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RESEARCH ARTICLE

The Impact of Toxic Cadmium Chloride on the Hematological Parameters in Fresh Water Fish *Cyprinus carpio* (Linnaeus)

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ABSTRACT

In this present experiment the toxicity of fish *Cyprinus carpio* were exposed in both lethal and sub lethal concentration of Cadmium Chloride for (24h, 48h, 72h & 96 hours) were exposed. The acute toxicity (LC_{50}) of Cadmium Chloride was found to be 14 mg/L. One fifth ($1/5^{th}$, i.e. 2.8 mg/L) of the acute toxicity value was selected as the sub lethal concentration for sub acute test. The present investigation was carried out to study the impact of toxic Cadmium Chloride on the haematological parameters in fresh water fish *Cyprinus carpio* in both lethal (1, 2, 3, and 4 days) and sub lethal (1, 5, 10, and 15 days) concentration. The blood parameters like red blood corpuscle (RBC), white blood corpuscle (WBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are commonly studied in fishes to assess the impact of Cadmium Chloride in aquatic biota. In red blood corpuscle at lethal concentration maximum reduction of -65.397% was observed on day 4 and in white blood corpuscle the maximum count 10.610 x 10^3 was observed on day 1 under lethal concentration, which later regressed on 2^{nd}, 3^{rd} and 4^{th} day. The anaemia produced due to Cadmium Chloride treatment might be the reasons for the decrease in the RBC count, haemoglobin percentage and haematocrit value at lethal concentration, which therefore clearly suggests the species response to Cadmium Chloride.

Key words: *Cyprinus carpio,* Cadmium Chloride, Lethal and Sub lethal, Haematological, Anaemia.

INTRODUCTION

Blood is a fluid connective tissue circulating in the body. It provides one of the methods of communication between the cells of different parts of the body. Haematological studies plays an important role in understanding variation of blood characteristics in relation to factors like phylogenetic position, ecological habitat, pollutants, food selection, etc. The regular monitoring of the fish blood is a diagnostic tool in establishing the health status of the fish in farm. It helps in evaluating the response of different types of blood cells and its components in the conditions of physiological stress due to toxicity, as it quickly reflect the poor conditions of fish than other commonly measured parameters. The blood composition of a fish reflects to some extent to metabolic and other physiological processes. Accordingly, haematology can be used as clinical tool for the investigations of physiological and metabolic alterations in fish caused by pollution of the aquatic environment Anand Kumar, (1994).

New agricultural practices, rapid industrialization and public health programmes have resulted in the release of several toxic chemicals into the nature. The possible ill effects of these chemicals on non target organisms have lead to an investigation of their influence on animals including fish. Foundation of the work on haematology of fishes was laid down in the early 20^{th} century when Krough studied respiratory function of blood in fishes. Since then, the work on haematology of fishes has been carried out by Munian and Veeraraghavan, (1999) and Shivakumar, (2004). Haematological variables of fish under stress are of great significance in assessing the impacts of pollutants in the biota of a particular ecosystem.

Blood is a vehicle for quickly mobilizing defence against trauma and diseases. Since fishes differ considerably in their activity patterns and respond

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to the pollutant. The blood parameters like red blood corpuscle (RBC), white blood corpuscle (WBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are commonly studied in fishes to assess the impact of Cadmium Chloride in aquatic biota.

RBC and Hb can he used as an index of anaemia and f1uid volume disturbances. Reduction in RBC and haemoglobin is the most prominent haematological effect of heavy metals. Anaemia may be due to an increase of plasma volume caused by disturbed water balance, decreased rate of RBC production, loss or destruction of RBC Saraswathi *et al.,* (2003). Haemoglobin is the main constituent of blood and plays an important role in the transport of oxygen throughout the blood capillaries. Lowered haemog1obin level might reduce the ability of the fish for seeking food and escaping from predators due to stress syndrome. Many workers have reported alteration in RBC and Hb level Garg *et al.,* (1989); Magare and Patil, (2000). In the present investigation an attempt has been made to study the lethal and sub lethal effect of Cadmium Chloride on haematological aspect of fish.

