ZIBELINE INTERNATIONAL PUBLISHING
ISSN: 2521-0912 (Print)
ISSN: 2521-0513 (Online)
CODEN: JCOLBF

Journal Clean WAS (JCleanWAS)
***DOI** : http://doi.org/10.26480/jcleanwas.02.2018.10.15*

RESEARCH ARTICLE

EFFECT OF CADMIUM CHLORIDE ON THE HAEMATOLOGICAL PROFILES OF THE FRESHWATER ORNAMENTAL FISH, CYPRINUS CARPIO KOI (LINNAEUS, 1758)

A. Jawahar Ali[1*], N. Jalaluddin Akbar[1], M.S. Arun Kumar[1], S. Vijayakumar[1], B. Akbar John[2]

[1]Unit of Aquaculture and Aquatic Toxicology, PG & Research Department of Zoology, The New College, Chennai 600014, India
[2]Institute of Oceanography and Maritime Studies (INOCEM), Kulliyyah of Science, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia
***Corresponding author:** jawahar_alidr@yahoo.co.in*

ARTICLE DETAILS

Article History:

Received 23 December 2018
Accepted 26 January 2019
Available online 4 February 2019

ABSTRACT

Haematological profile [Haemoglobin (Hb), White Blood Corpuscles (WBC), Red Blood Corpuscles (RBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin concentration (MCHC) and Platelets count (PLT)] of the freshwater ornamental fish, *Cyprinus carpio* koi exposed to 1/10th of 96hrs LC_{50} (92.5 ppm) sublethal concentration of Cadmium chloride for a period of 30 days were checked. Compared to control, significant decrease in Hb, RBC, PCV, MCH, MCHC and PLT were noted ($P < 0.05$) while WBC and MCV values were significantly increased ($P < 0.05$). Observation of haematological parameters allows the most rapid detection of alterations in fish after the exposure with xenobiotic compounds and thus, it could be concluded that the haematological indices could be considered as possible biomarkers for heavy metal pollution.

KEYWORDS

Cyprinus carpio koi, Cadmium chloride, Haematology, Heavy metal pollution

1. INTRODUCTION

Intensive agricultural operations and industrial activities release enormous amount of heavy metals into aquatic ecosystem. The heavy metals after reaching the aquatic habitats cause serious problem due to bioacummulation, biomagnifcation in the food chain and toxicity to the organisms [1-4]. The toxicity of heavy metals to aquatic organisms particularly on freshwater fishes is well documented [5-13]. Cadmium (Cd) is one of the biologically non-essential, most toxic heavy metal widely used in Ni-Cd batteries manufacture, metal and mining, dentistry etc. because of its noncorrosive nature [14].

Cadmium is usually rare in natural form and is concentrated in argillaceous and shale deposits as green rocks (Cds) or otavite ($CdCO_3$) and it is naturally associated with zinc, lead or copper in sulphide form [15]. It has been listed in the 'black list' of European community and classified as b-class (soft) metal [16,17]. Due to its non-biodegradable nature; it gets into the aquatic ecosystems and ultimately enters the human and animal's blood stream [18]. The major sources of contamination include electroplating, paper, PVC manufactures, Plastic, paint pigments, fumicides and ceramic industries [19]. It is also entering into aquatic bodies through sewage sludge and with runoff from agricultural fields, as it is one of the major components of the phosphate fertilizers [20]. In human, Cd has been found to cause wide range of biochemical and physiological dysfunctions as manifested in the forms of various diseases viz. Itai-itai, liver malfunction, inflammation, diabetes, Parkinson's disorder, respiratory tract cancer, kidney and prostate cancer [21-24]. In fishes and shrimps, it causes impairment of reproductive activity and disrupts endocrine functions when they exposed even at low concentrations [25,26]. The accumulation of Cd has also been well described in different tissues of fishes [27,28].

Haematological indices have been recognized as valuable tools for evaluation of fish physiological status, the changes of which depend on fish species, age, cycle of sexual maturity and diseases [29-35]. The toxicity of Cadmium to freshwater fishes has been well reported. For example, anaemic condition was reported in *Channa punctatus* and *Oreochromis mossambicus* exposed to different doses of Cadmium [36,37]. A researcher observed increased leucocytes, neutrophils and eosinophils (leukocytosis, neutrophilia and eosinophilia) in *Cyprinus carpio* exposed to sublethal concentrations of Cd [38]. Changes in serum biochemical parameters due to liver, gill and kidney dysfunction was reported by a researcher in *Oreochromis niloticus* exposed to Cadmium [39]. It also acts as an immunosuppressant in common carp (*C. carpio*), *Oreochromis aureus, O. niloticus* and *Ictalurus melas,* respectively [40-42].

