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Toxicity Evaluation and Tissue Damaging Effects of Cadmium in *Labeo rohita*

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ABSTRACT

Heavy metals are polluting the freshwater ecosystems with hazardous impacts on aquatic animals especially fish. Considerable amounts of Cadmium (Cd) in aquatic ecosystems are posing a serious threat to sustainable growth of aquaculture industry. **Objective:** To investigate acute toxicity of Cd (96-hour LC50 and lethal concentrations) was determined by utilizing probit analysis method for the freshwater fish *Labeo rohita*. **Methods:** Different organs of fish (liver, gills and muscles) were also analyzed for accumulation of Cd by inductively coupled plasma mass spectrometry (ICP-MS). **Results:** Acute toxicity of Cd (96-hour LC50) for *Labeo rohita* was calculated as 159.59 mg/L. Bioaccumulation of Cd in different tissues of fish was examined in the following order liver>gills> muscles. Comet assay was performed for the evaluation of DNA damage by different sub-lethal doses (1/2, 1/3, 1/4 of LC50) of Cd. **Conclusions:** This research will aid in control of heavy metals contamination and conservation of fish species Labeo rohita in natural aquatic habitats of Pakistan.

INTRODUCTION

Metals are non-recyclable major pollutants of our environment. These are major cytotoxic, mutagenic and carcinogenic in action [1]. Elements having density of more than 5 g/cm3 are termed as heavy metals. Heavy Metals (HMs) have emerged as major cause of concern for humans because of their harmful effects to nature [2]. Animals can accumulate heavy metals in their body directly or indirectly. Terrestrial animals mainly accumulate them by inhaling in polluted environment, by drinking polluted water or by consuming contaminated food. Human activities are mainly responsible for degradation of air and water quality by releasing toxic gases in air from factories and discharging harmful chemicals in water reservoirs as their waste products. These chemicals and gases are major source of heavy metals pollution [3]. Emissions of gasses (SO2 and NO2) result in acid rain which causes metal poisoning in soil and ground water [4, 2]. Soil attrition, water runoff, dust deposition, aerosol sprays and release of contaminated waste water are some other sources of heavy metals entrance to water ecosystems [5]. Cadmium (Cd) is a nonessential element in nature and regarded as one of the major toxic heavy metals. It is discharged into water bodies by industrial sources (plating processes), refining and mining of ores, Ni-Cd batteries, pigments and utilization of phosphate fertilizers [6]. Fishes are very good bioindicators of heavy metals contamination in aquatic ecosystems as they are very important part of all food chains and food webs. They are mostly used as delicacy in

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many parts of the world by humans especially in coastal areas [7]. High concentrations of heavy metals in any body part of the fish will induce changes in growth rates, physiology, mortality and reproduction rate, serum biochemical changes, histopathological fluctuations and other stresses may also be examined [8, 9]. Cadmium induces a variety of pathomorphological and structural changes in different organs of fish. The highest levels of cadmium were observed in the liver and kidneys of fish [10]. It also causes biochemical and morphological fluctuations in the gills of fish [11]. In humans one of the basic routes of exposure to Cd is through the consumption of fish contaminated with Cd. Bioaccumulation of Cd in human body can cause impairment of renal function, osteoporosis, obstructive lung disease, hypercalciuria and prostate cancer [12]. Acute toxicity tests (96 hours LC50 and lethal concentrations) allow us to measure the effects of different toxicants in a short time on aquatic organisms such as fish [13]. The genotoxic impacts of different environmental pollutants such as heavy metals can be detected using comet assay. It has been utilized for accessing genotoxicity induced by different toxicants in peripheral erythrocytes in many fish species [14]. It has advantage over other biomarker assays because of its sensitivity for monitoring low levels of DNA damage [15]. Bioaccumulation of different heavy metals like Cd in fish requires continuous surveillance and monitoring because of biomagnifying potential of these metals in human food webs [11].

Thus, the objectives of this investigation were to measure the acute toxicity of Cd (water-borne), in terms of 96 hours LC50 and lethal concentrations for the fish *Labeo rohita*. To compare bioaccumulation of metal in liver, muscle and gills of *Labeo rohita* and DNA damage caused by different doses of Cd in fish.