MATERIALS AND METHODS

Sample Collection, Maintenance and Acute Toxicity Test

For this study I have selected easily available fresh water fish *Cyprinus carpio* Common carp in which heavy consumption of human being life everyday throughout the world. The biological test system *Cyprinus carpio* weighing about 5 ± 2 g and measuring an average length of 4-5 cm were collected from the State Fisheries Department, Tumakuru, Karnataka, India and these fishes were kept in large aquarium. The experimental fishes were acclimatized to laboratory conditions for 15 days in tap water whose physico-chemical characters were analyzed by following the international guideline method APHA, (2005). Water media was renewed every day and maintained a 12-12h photoperiod during acclimatization and test periods. The fishes were fed regularly with commercially available fish food pellets during acclimatization and test tenures but feeding was stopped two days prior to treatment to test medium for acute toxicity test. Cadmium Chloride about 95% purity was procured from local market of Bangalore, Karnataka, India, under the trade name Thermo Fisher Scientific India Pvt Ltd., Supplied by Vasa Scientific Co., Bangalore, Karnataka, India. The Quantity of Cadmium Chloride at a concentration of (9mg/L, 11mg/L, 14mg/L, 18mg/L and 22mg/L) was prepared and exposed to ten fish per concentration along with 20 L of water for each concentration with control replicates and (LC_{50}) of Cadmium Chloride was found to be 14 mg/L. The LC_{50} value at (24h, 48h, 72h & 96 hours) was determined by following the method of Finney (1971). Based on the results of LC_{50}, the fishes were exposed sub lethal concentrations for (1, 5, 10 and 15 days) One fifth ($1/5^{th}$, i.e. 2.8 mg/L) of the acute toxicity value was selected as the sub lethal concentration for sub acute test. Renewal of the water medium after every 24h up to 96h was followed by the addition of the desired dose of the test compound. For LC_{50} calculation mortality was recorded every 24h and the dead fish were removed when observed, every time noting the number of fish death at each concentration up to 96h exposure periods.

Collection of Blood and Determination

Blood was collected by the method of Steuke and Schoettgr, (1970) by severing the caudal peduncle. Before considering the blood for hematological observation, a drop was placed on a clean slide and examined for the presence of parasites if any. Blood free from any infection was used to study the hematological parameters to avoid variation; the samples were taken at a particular time during early hours of the day. Ethylene diamine tetra acetic acid (EDTA) was used as an anticoagulant. RBC by Davidson and Henry (1969). WBC by Donald Hunter and Hennry, (1969). Hb by Dacie and Lewis, (1961). PCV by Schalm *et al.,* (1975). MCV, MCH and MCHC were calculated by the following formula:

$$\text{MCV} = \frac{\text{PCV} \times 10}{\text{RBC count (In millions per Cumm)}}$$

$$\text{MCH} = \frac{\text{Haemoglobin (grams/deci liter)}}{\text{RBC count}}$$

$$\text{MCHC} = \frac{\text{Haemoglobin (grams/deciliter)}}{\text{Packed cell volume}}$$

RESULTS

The results of the haematological parameters are found to be time dependent. The summary of the results after exposing fish, *Cyprinus carpio* to lethal (1, 2, 3, and 4 days) and sub lethal (1, 5, 10,

and 15 days) concentrations of Cadmium Chloride is as follows.

Red Blood Corpuscle (RBC)

Results of total erythrocyte count presented in the **(Fig. 1)** indicated alteration at both lethal and sub lethal concentrations. At lethal concentration maximum reduction of -65.397% was observed on day 4. At sub lethal concentration the reduction was observed only up to day 10, which later decreased on day 15. Under lethal concentration the count ranged from $0.605x10^6$ to $1.501x10^6$ and under sub lethal concentration of Cadmium Chloride the count ranged from $1.211x10^6$ to $1.618x10^6$.