Fishes were proved to be significant bioindicators of the aquatic environment so- called ecological integrity [43,44]. It can provide quantitative information on the ecological integrity and its health. As ornamental fishes contribute significantly to the freshwater aquaculture of India and due to their species richness, they have been selected as suitable bioindicators for heavy metal pollution. Amongst ornamental fishes, *C. carpio* koi has the highest market value and hence it has been selected as the bioindicator model for Cd pollution. Though several studies have been carried out to document the toxic effect of heavy metals in general and in Cd in particular on few ornamental and marine fishes, the studies on the effect of pollutants on the hematological profile is still scanty [45-47]. Hence, the present investigation was aimed to evaluate the toxic effects of $CdCl_2$ on the haematological indices of the freshwater ornamental fish, *C. carpio* koi.

2. MATERIAL AND METHODS

2.1 Experimental setup

The freshwater ornamental fish, *Cyprinus carpio* koi (11.56±1.28 cm length and approximately 25.84±6.0 g weight) were procured from Solai Aquarium fish farm, Kolathur, Chennai, Tamil Nadu, India. The test organisms were transported to the laboratory in plastic bags with least disturbance and were washed with 0.1% $KMnO_4$ solution to get rid of dermal infection. Healthy fishes were selected and acclimated to the laboratory conditions (12hrs light/ 12hrs dark) by introducing them in a plastic tub (80 × 30 × 40 cm) using chlorine free tap water with adequate aeration for a period of 15 days. The water quality conditions such as temperature (27±1º C) pH (7.5); Dissolved Oxygen (6.7 mg/l); Salinity (0.5 ± 0.05 ppt); Total hardness (255.0 mg/l); Nitrate (1.6 mg/l); Chloride (27.0 mg/l); Ammonia (0.058 mg/l); BOD (5.8 mg/l); COD (14.7 mg/l) and Total solid (1.7g/l) were maintained. Commercial fish feed (Ocean free) was provided thrice a day satiation. Water was exchanged 100% on daily basis; cleaning of aquaria was carried out to remove fecal matter and food remains.

2.2 Chemical

Analytical grade Cadmium chloride ($CdCl_2$) was obtained from Merck (Merck Company, Darmstadt, Germany, Glaxo India Limited, Bombay, India (No. 17584) and used without further purification.

2.3 Experimental exposure

The LC_{50} values of Cadmium chloride was calculated by Probit's method [48]. The 96hrs LC_{50} for $CdCl_2$ was found to be 925 ppm to *C. carpio* koi. After determining the LC_{50} values, 20 fishes of two batches were introduced into 35 L plastic tubs containing 25 L of water; 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 96hrs LC_{50} (92.5 mg/L)) sublethal concentration of $CdCl_2$. The concentration of the toxicant in the water was renewed daily. During the period of continuous exposure to the $CdCl_2$, the fish were fed with commercial feed on every alternate day at a rate of 3% body weight. The control and treated fishes were kept under continuous observation for 30 days under aerated condition. At the end of 15th and 30th days, 10 fishes were collected from each tub and the blood samples were collected through cardiac puncture using 1 ml dispovan heparinized syringes and stored in EDTA vials.

2.4 Haematological analysis

Haematological profiles were estimated by following the standard procedures of a scholar [49]. Haemoglobin (Hb) was estimated by the method of another scholar [50]. Red Blood Corpuscles (RBC) and White Blood Corpuscles (WBC) were enumerated in the Neubauer Haemocytometer (Improved Neubauer Weber Scientific Ltd.) following the method of a previous researcher [51]. Packed Cell Volume (PCV) was determined adopting the method of another researcher [52]. The red blood cell indices that include Mean Corpuscular Volume (MCV), Mean Corpuscular Haemaglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using the formula of a researcher [53]. Platelet (PLT) count was performed following the method of [54].

2.5 Statistical analysis

Significant differences between the control and experimental group were calculated by using Analysis of Variance (ANOVA) test followed by Tukey's post-hoc test. Data expressed in Mean±SD. All analysis was done using IBM SPSS Statistical Package (Ver. 20).

3. RESULTS

The exposure of freshwater ornamental fish, *Cyprinus carpio* koi to sublethal concentration of $CdCl_2$ for 15 and 30 days caused significant alterations in haematological profiles as shown in Table-1.