METHODS

In Acute Toxicity Assay the live fingerlings of *Labeo rohita* (300 samples) were procured from Himalaya Fish Hatchery, Muridke and transported to Fisheries Research Farms, Department of Zoology, University of Gujrat, Pakistan and acclimatized for one week to laboratory conditions. Fish were kept in chlorine free tap water in cemented tanks supplied with filters and aerators. During this period fish (mean length: 15 cm) were selected and fed (35% protein and 2.90 kcal/g) once daily. However, no feed was provided for 24 hours before acute toxicity test. Laboratory tests were performed in aquaria with 40-liter water capacity. Analytical grade chloride of Cd ($CdCl_2.H_2O$) was used in stock solution preparation. Acute toxicity experiments (96-hour LC_{50} and lethal concentrations) were performed at constant physicochemical variables e: g water temperature (28°C), pH (7), total hardness (200 mg/L). All physicochemical parameters of water were analyzed by following APHA [16]. Three replications were employed for each test dose. A group of ten (10) fish were selected for each test concentration. Supply of fresh air was made possible through a pump to each aquarium for fish respiration. The test concentration of Cd was started from zero and increased slowly with an increment of 0.01 and 0.1 mg/L for both low and high concentrations, up to the full toxicant concentration in six hours. No mortality was noted among control group. Fish mortality data of fish was compiled and analyzed by using probit method [17]. In Bioaccumulation Assay the dead fish were separated at each 96-hours LC_{50} and lethal concentration of heavy metal (Cd) and lightly blotted dry. Fish were dissected and their desired organs (liver, muscles, gills) isolated and cleaned with distilled water. The samples were placed in aluminum foil and stored them in ultralow freezer at -20°C. Fish tissues were weighed 0.5 g and digested in a mixture of deionized water (3 ml), HNO_3 (7 ml) and H_2O_2 (1 ml) in a microwave digestion system for 31 min [18]. Digested samples were filtered with 0.45µm membrane filter paper. Samples were then analyzed by inductively coupled plasma mass spectrometry ICP-MS for Cd concentration [19]. To observe DNA damage using the Comet Assay in blood erythrocytes of Labeo rohita, the fingerlings were treated with three sub-lethal concentrations (1/2, 1/3, 1/4 of 96-hr LC_{50}) of Cd for 30 days in aquarium. Three replications for each concentration were used, whereas, control group was not treated with heavy metal. Blood sample (100 µl) were taken from caudal vein and processed for comet assay by following the protocol [20]. After electrophoresis, the slides were rinsed in 0.4 M Tris neutralization buffer (pH = 7.5). Slides were stained with ethidium bromide and examined for comet tails under fluorescence microscope (Nikon AFX-1 Opti phot). Images of randomly selected cells (100 cells) per sample were analyzed. Intact nuclei (without tail) were observed in the cells without any DNA damage, whereas, cells showing DNA damage appeared like a comet with tail. Length of DNA migration in the comet tail was considered as DNA damage [21]. Apoptotic cells (cells with dispersed heads or no heads) were not considered for analysis. All statistical analyses were performed by using Microsoft Excel, SPSS version 22.0, MINITAB (Probit analysis). Means were calculated by Tukey's Student Newman-Keul tests and a value range of $p<0.05$ was considered as statistically significant. And DNA damage was evaluated by comet score and software comet 1.1 version.

RESULTS

The mean 96-hr LC_{50} value of Cd for fish (*Labeo rohita*) was recorded as 159.59 mg/L (Figure 1).

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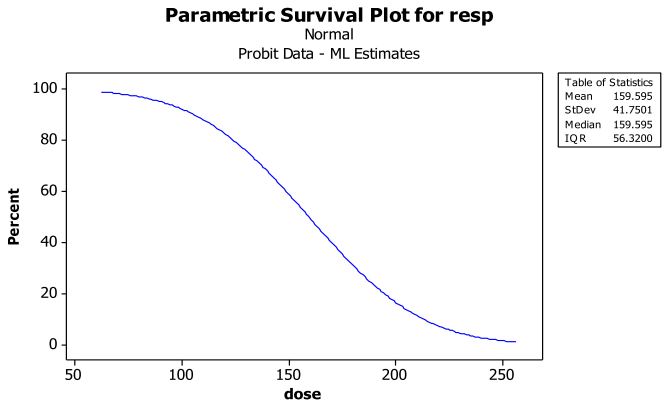

