTION

The environment is plagued with different kinds of pollutants. Industrial effluents contain the heavy metals and are released to aquatic ecosystem. The heavy metals due to bio magnification cause serious problem to aquatic organisms (Yousafzai *et al.* 2012, Ibemenuga and Nwamaka 2013). The toxicity of heavy metals to aquatic organisms particularly on freshwater fishes is well documented (Placios *et al.* 2000, Karatas and Kaley 2002) among them Cadmium chloride is toxic heavy metal and widely used in Ni-Cd batteries manufacture, metal and mining, dentistry (Shoba *et al.* 2007). The Cadmium chloride exposure in fishes and shrimps, causes impairment of reproductive physiology and disrupts endocrine functions when exposed at low concentrations (Tuteja *et al.* 2021, Revathi *et al.* 2011). The accumulation of Cd has also been well described in different tissues of fishes (Jayakumar and Paul 2006, Maisunko 2015). Hematological parameters have been used as indicators of fish physiological status. The toxicity of Cadmium to freshwater fishes and has been well documented in *Oreochromis mossambicus* anemic condition was reported when exposed to different doses of Cadmium (Karuppasamy *et al.* 2005, Wu and Deng 2006) and increased leucocytes, neutrophils and eosinophils (leukocytosis, neutrophilia and eosinophilia) in *Cyprinus carpio* exposed to sub lethal concentrations of Cd (Schuwerack *et al.* 2003). Changes in serum biochemical parameters due to liver, gill and kidney dysfunction was also reported in *Oreochromis niloticus* and acts as an immune suppressant in common carp (*C. carpio*), *Oreochromis aureus*, *O. niloticus* and *Ictalurus melas* respectively exposed to Cadmium (Oner *et al.* 2008, Patrick *et al.* 1993). *Channa punctatus* (Bloch) is a freshwater common snake-headed fish belonging to the family Channidae of the order Perciformes. *C. 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 (Tabrez *et al.* 2021). Though several studies have been carried out to document the toxic effect of Cadmium chloride the toxic studies on the hematology is lacking. Hence, the present investigation was aimed to evaluate the toxic effects of $CdCl_2$ on the hematological parameters of the freshwater murrel *Channa punctatus* (Bloch).

MATERIALS AND METHODS

Chemical

Technical-grade Cadmium chloride, monohydrate, AR (CdCl2; 98.0% EC, maximum limits of impurities, iron 0.0005% and sulfate 0.005%) manufactured by HiMedia Laboratories Pvt Ltd Mumbai, India, was procured and used for the study.

Experimental setup

Freshwater fish *Channa punctatus* (Bloch) were collected from the river streams and dams of Thippagondanahalli and Manchanbele with the help of local fisher men Bengaluru, South taluka, Bengaluru District. Fishes were rinsed with 0.1% $KMnO_4$ solution to avoid infection and were acclimatized to laboratory conditions for two weeks prior to experiments. Healthy fishes were selected and were introduced to glass aquaria using chlorine free tap water with adequate aeration. Water was exchanged on daily basis and regular cleaning of aquaria was done to remove fecal matter and food remains. The LC_{50} values of Cadmium chloride to *Channa punctatus* for 96 h was calculated by Probit's method (Finney and Probit 1964). The 96 h LC_{50} for $CdCl_2$ was found to be 559.23µg/l experiments were carried out in triplicates. Group I with 20 fishes was kept as control and Group II fishes (N=20) were exposed to 1/10th of 96 h LC_{50} (55.92 µg/L) for 48 and 96 h. During the period of exposure, the fish were fed with commercial feed at the end of 48 and 96 h of treatment, 10 fishes were collected and the blood samples were collected and stored in EDTA vials.

Hematological parameters

Hematological profiles were estimated by following the standard procedures as described by Blaxhall and Daisley (1973). Hemoglobin (Hb) was estimated by the method described by Brown (1980). Red Blood Corpuscles (RBC) and White Blood Corpuscles (WBC) were enumerated in the Neubauer Hemocytometer (Improved Neubauer Weber Scientific Ltd) following the method as described by Baker and Silverton (1982). Packed Cell Volume (PCV) was determined adopting the method of Snieszko (1960). The red blood cell indices that include Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated using the formula as described by Daicie and Lewis (2001). Platelet (PLT) count was performed following the method described by Seiverd (1983).

Statistical analysis

Significant differences between the control and experimental group were calculated by using Analysis of Variance (ANOVA) followed by Tukey's post-hoc test.

RESULTS AND DISCUSSION

The exposure of freshwater *Channa punctatus* to sublethal concentration of Cadmium chloride at 48

Table 1. Hematological profiles of *Channa punctatus* exposed to sublethal concentration of Cadmium chloride. Values are expressed as Mean±SD of ten individual observations, -: Percent decrease over control, * Indicates significant differences over control at $p < 0.05$.

