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**Original Article**

# Effect of cadmium exposure on hematological and morphological changes in fresh water fish (*Labeo rohita*)

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**ABSTRACT**

Cadmium (Cd) is a well-known and highly poisonous metal which presents a major health concern in exposed organisms. It is a naturally occurring metal in the environment which comes from industrial and agricultural process. Cadmium toxicity has been recorded in different countries across the globe. Therefore, this study executed to investigate the hematological, nuclear changes (micronuclei) and morphological alterations in erythrocytes of fresh water fish (*Labeo rohita*) exposed to sub-lethal dose (1.495 mg/L; 1/3rd $LC_{50}$) of cadmium for 28 days. The results on blood investigation revealed that various blood parameters like erythrocyte, hemoglobin (Hb), hematocrit (Hct), and lymphocytes exposed to heavy metal showed significantly decreased values in treated group except white blood cells (WBCs) that showed significant increase in treated group. Various morphological alterations (sphrerocyte, dividing erythrocyte, microcyte, leptocyte, pear shape erythrocytes, tear shaped erythrocyte and leptocyte) were increased in cadmium treated as compared to unexposed group. The findings of current experiment work report for the first time the hematological and morphological abnormalities in the blood of fish due to Cd toxicity which may be considered as the biomarkers of cadmium toxicity in other experimental species.

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## Introduction

The prevalence of intoxication has tremendously risen during last few decades due to the widespread availability of numerous chemical compounds (Rahimzadeh et al. 2017; Akram et al. 2021; Ghaffar et al. 2021). Contamination of freshwater with diverse pollutants due to industrialization has led to ubiquitous exposure of aquatic biota to different metals from last few decades (Vander Oost et al. 2003; Jabeen and Javed 2012; Akram et al. 2021). Long term exposure to these pollutants (cadmium, mercury, lead, arsenic and chromium) causes countless abnormalities and reduces the life expectancy of organisms (Jabeen et al. 2012; Khan et al. 2012; Naz et al. 2020).

Cadmium (Cd) particularly is a dangerous metal due to its widespread prevalence in the environment. In rivers and lakes, Cd concentrations range from 1.120 to 8 g/L, and can even exceed to 0.8 mg/L, which is significantly higher than in typical freshwater. Hence, Cd poisoning poses a constant hazard to aquatic species (Guo et al. 2018). Cadmium is deliberately reported as one of toxic metals and associated with hematologic toxicity in a number of fish species. Fish blood is extensively used as a biomarker of hematological parameters for heavy metal intoxication (Yamin et al. 2020; Ghafar et al. 2021).

In aquatic ecosystems, Cd and other heavy metals discharge come from sewage effluent, mining and processing of metals (Ghaffar et al. 2016; Ghaffar et al. 2021). Even though studies have determined that Cd is continuously released into aquatic and terrestrial ecosystems resulting in deposition of this metal into the body of various aquatic and terrestrial organisms (Abdel-Tawwab and Wafeek 2017). Reports have investigated that aquatic organisms particularly fish are the major part of food of human and consumption of meat of contaminated fish with heavy metal may cause adverse effects in exposed organisms (Yamin et al. 2020). Different studies have

investigated the presence of different heavy metals in commercial fish (Begum et al. 2005). It has been observed that contaminated fish can be a major health problem (Castro-Gonzales et al. 2008; Ghaffar et al. 2018; Akram et al. 2021). Fish accumulates Cd directly from water-borne and dietary sources. Cadmium is directly engrossed by fish in its free ionic form ($Cd^{2+}$) due to chronic sub-lethal water-borne exposure that can lead to subsequent ingestion of Cd-contaminated fish by consumers like humans (Drexler et al. 2003). Thus, measuring of the lethal impacts of metals on aquatic organisms is of vital importance for the screening hazardous substances.

Biomarkers, such as blood profile and different enzymes involved in normal physiological metabolism might be utilized to assess physiological changes in organisms (Hussain et al. 2019). Hematological characteristics might be utilized as markers of fish health status under endogenous or external stresses, according to certain studies (Li et al. 2021). Therefore, we performed this research to know the sub-lethal effects of Cd on fish hematology to reduce physiological impairments and optimum functioning.

## Materials and Methods

### *Experimental design*

A total of 36 freshwater fish (*Labeo rohita*) weighing about 6.20±2.38g and measuring 9.60±1.84cm in length were collected form commercial hatchery in plastic bag containing appropriate oxygen. After acclimatization, fish were distributed into two groups, control group containing normal tap water with continuous aeration and treatment group were subjected to sub-lethal dose (1/3 $LC_{50}$, 1.495 $mgL^{-1}$) of Cd based on pre-determined value of $LC_{50}$ (Tiwari et al. 2011). The experiment was conducted by employing a semi-static system.