3.1 Estimation of Haemoglobin

As shown in Table 1, haemoglobin contents of control fishes after 15 and 30 days were 8.40±0.10 and 10.20±0.20 g/dl, respectively, whereas in treated fishes, the Hb contents after 15 and 30 days were 7.50±0.10 and 5.36±0.05 g/dl, respectively. The percentage changes over control were -10.71 and -47.45%, respectively. One-way ANOVA followed by Tukey's test revealed that after 15th and 30th days of exposure there was significant difference ($P < 0.05$) between control and treated groups.

3.2 Total WBC count

The WBC counts in control fishes after 15 and 30 days showed an average number of 1.69±0.02 and 1.87±0.01 thousand cells/mm^3 of blood, respectively, whereas the treated fishes showed a significant increase in WBC count ($P < 0.05$). The WBC counts of treated fishes after 15 and 30 days were 2.19±0.05 and 3.16±0.01 thousand cells/mm^3, respectively. The percentage variations from the control group were +29.58 and +68.98% of cells, respectively.

3.3 Total RBC count

The average number of RBCs in control fishes after 15 and 30 days were estimated to be 1.49±0.01 and 2.10±0.1 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 15 and 30 days were 1.21±0.02 and 0.88±0.01 million cells/mm^3 of blood respectively. The percentage changes over the control were-18.79 and -58.09% of cells, respectively.

3.4 Packed Cell Volume (PCV)

PCV levels of control fishes after 15 and 30 days were found to be 10.46±0.15 and 12.62±0.01%, respectively. In treated fishes, the PCV levels after 15 and 30 days were 8.43±0.11and 6.27±0.05%, respectively. The percentage changes over the control were -19.40 and -50.31%, respectively. One-way ANOVA followed by Tukey's test revealed that after 15th and 30th days of exposure, there was a significant difference ($P < 0.05$) between control and $CdCl_2$ treated group.

3.5 Mean Corpuscular Volume (MCV)

MCV levels of control fishes after 15 and 30 days were 82.36±0.01 and 85.23±0.01 fl, respectively. The treated fishes showed a significant increase in MCV levels ($P < 0.05$). The MCV levels of treated fishes after 15 and 30 days were 84.63±0.02 and 90.35±0.01 fl, respectively. The percentage changes over the control were +2.75 and +3.67%, respectively.

3.6 Mean Corpuscular Haemaglobin (MCH)

The MCH levels of control fishes during 15 and 30 days were 32.84±0.01 and 35.48±0.005pg, respectively. In the treated fishes, the MCH levels were 30.22±0.01 and 27.62±0.02pg, respectively. The percentage changes over the control were -7.97 and -22.15%, respectively. One-way ANOVA followed by Tukey's test revealed that after 15th and 30th days of exposure, there was a significant difference between control and $CdCl_2$ treated group ($P < 0.05$).

3.7 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC levels of control fishes after 15 and 30 days were 40.66±0.01 and 44.56±0.02%, respectively. The treated fishes showed a significant decrease in MCHC ($P< 0.05$). The MCHC levels of treated fishes after 15 and 30 days were 39.77±0.01 and 37.50±0.2%, respectively. The percentage changes over the control were -2.18 and -15.85%, respectively.

3.8 Platelet Count

The platelet (PLT) counts of control fishes after 15 and 30 days were 5.95±0.005 and 7.87±0.011 10^9/l, respectively. In the treated fishes, the platelet counts after 15 and 30 days were 3.96±0.005 and 3.53±0.015 10^9/l, respectively. The percentages changes over the control were - 33.44 and -55.14%, respectively. One-way ANOVA followed by Tukey's test revealed that there was a significant difference between control and $CdCl_2$ treated group ($P < 0.05$).

4. DISCUSSION

Hematological parameters can be used as a diagnostic tool in toxicology to detect the fish health status under different stress conditions like diseases, hypoxia and exposure to heavy metals and pollutants [55-59]. Heavy metals have a tendency to accumulate in different tissues of fish resulting in the reduction of DNA, RNA, protein, carbohydrate and lipids contents of the cell [60-63]. In the present investigation, the haematological indices such as Hb, WBC, RBC, PCV, MCV, MCHC and PLT levels were observed in the fish (*C. carpio* koi) exposed to $CdCl_2$. The haematological parameters like Hb, RBC, PCV, MCH, MCHC and PLT were significantly ($P < 0.05$) decreased whereas the profiles like WBC and MCV values were significantly ($P < 0.05$) increased when compared to that of control (Table 1).

