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Original Research

Effects of Sublethal Cadmium Exposure on Hemato-Biochemical Parameters and Tissue Accumulation in *Wallagu attu*

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Abstract

This research investigates the effects of sublethal cadmium (Cd) exposure on *Wallagu attu*, focusing on physiological impacts. Seventy fish, averaging 144.8±24 g, were divided into control and Cd-exposed groups (35 fish each) in 130 L fiberglass tanks. The Cd-treated group was exposed to one-third of the lethal concentration (LC_{50}) for 1, 20, and 40 days. The study assessed metal accumulation and hemato-biochemical responses post-exposure. Results showed a significant increase in metal accumulation in Cd-exposed fish, with the highest Cd levels in the kidneys, followed by the gills and intestines. Catalase and Superoxide Dismutase activities significantly decreased in the Cd-treated group, particularly on day 40. On days 20 and 40, Cd-exposed fish exhibited significant reductions in red blood cells, hemoglobin, hematocrit, and total protein. Conversely, glucose and cortisol levels

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increased significantly with prolonged Cd exposure. No mortality was recorded up to day 20, but by day 40, the mortality rate reached 11.43%. This study underscores the significance of managing sublethal Cd-induced stress to mitigate heavy metal impacts and protect aquatic ecosystems.

Keywords: metal exposure, *Wallagu attu*, metals contamination, cadmium lethal concentration, toxicology

Introduction

Heavy metals are persistent environmental pollutants that pose challenges for removal through natural processes [1]. Freshwater ecosystems, particularly affected by untreated waste from human activities, agriculture, and industries, suffer from heavy metal pollution [2, 3]. This is a significant concern due to its negative influence on aquatic life [4]. Cadmium (Cd), a highly toxic heavy metal found in liquid waste, is known to harm the body's organs [5]. Previous research indicates that Cd exposure in fish results in biochemical alterations and oxidative stress in organs like the kidneys, brain, and liver [6]. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione levels, and lipid peroxidation are key biomarkers used to assess water pollution and its effects on aquatic organisms [7, 8]. Pollutants can infiltrate fish through several routes, including intake of contaminated food, absorption via the skin and gills, interaction with non-food particles, and consumption of polluted water. When fish consume these pollutants, heavy metals accumulate in their organs at varying levels [9]. Once absorbed, these metals travel through the bloodstream to various organs, including the liver, where they are transformed, detoxified, and stored [10]. Thus, evaluating heavy metal concentrations in commercial fish species is essential for understanding and assessing the potential risks of fish consumption [11].

The *Wallagu attu*, a freshwater catfish in South and Southeast Asia, is crucial for the ecosystem, contributing to nutrient cycling and supporting local fisheries [3, 12]. However, similar to other freshwater species, it faces challenges from habitat destruction, excessive fishing, and pollution [13]. Understanding how *W. attu* responds to pollutants, particularly heavy metals, is essential for effective conservation. Pollutant-contaminated rivers in Pakistan pose a significant threat to *W. attu*, pushing it toward becoming a threatened species [14]. Limited research has focused on understanding how heavy metals accumulate in aquatic animals' tissues and impact their health under controlled conditions. This study investigates Cd accumulation and its physiological impacts on fish over 40 days, highlighting short- and long-term effects. Analyzing tissue metal levels, blood, and biochemical markers at intervals provides insights into heavy metal exposure, accumulation, and fish health responses.

Materials and Methods

Study Design and Sampling Methodology

The study was carried out at the Laboratory of Animal Sciences in Sargodha, Pakistan, involving *W. attu* (total of 70 specimens) averaging 144.8±24 g in body weight and 26±2.6 cm in length. The specimens were sourced from the Indus River in Mianwali, Punjab, Pakistan, using various nets with the assistance of local fishermen. Upon acquisition, they were housed in 500 L water tanks fitted with aeration systems. A 14-day acclimatization period (12:12 light:dark) was observed in fiberglass tanks upon arrival at the laboratory. Throughout this period, the fish were given a basal diet, comprising 40.42±1.3% (crude protein), 48.9±0.14% (crude lipid), 24.73±0.17% (moisture), and 14.22±0.7% (ash). The feeding regimen consisted of twice-daily meals (morning and evening), amounting to 3% of body weight. Post-acclimatization, the fish were chosen and allocated into two groups of 35 each, placed in 130 L fiberglass tanks. The control group was kept in dechlorinated tap water, while the other group was exposed to Cd. The fish were treated to a sub-lethal concentration of cadmium chloride ($CdCl_2$), specifically one-third of the LC_{50} as determined by Batool et al. [15], for a duration of 1, 20, and 40 days. During the study, water parameters were consistently monitored following the APHA [16] method, maintaining a stable water temperature of 26.95±0.04ºC. Dissolved oxygen (DO) levels were maintained at 5.87±0.15 mg/L, total hardness at 137.7±2.14, pH at 7.08±0.04, total ammonia at 0.21±0.04, nitrite at 0.13±0.03, and nitrate levels at 2.38±0.04 mg/L. Both the control and experimental tanks were kept aerated continuously with a capillary system linked to an air pump [9].