Figure 1: Regression Line between the Probit kill of *Labeo rohita* and log Concentration of Cadmium Chloride ($CdCl_2$)

For the determination of bioaccumulation of Cd in different organs of fish further treatments were prepared as 1/2 (80 mg/L), 1/3rd (53 mg/L) and 1/4th (40 mg/L) of 96-hr LC_{50} of Cd. It was observed that by increasing the doses of Cd, bioaccumulation of heavy metal in all targeted organs (gills, liver, muscles) increased. Among all the organs liver showed higher tendency to amass Cd followed by gills and muscles at all sub-lethal concentrations of metal (Figure 2)

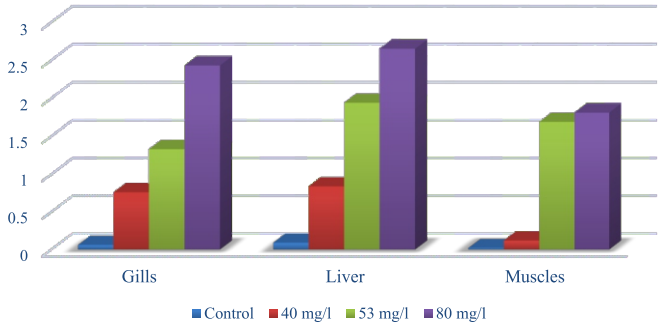

Figure 2: Accumulation of Cadmium (µg/g) in Gills, Liver and Muscles of Fish *(Labeo rohita)*

In Table 1, the accumulation of cadmium in different tissues (gills, liver, and muscles) of fish exposed to various doses of cadmium. The data were expressed as the mean concentration of cadmium (µg/g) along with the standard deviation.

Table 1: Accumulation of Cadmium (µg/g) in Gills, Liver and Muscles of Fish

Doses (mg/L)	Gills (µg/g) (Mean ± SD)	Liver (µg/g) (Mean ± SD)	Muscles (µg/g) (Mean ± SD)
00	0.0000 ± 0.00000^{a}	0.0000 ± 0.00000^{a}	0.0000 ± 0.00000^{a}
40	0.7633 ± .01528^{b}	0.8400 ± .02000^{b}	0.1200 ± .01000^{b}
53	1.3267 ± .03055^{c}	1.9400 ± .04583^{c}	1.6900 ± .03000^{c}
80	2.4267 ± .04041^{d}	2.6533 ± .02082^{d}	1.8067 ± .02082^{d}

For the determination of DNA damage caused by different doses of Cd three (3) comet parameters were evaluated. According to results a positive correlation was observed between comet tail length and exposure concentrations of Cd. Enlargement of comet tail was noticed by increasing the concentration level of heavy metal. Maximum length of comet tail was examined at highest sub-lethal dose of Cd (80 mg/L). Similarly, values of Tail DNA escalated with slight increment in exposure concentrations of Cd, whereas, Head DNA showed negative relationship with exposure concentrations of Cd and values of Head DNA decreased by increasing the concentration of doses of Cd (Figure 3).

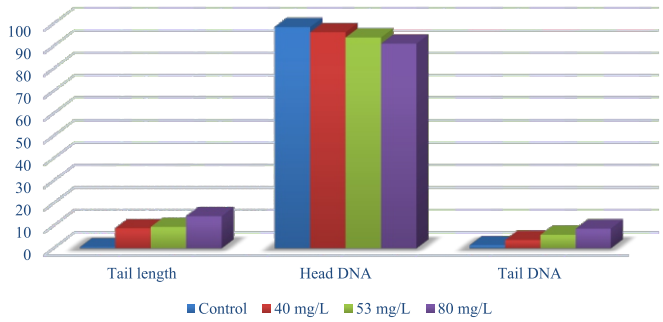

Figure 3: Analysis of Various Comet Parameters in Blood of *Labeo rohita* After Exposure to Different Doses of Cd