White Blood Corpuscle (WBC)

The WBC count altered specifically. Maximum count 10.610 x 10^3 was observed on day 1 under lethal concentration, which later regressed on 2^{nd}, 3^{rd} and 4^{th} day. In sub lethal concentration of Cadmium Chloride, WBC count increased from day 1 to day 10 and later decreased on day 15 showing normalcy. The results are presented in the **(Fig. 2)**.

Haemoglobin (Hb)

A result of haemoglobin presented in the (**Fig. 3**) suggests the progressive reduction at lethal concentration. The decrease was -57.588% up to day 4. At sub lethal concentration of Cadmium Chloride on day 1, 5, 10 and 15 registered continuous decrement.

Packed Cell Volume (PCV)

The trend of PCV value was like that of RBC and Hb at lethal and sub lethal concentration. The results are presented in **(Fig. 4)**. In the lethal concentration of Cadmium Chloride decrease was up to -57.657% on day 4. During sub lethal concentration decrease from -5.339% to 25.416% was observed up to 10^{th} day. While approaching towards 15^{th} day decrease of -23.768% was observed as compared to day 10.

Mean Corpuscular Volume (MCV)

MCV value calculated on the basis of PCV and RBC values and presented in the **(Fig. 5)**. The values exhibited enhancement at lethal concentration of Cadmium Chloride. At lethal concentration the percent elevation ranged from 7.021% to 30.684%. In contrary at sub lethal concentration the elevation was seen up to day 5, which later decreased on day 10 and 15. The maximum elevation was 12.742% on day 5.

Mean Corpuscular Haemoglobin (MCH)

MCH represents the average weight of haemoglobin in RBC. MCH exhibited increase in all exposure periods of lethal concentration of Cadmium Chloride. Maximum increase was observed on day 4 (23.725%). At sub lethal, the increase was up to 21.871% on day 10. The values are presented in **(Fig. 6)**.

Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC is the indication of average concentration of haemoglobin in RBC cells, calculated based on Hb and PCV. Decrease in Hb and PCV coupled with increase in MCV might be the reason for the decreased MCHC. At lethal concentration of Cadmium Chloride decrease in value was registered with a maximum decrease of -16.103% on day 4. The maximum decrease registered was -15.580% at 5^{th} day of the exposure in sub lethal concentration. The results are presented in **(Fig. 7)**.

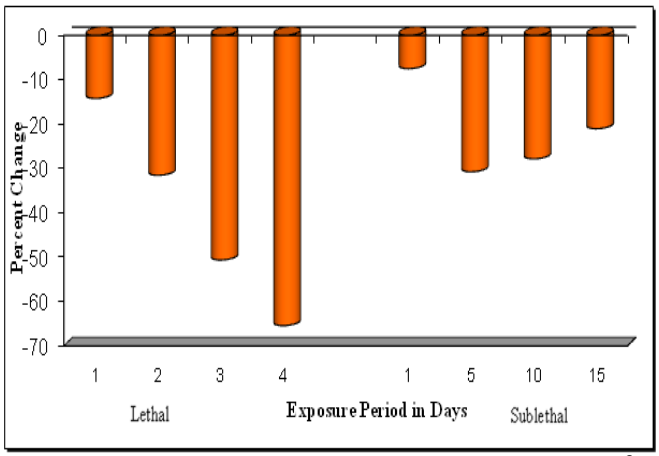

Figure 1: Percent change over control in RBC count (x 10^6 / mm^3) in the blood of the fish, *Cyprinus carpio* on exposure to lethal and sub lethal concentrations of Cadmium Chloride