Sampling of Fish Tissues and Analysis of Biochemical and Metals

At different intervals (1, 20, 40), five fish from each group underwent sacrificial procedures. The gills, liver, kidneys, and intestines were precisely dissected using sterile instruments [3]. The liver tissue was subsequently rinsed with phosphate-buffered saline (PBS) at pH 7.4 to clear away any erythrocytes or clots. To prepare a 10% homogenate, a measured portion of the tissue was minced and homogenized with ice-cold (0.1 M phosphate buffer at pH 7.4). Post-homogenization, the samples were centrifuged at 4000 rpm at 4ºC. The supernatants were then collected and preserved at -80ºC for future analysis of enzymes [catalase (CAT),

superoxide dismutase (SOD), and glutathione (GSH) levels], utilizing diagnostic kits from Biodiagnostics Co. Cd levels in the tissues were assessed using a PerkinElmer 3300 graphite furnace atomic absorption spectrophotometer (AAS).

Examination of Hematological and Biochemical Parameters

Blood sampling was done from both the control and treated fish tanks at 1, 20, and 40 days. The sampling process involved randomly selecting fish (n = 5) from each tank. The fish were fasted for at least 24 hours before sampling. To ensure the fish were unconscious and minimize stress during handling, MS-222 (dose 40 mg/l) was used. Blood was swiftly collected from the caudal vein using a sterile syringe, shortly after the fish were rendered unconscious. The blood samples were placed in tubes with EDTA, an anticoagulant, for subsequent analysis. The red blood cell (RBC) count was performed with a Neubauer counting chamber, following the approach detailed by Habib et al. [17]. The concentration of hemoglobin (Hb) was determined using the cyanomethemoglobin method [18], while the hematocrit (Hct) value was evaluated using the method specified by Britton and Whitby [19].

Plasma was isolated for further analysis of biochemical parameters; the blood was centrifuged at 3000 rpm (for 12 min). The extracted plasma was then preserved in a deep freezer. Blood plasma glucose levels were analyzed using an assay kit (Fluorometric Assay Kit) and following the procedure of Trinder [20]. Cortisol levels were determined with an ELISA kit provided by Alpha Diagnostic International (San Antonio, TX, USA). The total protein content was quantified calorimetrically according to the procedure established by Henry [21].

Data Analysis

The data were analyzed using one-way analysis of variance (ANOVA) to compare different time intervals. Pairwise comparisons were performed with the Tukey test, and the Duncan multiple-range test was applied to the means. Results are expressed as mean±standard error, with statistical significance set at $P<0.05$. All analyses were carried out using Prism GraphPad (version 8.1.2).