As shown in Table 1, the Hb content shows a decline in the heavy metal treated fishes. It was found to be corresponding to the lowering of RBC and might be due to the impairment of haemopoietic system as a result it leads to anemia associated with erythropenia. The results of the present study support the 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 [64]. According to a group of researchers, 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 [65,66]. A researcher suggested that the decline in Hb content in fish exposed to toxicants might also be due to the inhibitory effect of the toxic substance on the enzyme system responsible for the Hb synthesis [67]. Prolonged decrease in Hb content impaired an oxygen supply, blood dyscrasia and degeneration of the erythrocytes leading to hypoxia conditions in fishes exposed to toxicants [68-71]. Significant reduction in the RBC and Hb in fishes exposed to heavy metals was also reported by earlier studies [72-75].

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 lymphomyeloid tissue to combat the toxicant stress. Similarly, the findings of several researchers suggested that 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 [76-84].

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 [85]. In contrast, a researcher 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 [86]. In general, the reduction in the levels of RBCs and PCV (due to the destruction of RBC or erythroblastosis) results in anaemia [87].

The anaemic condition in fish may also be attributed to low number of RBCs or too low levels of Hb in the RBCs. A researcher suggested that the anemic condition was probably not due to the increased destruction of erythrocytes, since the spleen size was unchanged but might be due to a reduced RBC synthesis or release of blood cells into the circulation [88]. A previous researcher noticed a decrease in the number of erythroblasts-proerythrocytes when goldfish, *C. auratus* and rainbow trout, *Salmo gairdneri* were exposed to Cadmium [89]. It was also noticed that the cadmium caused a decreased erythropoiesis and impaired the production of RBCs. Additionally, a researcher stated that the haemolysis of blood took place by stimulatory effects of toxicants in RBCs, which altered the permeability of the cell membrane or increased mechanical fragility [90]. The decline in the level of Hb, PCV and platelet count of *C. carpio koi* during exposure to $CdCl_2$ clearly implied a haemodilution 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 a previous researcher [91-94].

The haematological 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. This falls in line with the findings of a researcher, who had recorded similar changes in MCV, MCH and MCHC of *L. rohita* treated with Chromium. Significant increase in MCV and WBC count implies that the anemia is of Macrocytic type [95,96]. The increase in MCV may be due to swelling of RBC as a result of hypoxia condition or Macrocytic anaemia in the fishes exposed to heavy metals as suggested by a researcher [97]. 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 haemoglobin in circulation. Meanwhile, the significant decline in MCH and MCHC values might be due to Hypochromic anemia.

5. CONCLUSION

This study clearly indicates that $CdCl_2$, a biologically non-essential toxic heavy metal, caused severe anemia and alterations in haematological profiles under laboratory conditions. Therefore, the haematological parameters are considered as most sensitive in monitoring toxicity of Cadmium and other heavy metals especially at sublethal doses. Further, the fish *C. carpio* koi may be considered as a suitable model to detect the toxicity of xenobiotics drained or contaminated in the aquatic ecosystems. The present basic information of the haematological parameters would serve as a useful tool for further ecological assessment and monitoring of these aquatic organisms, which is considered to be an important food source for human beings.

ACKNOWLEDGEMENTS

Authors are thankful to Management, Principal and Head of the Department of Zoology, The New College, Chennai-14, Tamil Nadu, India for providing necessary facilities.

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Table 1: Haematological profiles of *Cyprinus carpio* var. koi exposed to sublethal concentration of $CdCl_2$.

	Exposure periods					
Haematological profiles	15 Days			30 Days		
	Control	Treatment	% changed	Control	Treatment	% changed
Hb(g/dl)	8.40±0.10	7.50±0.10*	-10.71	10.20±0.20	5.36±0.05*	-47.45
WBC (thousand cells/mm^3)	1.69±0.02	2.19±0.05*	29.58	1.87±0.01	3.16±0.01*	68.98
RBC (million cells/mm^3)	1.49±0.01	1.21±0.02*	-18.79	2.10±0.1	0.88±0.01*	-58.09
PCV (%)	10.46±0.15	8.43±0.11*	-19.40%	12.62±0.01	6.27±0.05*	-50.31
MCV (fl)	82.36±0.01	84.63±0.02*	2.75	85.23±0.01	88.36±0.01*	3.67
MCH (pg)	32.84±0.01	30.22±0.01*	-7.97	35.48±0.005	27.62±0.02*	-22.15
MCHC (%)	40.66±0.01	39.77±0.01*	-2.18	44.56±0.02	37.50±0.2*	-15.84
PLT (10^9/l)	5.95±0.01	3.96±0.05*	-33.44	7.87±0.011	3.53±0.015*	-55.14

C: Control, T: exposed. Values are expressed as Mean±SD of ten individual observations, -: Percent decrease over control, * indicates significant changed at P < 0.05.