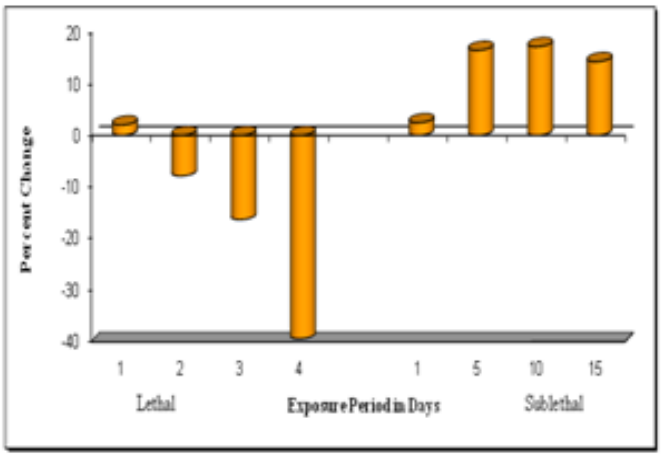

Figure 2: Percent change over control in WBC count (x 10^3/mm^3) in the blood of the fish, *Cyprinus carpio* on exposure to lethal and sub lethal concentrations of Cadmium Chloride

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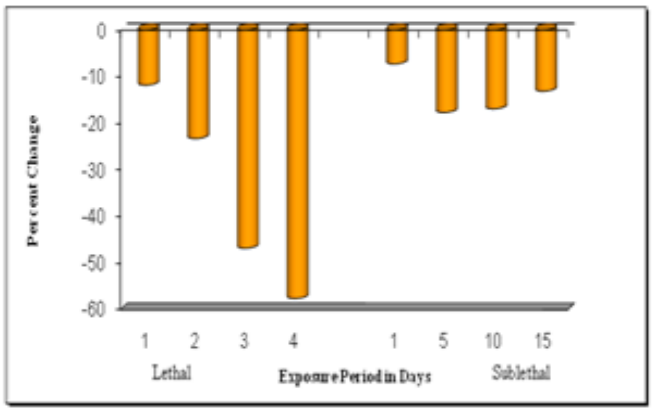

Figure 3: Percent change over control in haemoglobin level (g/100 ml) in the blood of the fish, *Cyprinus carpio* on exposure to lethal and sub lethal concentrations of Cadmium Chloride

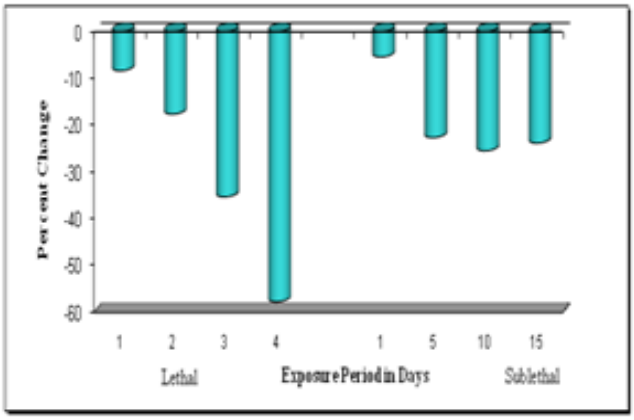

Figure 4: Percent change over control in PCV level (%) in the blood of the fish, *Cyprinus carpio* on exposure to lethal and sub lethal concentrations of Cadmium Chloride

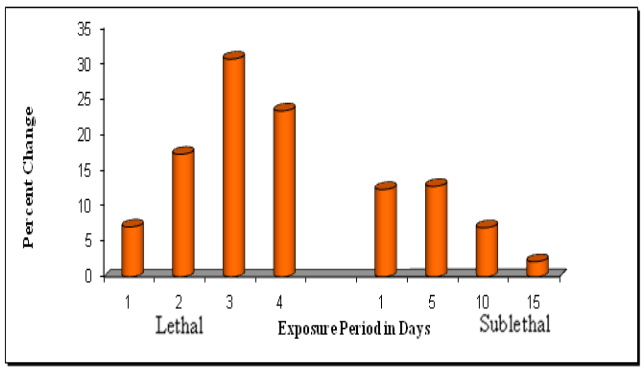

Figure 5: Percent change over control in MCV level (cu mm) in the blood of the fish, *Cyprinus carpio* on exposure to lethal and sub lethal concentrations of Cadmium Chloride