Results and Discussion

Heavy metals such as Cd enter aquatic ecosystems from multiple sources, including industrial processes, agricultural practices, and human activities. The toxicity of these metals forces fish to adapt by modifying their behavior and physiology to manage the oxidative stress imposed upon them [22]. The levels of Cd concentration in fish tissues (gills, intestine, and kidney) following exposure over varying durations (1, 20, and 40 days) are presented in Fig. 1 (a-c). Remarkably higher Cd concentrations were detected in the tissues (gills, intestine, and kidney) of the metal-exposed groups than the control across all intervals, reaching statistical significance at $P<0.05$. As the exposure duration increased from day 1 to day 40, there was a consistent increase in Cd concentration across all fish tissues. On day 40, the level of Cd was significantly $P<0.05$ higher than at earlier intervals (1 and 20). This pattern of metal bioaccumulation over time mirrors trends observed in other research, which similarly document metal buildup in fish from their aquatic surroundings. For instance, Malarvizhi et al. [23] observed higher Cd levels in the kidney, gills, and liver of *Cirrhinus mrigala* after a 30-day exposure. Similarly, Javed et al. [24] found increased Cd accumulation in *W. attu* over the same duration. Furthermore, Al-Kshab and Yehya [25] documented a rise in Pb concentrations in *Gambusia affinis* tissues with extended exposure. In this study, the trend indicated a rise in Cd concentration with prolonged contact time. The fish tissue (kidney) consistently exhibited the highest absorptions, followed by the fish gills. The kidneys are crucial for detoxifying heavy metals, and Cd accumulation in renal tissues can result in nephrotoxicity [26]. This can result in reduced kidney function, histopathological changes, and disruptions in excretory processes [27]. As a primary site for Cd accumulation, the gills are susceptible to structural and functional disruptions at high metal concentrations, impacting respiratory functions [28, 29]. Cd disrupts ion transport processes and damages the gill epithelium, impairing overall gill function [28]. The buildup of heavy metals in the fish intestine can compromise nutrient absorption and disturb the gut microbiota, potentially resulting in histopathological issues like inflammation and changes to the intestinal structure [3, 30].

Fig. 1d) illustrates the CAT activity in fish after exposure to Cd across various time intervals. No significant variation in CAT activity was observed within the control group across different time points. However, in the metal-exposed group, the CAT activity was considerably ($P<0.05$) higher on day 1 compared to days 20 and 40. As the exposure duration extended, there was a decreasing trend in CAT activity, with the lowest levels observed on day 40, indicating a more pronounced reduction in activity over time. Fig. 1e) illustrates the SOD activity in fish after exposure to Cd at different time intervals. No significant difference in SOD activity was noted within the control group across the various durations. In contrast, within the Cd-treated group, SOD activity was significantly higher on day 1 compared to days 20 and 40, as indicated by a significance level of $P<0.05$. As the exposure duration extended, there was a declining trend in SOD activity, reaching the lowest levels on days 20 and 40, with no significant differences observed between these two time points. The decrease in enzymatic activities