Hematological profiles		48 h	96 h	Increase/ decrease over control (48 h)	Increase/ decrease over control (96 h)
Hb (g/dl)	Control	08.47 ± 0.20	11.10 ± 0.10	-11.68	-43.51
	Treatment	07.48 ± 0.81*	06.27 ± 0.03*		
WBC (thousand	Control	01.65 ± 0.03	01.72 ± 0.02		
cells/mm^3)	Treatment	02.21 ± 0.07*	03.12 ± 0.10*	33.93	81.39
RBC (million	Control	01.51 ± 0.05	02.45 ± 0.21		
cells/mm^3)	Treatment	01.22 ± 0.03*	0.95 ± 0.51*	-19.2	-61.22
PCV (%)	Control	10.51 ± 0.12	12.82 ± 0.03		
	Treatment	08.34 ± 0.21*	06.32 ± 0.06*	-20.6	-50.70
MCV (F_l)	Control	83.12 ± 0.07	85.42 ± 0.02		
	Treatment	85.13 ± 0.06*	89.12 ± 0.04*	2.41	4.33
MCH (pg)	Control	33.02 ± 0.04	36.23 ± 0.02		
	Treatment	29.83 ± 0.01*	27.58 ± 0.01*	-9.66	-23.87
MCHC (%)	Control	41.71 ± 0.03	45.17 ± 0.10		
	Treatment	38.72 ± 0.10*	36.89 ± 0.32*	-7.16	-18.33
PLT (10^9/l)	Control	05.81 ± 0.30	07.76 ± 0.12		
	Treatment	03.72 ± 0.07*	03.62 ± 0.13*	-35.97	-53.35

and 96 h revealed that there was a significant variation ($p \leq 0.05$) in hematological profiles as shown in Table 1.

Estimation of hemoglobin

Hemoglobin contents of control fishes after 48 and 96 h were 8.47 ± 0.20 and 11.10 ± 0.10 g/dl, respectively, whereas in treated fishes, the Hb content were 7.48 ± 0.81 and 6.27 ± 0.03 g/dl, was recorded after 48 and 96 h respectively. The percentage changes over control were –11.68 and –43.51%, respectively. In the present study Hb content declined, it was found to be corresponding to the lowering of RBC and might be due to the impairment of hemopoietic system as a result it leads to anemia associated with erythropenia. The results of the present study were in accordance with work carried out by a researcher who observed the decrease in Hb concentration in the blood of Eel and Perch after short-term and long-term exposure to Cadmium (Brahan *et al.* 2017). According to Carvalho and Fernanles (2019), Joshi *et al.* (2002) the fish exposed to Cadmium shows a significant decrease in blood iron level caused by the deficiency of intestinal absorption which might be the reason for reduction in Hb concentration. As per the Singh *et al.* (2008) Hb%, RBC and PCV% decreased significantly after 15, 30 and 45 days of exposure periods, respectively, in comparison with control. The exposure of *Channa punctatus* to sub-lethal concentration of copper significantly decreased Hb%, RBC count and PCV% values leading to anemia.

Total WBC count

The WBC counts in control fishes after 48 and 96 h revealed 1.65±0.03 and 1.72±0.02 thousand cells/mm^3 of blood, respectively, whereas in treated fishes showed a significant increase in WBC count ($p < 0.05$). The WBC counts of treated fishes after 48 and 96 h were 2.21±0.07 and 3.12±0.10 thousand cells/mm^3, respectively. The percentage variations from the control group were +33.93 and +81.39% cells, respectively. The sudden spurt in WBCs of the treated fish in the present work might be due to the stimulation of lymphopoiesis and release of lymphocytes from lymph myeloid tissue to combat the toxicant stress. When the pollutant enters the animal tissues, it could have combined with biochemical constituents of the cells and form xenobiotics, due to these reactions, the production of WBCs may be increased and there by the process of elimination of toxic substances from the tissues would have been attempted to prevent the animals from fatal condition

(Rombout *et al.* 2005, Chakrabarti *et al.* 2014, Naz *et al.* 2021). Many researchers previously reported similar results with significant reduction of RBC and Hb% content in fishes exposed to different heavy metals (Goel and Kalpana 1985).

Total RBC count

The number of RBCs in control fishes after 48 and 96 h were estimated to be 1.51±0.05 and 2.45±0.21 million cells/mm^3 of blood, respectively. The treated fishes showed a significant decrease in RBC counts ($p< 0.05$). The RBC counts of treated fishes after 48 and 96 h were 1.22±0.03 and 0.95±0.51 million cells/mm^3 of blood respectively. The percentage changes over the control were –19.2 and –61.22% cells, respectively. The decrease in RBC count noticed in the present study might be due to the destructive action of heavy metal on the peripheral red cells. As a result, the viability of cells is affected. Similar observations were also reported in the gold fish *Carassius auratus* exposed to Nickel (Moosavi and Shamushaki 2015). In contrast, Singh *et al.* (2008) observed an increase in RBCs and Hb in freshwater fish, *Prochilodus scrofa* exposed to Copper. However, the damaging effects on the RBCs may be secondary, resulting from primary action of the toxicant on the erythropoietin tissues due to which there exists a failure in red cell production. According to Das and Goswami (2021) exposure of *C. punctatus* to sub lethal concentration of sodium arsenite exhibited significant decrement in RBC count that might have led to anemia.