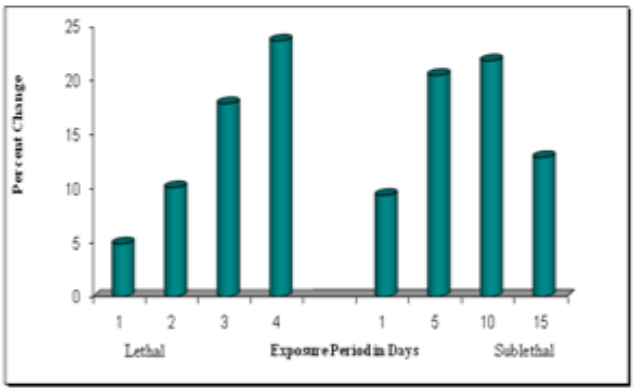

Figure 6: Percent change over control in MCH level (pg) in the blood of the fish, *Cyprinus carpio* on exposure to lethal and sub lethal concentrations of Cadmium Chloride

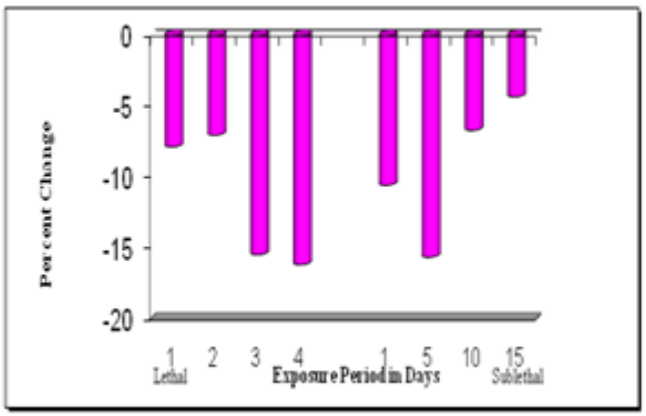

Figure 7: Percent change over control in MCHC level (%) in the blood of the fish, *Cyprinus carpio* on exposure to lethal and sub lethal concentrations of Cadmium Chloride

DISCUSSION

A haematological study plays a vital role in monitoring fish health, due to pollution load, stress and disease. Therefore, it is an important index to study the impacts of toxins on fishes. In the present investigation fish, *Cyprinus carpio* treated to the lethal and sub lethal concentrations of Cadmium Chloride, showed considerable alteration in the level of different blood parameters. Decrease in total erythrocyte count, haemoglobin percentage, PCV values indicates the occurrence of anaemia associated with erythropenia. Various workers Agarwal, (1983); Sen *et al.*, (1993) have also obtained similar results while investigating with different toxicants. The erythrocytes also show deformity and this may be the possible cause of decrease in Hb percentage. Different workers have reported reduction in RBC and Hb in different fishes exposed to different toxicants. *Cyprinus carpio* exposed to Cadmium Chauhan *et al.*, (1994), H. fossilis exposed to dimecron Anand Kumar *et al.*, (2001). Very few workers have reported increase in RBC and Hb in fishes exposed to pollutants: *Puntius conchohius* exposed to mercury Gill *et al.*, (1988). They suggested that, the increment was might be due to the enhancement of erythropoiesis, which triggered as a typical stress response. Significant decrease in RBC count, haemoglobin percentage and haematocrit value reflects the anaemic state of fish. Anaemia associated with decreased RBC, Hb percent and haematocrit value has been recorded to occur in response to heavy metals Christensen *et al.*, (1972); Garg, *et al.*, (1989). Web, (1966) attributed the decrease in RBC count and haemoglobin concentration to haemolysis. The reduced observation in the RBC count and haemoglobin concentrations suggests that an acute anaemia was induced in the fishes under Cadmium Chloride intoxication. The subsequent

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rise in RBC count and haemoglobin concentration, which occurred, on exposure to sub lethal concentration of Cadmium Chloride beyond 10 days was probably due to enhanced erythropoiesis.