DISCUSSION

Heavy metals are always toxic in action even at very low concentrations. With the increase in concentration of metal, its impact also multiplies [22]. LC_{50} values are very useful in probing the lethal concentration of a metal. We employed Finney's probit method for the determination of acute toxicity (96-hr LC_{50}) of Cd in fish, and it gave the value of 159.59 mg/L [23]. In case of fish *Salmo gairdneri*, the 96-hr LC_{50} value of Cd was 80 mg/L [24]. Acute toxicity of Cd (96-hr LC_{50}) changed in different species. Its mean value was 101.25 mg/L for *Channa marulius*, whereas, it was 69.57 mg/L for *Wallago attu*. Similarly, length of fish species also alters its sensitivity to heavy metal like in 50 mm *Channa marulius*, 96-hr LC_{50} was 86.74 mg/L, whereas in 150 mm fish acute toxicity was measured as 120.01 mg/L. The acute toxicity values of Cd reported in previous studies were different from present study due to the use of different species, age group, length, size, water quality parameters and test methods [25, 26]. Bioaccumulation of different heavy metals in a particular tissue is primarily dependent upon the exposure period and concentrations of metals in surrounding water [27]. When the absorption rate of metal exceeds its elimination rate then metal starts to accumulate in the fish body. Cd accumulation usually varies in different species of fish and it mainly depends on exposure duration. In our findings, liver showed maximum bioaccumulation of Cd followed by gills and muscles. Our results were also described in previous investigations, where liver exhibited maximum bioaccumulation of metal as compared to other organs [28, 29]. Bioaccumulation of metals such as Cd takes place in the liver because it works

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as a primary site for regulation and detoxification of toxic metals by producing metallothionein [30]. Higher content of metal in liver promotes liver damage by disturbing the normal range of different biochemical parameters [31, 32]. According to previous studies on metal accumulation liver appears to be the most vital organ for Cd sequestration [33]. In fish, uptake of heavy metals by gills is usually avoided by mucous secretion, however, mucous bonded heavy metals gain entry into gills and high levels of their accumulation in this tissue can be observed [34]. Gill is a primary site for the uptake of metals. Long term exposure to metals causes them to accumulate in this tissue which promotes gill damage [35]. In *Tilapia nilotica*, Cd accumulation was higher in gill, liver and muscle as compared to Zn, when it was exposed over a long period to sublethal concentrations of both metals Cd and Zn [36]. In general, heavy metals do not tend to distribute evenly in different body tissues and accumulate in specific target organs, whereas, in case of muscle tissues metals spread evenly over the muscles. That is why bioaccumulation of Cd was observed minimum in muscles as compared to liver and gill [37]. Metallic ions move from blood and tissues towards liver for the detoxification of tissues which was evident by low levels of Cd in fish muscles [32]. Variations in bioaccumulation of metals in different fish organs relies upon their compatibility to uptake particular metals. Comet assay is a very efficient and reliable technique for the evaluation of DNA damage in animals. In present investigation, the fish specimens exposed to 40 mg/L, 53 mg/L and 80 mg/L of CdCl2 manifested more DNA damage in blood cells as compared to control samples. A gradual increase in exposure concentrations of Cd exacerbated the DNA damage in blood cells of *Labeo rohita*. These results revealed vulnerability and sensitivity of *Labeo rohita* to Cd by displaying higher comet tail length and tail DNA. Previous findings conducted on metal induced DNA damage in various fish species also recorded similar results [38, 14]. Fish *Oreochromis mossabicus* exposure to different concentrations of Arsenic (As) caused DNA damage in gill, liver and blood tissues. DNA damage was concentration dependent and maximum comet tail DNA (%) was observed in liver tissues [39]. Three comet assay parameters (comet tail length, head DNA, tail DNA) were used for the evaluation of DNA damage in erythrocytes of *Labeo rohita* [40]. An increase in comet tail length and tail DNA was noticed by increasing the exposure concentrations of Cd, whereas, a gradual decrease was observed in head DNA values. This pattern is also reported in earlier studies in different organisms [41, 42].

CONCLUSIONS

In conclusion, revelations about Cd toxicity in fish were crucial for conservation of environment and human health. The tendency of *Labeo rohita* to accumulate Cd in different body tissues followed the order: liver > gill > muscles. DNA damage in blood cells showed concentration dependent pattern. By considering the genotoxic potential of Cd in fish, it is pertinent to control the use of Cd for the growth of aquaculture industry in Pakistan.

Authors Contribution

Conceptualization: SAH
Methodology: MAR
Formal analysis: AH
Writing, review and editing: SAH, SF, AM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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