### *Blood collection for hematological studies*

After 28 days, blood samples from the control and Cd treated fish were obtained from caudal peduncle with the help of syringe and transferred to eppendorf tubes containing EDTA as anticoagulant (Akram et al. 2021).

Total count of RBC and WBCs were estimated according to previous literature using Neubaur haemocytometer (Hussain et al., 2018, 2019; Ghaffar et al. 2021). Different other blood parameters were measured according to the methods as reported earlier (Ghazanfar et al. 2018; Bojarski et al. 2021; Ghaffar et al. 2021).

### Morphological changes and genotoxic Studies

For morphological changes and genotoxic investigation, thin blood smears were prepared from all the experimental fish in both groups.

### Statistical analysis

Values were analyzed and statistically data were compared with the help of SPSS at 95% confidence level and level of significance was set at $p<0.05$. All slides for morphological changes were observed under an optical microscope (Optika B-150).

## Results

### *Hematological profile*

Erythrocytes count (RBC) of *Labeo rohita* treated with sub lethal dose of Cd showed statistically highly significant variation between exposed and unexposed fish (Table 1). Haematological analysis showed significant decreased level of hemoglobin and hematocrit values in treated fish. The mean values of total leucocytes increased significantly in treated group as compared untreated group. The platelet count of the treated fish also increased in Cd treated fish. The lymphocytes decreased in Cd treated fish in comparison to untreated group. Monocytes of treated group manifested a significant decrease in number as compared to non-treated group. A prominent variation was observed in the quantity of neutrophils and a notable increase was recoded in Cd treated fish (Table 1).

### *Morphological and nuclear changes*

Peripheral blood smears examination showed normal and homogenized appearance of cytoplasm in red blood cells of control group. The shape and nucleus of red blood cells of untreated control fish was normal while in treated fish different morphological and nuclear abnormalities were recorded in Cd treated fish. Morphological and nuclear variations examined in the treated group indicated increased frequency of red blood cells with morphological and nuclear alterations. During present investigations, morphological abnormalities in the erythrocytes of the fish exposed to Cd included increased percentile rate of sphrerocyte, dividing erythrocyte, microcyte, leptocyte, pear shape erythrocytes, tear shaped erythrocyte and leptocyte (Table 2) when compared to non-treated fish.

**Table 1:** Blood profile of freshwater fish (*Labeo rohita)* exposed to cadmium

| Hematological Parameters | Treatment/Metal exposure | |
|---|---|---|
| | Control Group | Treated group |
| Erythrocyte (106/mm3) | 4.39±0.13 | 3.43±0.07* |
| Hemoglobin (g/dl) | 10.11±0.11 | 7.13±0.05* |
| Pack cell volume (%) | 33.31±1.03 | 24.27±1.11* |
| WBC Count ($x10^3/\mu L$) | 9.11±1.69 | 12.5±7.32* |
| Neutrophils (%) | 31.1±8.05 | 53.8±4.74* |
| Lymphocytes (%) | 31.73±3.41 | 22.8±04.4* |
| Monocytes (%) | 4.4±0.36 | 3.2±0.98* |
| Eosinophils (%) | 2.4±1.36 | 2.25±1.18 |

Values (mean ± SE) having asterisks are significantly different ($P \leq 0.05$) from normal fish

**Table 2:** Different morphological alterations in red blood cells of fresh water fish (*Labeo rohita)* exposed to cadmium

| Morphological changes | Treatment/Metal exposure | |
|---|---|---|
| | Control Group | Treated group |
| Hypochromia | - | **++++** |
| Microcytosis | - | **+++** |
| Anisocytosis | - | **++** |
| Poikilocytosis | - | **++** |

Semi-quantitative scoring for the evaluation of morphological changes. No distinct morphological changes (-), mild (++), moderate (+++) and severe morphological changes (++++)

**Discussion**

Stress due to heavy metal (Yamin et al. 2020) and different toxicants including industrial waste (Aram et al. 2021) and pesticides (Ghaffar et al. 2020, 2021; Hussain et al. 2020) adversely affects the fish health by causing hematological impairments, hemolysis (erythrocytes) and leukocytosis (Ghaffar et al. 2016). In the present investigation, *Labeo rohita* exposed to Cd concentration (1.495mgL$^{-1}$) for 28 days depicted changes in behavioral patterns and haematological indices of treated group as compared to the control group.