A significant decrease in erythrocyte (RBC) counts, hemoglobin (Hb), an increase of White Blood Corpuscles (WBC), in the fresh water fish *Channa punctatus* from polluted waters can definitely be related to the pollution due to slaughter house wastes (Rao and Hemavathi 2000).

Packed cell volume (PCV)

Packed cell volume (PCV) levels of control fishes after 48 and 96 h were found to be 10.51±0.12 and 12.82±0.03%, respectively. In treated fishes, the PCV levels after 48 and 96 h were 8.34±0.21and 6.32±0.06%, respectively. The percentage changes over the control were -20.6 and -50.70%, respectively. The decline in the level of Hb, PCV and platelet count of in the present study clearly implied a hemodilution mechanism probably due to impaired osmoregulation or gill damage. Similar results with significant decrease of RBCs, Hb, PCV and platelet counts in fishes exposed to different heavy metals have been reported earlier by Hussain *et al.* (2018), Javed *et al.* (2017).

Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV) levels of control fishes after 48 and 96 h were 83.12±0.07 and 85.42±0.02 fl, respectively. The treated fishes showed a significant increase in MCV levels ($p < 0.05$). The MCV levels of treated fishes after 48 and 96 h were 85.13±0.06 and 89.12±0.04 fl, respectively. The percentage changes over the control were +2.41 and +4.33% respectively.

Mean corpuscular hemoglobin (MCH)

The Mean corpuscular hemoglobin (MCH) levels of control fishes during 48 and 96 h were 33.02±0.04 and 36.23±0.02 pg, respectively. In the treated fishes, the MCH levels were 29.83±0.01 and 27.58±0.01 pg, respectively. The percentage changes over the control were -9.66 and -23.87%, respectively. MCHC levels of control fishes after 48 and 96 h were 41.71±0.03 and 45.17±0.10% respectively. The treated fishes showed a significant decrease in MCHC ($p< 0.05$).

Mean corpuscular hemoglobin concentration (MCHC)

The Mean corpuscular hemoglobin concentration (MCHC) levels of treated fishes after 48 and 96 h were 38.72±0.10 and 36.89±0.32% respectively. The percentage changes over the control were -7.16 and -18.33% respectively. The hematological indices like MCV, MCH and MCHC provide more information on size, relationship, form and Hb constants of erythrocytes. Furthermore, these indices serve as criterion for morphological studies of anemia belongs to Normocyte, Macrocyte or Microcytic type. The elevation of MCV and reduction of MCH and MCHC in the present study might be due to defensive mechanism against the Cadmium toxicity. The increase in MCV may be due to swelling of RBC as a result of hypoxia condition or Macrocytic anemia in the fishes exposed to heavy metals as suggested by Jastrzebska and Protasowickiz (2005). The MCH and MCHC were considered as good indicators for red blood swelling. In the present study, the decreased MCH and MCHC might be due to the release of proerythrocytes containing low hemoglobin in circulation. Meanwhile, the significant decline in MCH and MCHC values might be due to Hypochromic anemia (Makkawy *et al.* 2011, Shalaby 2007).

Platelet count

The platelet (PLT) counts of control fishes after 48 and 96 h were 5.81±0.30 and 7.76 ±0.12 109/l, respectively. In the treated fishes, the platelet counts after 48 and 96 h were 3.72±0.07 and 03.62±0.13 109/l, respectively. The percentages changes over the control were 35.97 and 53.35% respectively. The decline in the level of Hb, PCV and platelet count in the present study indicated a hemodilution mechanism probably due to impaired osmoregulation or gill damage. Similar results with significant decrease of RBCs, Hb, PCV and platelet counts in fishes exposed to different heavy metals have been reported earlier by Sinha *et al.* (2003), Afaq and Rana (2009).

CONCLUSION

Blood offers important profile to study the toxicological impact on animal tissues. Different blood parameters are often subjected to change depending on stress condition and environmental factors. The study indicates that Cadmium chloride caused severe anemia and alterations in hematological profiles there was a significant decrease in Hb, RBC, PCV, MCH, MCHC and PLT were noticed and WBC and MCV was significantly increased. Further, the fish *Channa punctatus* may be considered as a suitable model to detect the toxicity of heavy metals contaminated in the aquatic ecosystems. The present basic information would serve as a useful tool for ecological assessment and monitoring.

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