In the present investigation WBC count under lethal concentration increased on first day of exposure period, which later decreased consecutively. But under sub lethal the level increased from day 1 to day 10, which decreased on day 15 showing normalcy. The initial increase in the WBC count was might be the result of direct stimulation of immunological defence due to the presence of toxic substance or may be associated with induced tissue damage. Decrease in WBC count in later days might be due to series of changes in the immunological set up of the fish under heavy metal stress Anand Kumar, (1994). Kalavathy *et al.,* (2001) suggested that decrease in WBC count could be because of autolysis caused due to haemolytic enzymes leaked out by cells under toxicants. Few workers reported increased WBC count throughout the exposure period Gautam and Kalpana, (2002); Kalavathy *et al.,* (2001).

Haemoglobin is synthesized in the body of an organism form protein and iron. Liver is the storehouse of iron in the body Anand Kumar, (1994). This might have led to the non-availability of sufficient iron in the body of the exposed fish leading to a decrease in Hb percentage. A reduced Hb percent could have led to a decrease in oxygen carrying capacity of the blood. Haematocrit (PCV) values of the fish in the present study showed decreasing trend at both lethal and sub lethal concentration of Cadmium Chloride. Drop in PCV in present investigation could be attributed to low RBC count or haemodilution. Renal tissue damage due to cadmium stress might have led to low erythropoietin level leading to decrease in RBC count and hence a drop in PCV value Anand Kumar, (1994). Reduction in PCV level was observed in *Clarias batrachus* exposed to endosulfan and kelthane Venkateshwaralu *et al.,* (1990). In parallel to present study Kumar and Agarwal, (1993) and Janardan Reddy *et al.,* (1991) has observed increased haematocrit values in fish, *Clarias batrachus* and *Oreochromis mossambicus* exposed to mercuric chloride and phosalone. They suggested it to be due to oxygen tension that promotes the production of erythropoietin. MCV values exhibited gradual decrease at sub lethal concentration. But in sublethal concentration the increase was registered up to day 5, which later decreased. Anand Kumar, (1994) suggested increase in MCV to be because of endosmosis. Endosmosis leads to the passage of solvent from less concentrated solution to more concentrated one. This results in haemodilution, further increasing the MCV value. Workers like Garg *et al.,* (1989), Anand Kumar, (1994), Dhanapakiam and Ramasamy, (2001) reported increase in MCV of fishes exposed to toxicants. Few workers Nath and Banerjee, (1996) reported decrease in MCV value in fish. Janardan Reddy *et al.,* (1991) reasoned decrease in MCV for the variation in red cell volume that attributes the occurrence of exosmosis indicated by increased electrolyte concentration inside the red cell after insecticide treatment.

David (1995) observed a decrease and then a subsequent increase of MCV in *L. rohita* exposed to low and high sublethal concentration of fenvalerate. Janardan Reddy *et al.,* (1991) observed increase in MCV of *Tilapia mossambica* exposed to sublethal concentration of sumithion. As MCH and MCHC are derived from Hb and RBC any sort of alteration in the levels of Hb and RBC would result in the alteration of MCH and MCHC.

CONCLUSION

In this present investigation was carried out to study the impact of toxic Cadmium Chloride on the hematological parameters in fresh water fish *Cyprinus carpio*. And the present study thus confirmed that hematological parameters are very sensitive indicators of fish organisms and also shows that the anaemia produced due to Cadmium Chloride treatment might be the reasons for the decrease in the RBC count, haemoglobin percentage and haematocrit value at lethal concentration, which therefore clearly suggests the species response to Cadmium Chloride and it is an evidence to carry the experiment further.

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