Different studies have highlighted that hematological profile including lymphocyte and monocytes are valuable in monitoring toxicity in different animals especially fed with different chemical compounds in feed and water (Oyawoye and Ogunkunle 2004; Hussain et al. 2019; Ghaffar et al. 2021).

The decline in the RBC count during the current investigation might be due to negative impact of Cd resulting in the inhibition of RBC during the hematopoisis process. Leukocytes are mainly classified into a granulocytes (lymphocytes and monocytes) and granulocytes (neutrophils, basophils and eosinophils). The leucocytes count of treated fish in this study showed significantly increased values suggestive of injuries to different tissues of fish due to Cd toxicity. The abrupt increase in the WBC count is considered as a response to injurious stimuli and any stress condition. The decreased values of monocytes and lymphocytes in treated fish exposed to Cd might be due to the weaker immunological response of fish. The lower hematological parameters (hemoglobin and hematocrit) can be related to toxic impact of Cd on hematopoiesis process.

Similarly, decline in RBCs levels was reported in different earlier studies (Chandanshive et al. 2012; Ghaffar et al. 2016, 2021) after exposure to different toxicants including heavy metals (Ghaffar et al. 2016; Yamin et al. 2020). Previously, a study investigated that reduction in total RBC count occurs due to exposure to heavy metals which may be associated with impaired intestinal absorption of iron (Joshi et al. 2002). Similarly decreased number of RBC in fish caused by toxicant exposure has been recorded (Chowdhury et al. 2004; Ghaffar et al. 2021).

In the present study, leukocytosis in Cd treated fish may be due to the increased accumulation of Cd in liver and different tissues of exposed fish. Shah and Altindag (2005) reported an increase in leucocytes number as a counteracting mechanism of lymphoid tissues to obliteration of circulating lymphocytes. Leukocytosis (increase in WBC number) is attributed to stress and injury as a result of direct stimulation of immune system for fish survival exposed to heavy metal stress. The increase in WBC count in Cd treated fish could be injurious stimuli to various visceral organs. Previously, decreased levels of different blood parameters in *C. gariepinus* treated with lead have also been reported (Adeyemo 2007). It is reported that leucocytosis is a compensatory response under pathological conditions and heavy metal stress. In the present study, the decreased hemoglobin contents of treated fish are in agreement with earlier findings (Chen 2002; Vinodhini and Narayanan 2009). Earlier study has determined lower amount of haemoglobin in fish exposed to toxic chemical (Allin et al. 2000).

Hematocrit and reduced hemoglobin contents are suggestive of anemia in treated fish which could be due to toxic effects of Cd on blood producing organs (Karuppasamy et al. 2005; Ayandiran et al. 2010). Senthamilselvan et al (2012) investigated haematological disturbances in *Lates calcarifer* in response to Cd toxicity. The investigation of different morphological and nuclear changes in erythrocyte of avian species play crucial role in determination of oxidative stress and toxic effects of different environmental contaminants. In this study, increased morphological alterations in red blood cells including sphrerocyte, dividing erythrocyte, microcyte, leptocyte, pear shape erythrocytes, tear shaped erythrocyte and leptocyte could be related to increase oxidative stress by Cd exposure. Previously, similar morphological alterations in red blood cells of fish due to heavy metals (Yamin et al. 2020), different synthetic and naturally occurring chemicals (Ghaffar et al. 2015, 2016; Akram et al. 2021), different pesticides have also been observed (Hussain et al. 2014, 2016, 2018, 2021). Different nuclear alterations in red blood cells of treated fish observed in this study might be due to abnormal interaction of Cd.

In addition, formation of micronuclei and other nuclear abnormalities in this experimental study could be due to DNA damage in erythrocytes and different other genic changes caused by Cd toxicity resulting to mitochondrion dysfunction via activation of physiologically abnormal nuclear proteins (Hussain et al. 2012, 2014; Akram et al. 2021).

**Conclusion**

In conclusion, it can be suggested that heavy metal stress seriously damages metabolic functions of fish as revealed in terms of as adverse impacts of cadmium on hematological and different morphological and nuclear changes in red blood cells. The present data confirmed that evaluation of changes in hematological parameters of fish is a sensitive aquatic toxicity biomarker for fish survival, fisheries management and aquaculture. Heavy metals usage without proper mitigation measures can induces variety of disorders in aquatic animals and public health.

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