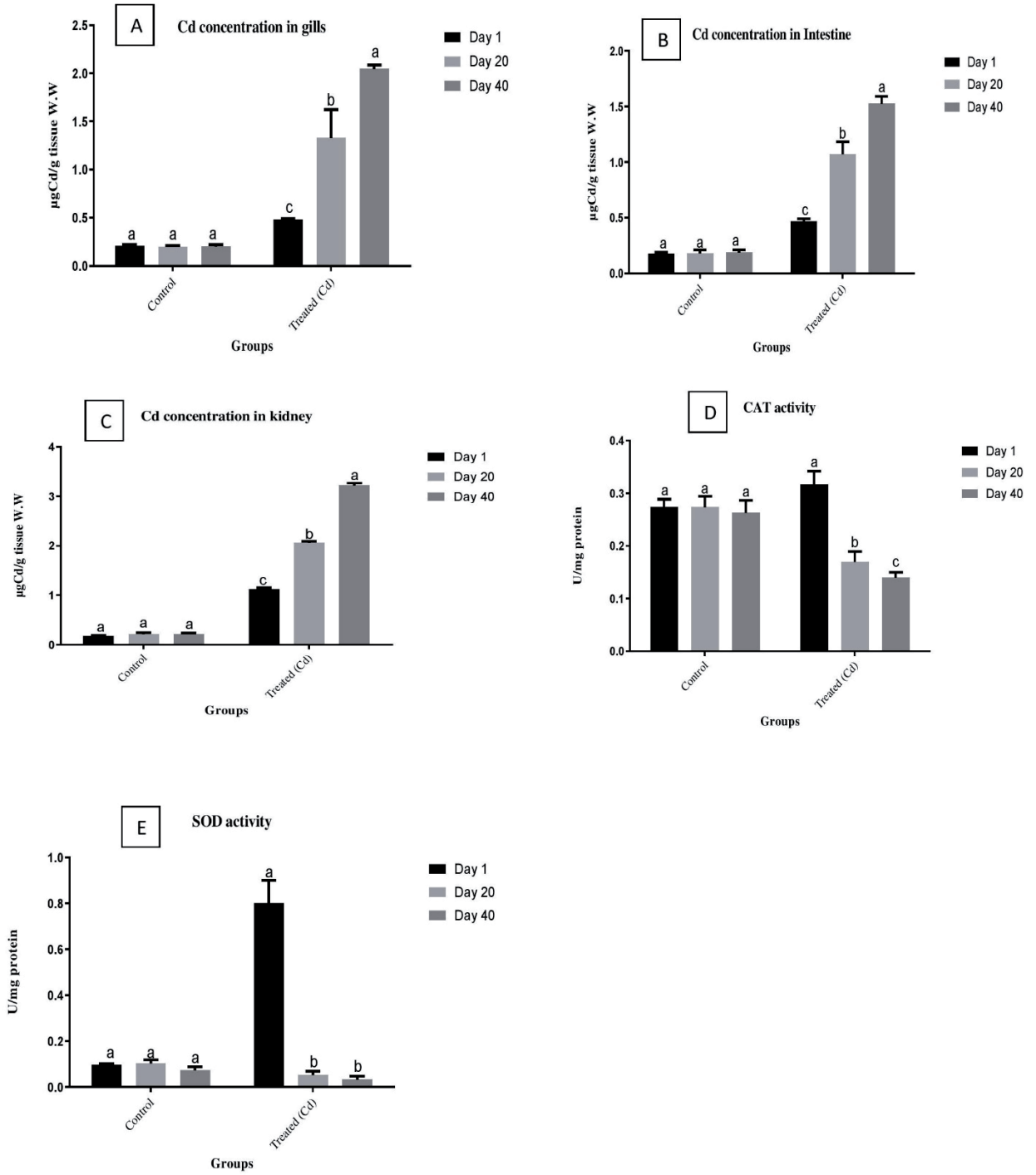

Fig. 1: Effect of Cd on control and treated groups of *Wallagu attu* at different intervals. a) Gill Cd concentration, b) Intestine Cd concentration, c) Kidney Cd concentration, d) Catalase (CAT) activity, e) Superoxide Dismutase (SOD) activity.

in the current study is consistent with the findings of Atli et al. [31] and Saliu and Bawa-Allah [32], who reported reduced CAT and SOD activities in various fish species exposed to sublethal levels of Cd. The declining enzyme activities with increasing exposure duration highlight the dynamic responses of the antioxidant defense system under chronic metal stress. This suggests that the fish's initial adaptive mechanisms may become less effective over prolonged Cd exposure, highlighting a time-dependent decline in their capacity to handle escalating oxidative stress caused by heavy metals. Extended exposure diminishes antioxidant defense mechanisms, reducing their effectiveness in combating oxidative stress [33]. If the antioxidant defense system in fish becomes compromised, they could become more vulnerable to various stressors, which may negatively affect their overall well-being. This weakened state could have cascading effects on their health, leading

Table 1. Hemato-biochemical parameters of *Wallagu attu* on exposure to Cd at different intervals.

Parameters	Pairwise			ANOVA
	Day 1	Day 20	Day 40	
	Control			
RBC count (10^6 mm^{-3})	1.85[a]	1.86[a]	1.88[a]	*P = 0.32*
Hct (%)	33.26[a]	35.32[a]	34.52[a]	*P = 0.16*
Hb (g/100 mL)	7.44[a]	7.53[a]	7.22[a]	*P = 0.12*
Cortisol (ng/l)	15.41[a]	16.62[a]	16.23[a]	*P = 0.87*
TP (g/100 mL)	3.21[b]	4.03[a]	4.52[a]	*P = 0.03*
Glucose (mg/L)	61.23[a]	62.71[a]	62.03[a]	*P = 0.83*
Mortality rate (%)	0.00			
Cd				
RBC count (10^6 mm^{-3})	1.83[a]	1.52[b]	1.21[c]	*P = 0.0002*
Hct (%)	32.22[a]	25.62[b]	21.47[c]	*P = 0.0002*
Hb (g/100 mL)	6.05[a]	5.13[b]	3.12[c]	*P<0.0001*
Cortisol (ng/l)	32.37[c]	39.42[b]	46.38[a]	*P<0.0001*
TP (g/100 mL)	2.27[a]	1.83[b]	1.52[b]	*P<0.0001*
Glucose (mg/L)	74.25[c]	90.32[b]	97.19[a]	*P<0.0001*
Mortality rate (%)	11.43			

to diminished reproductive success and potentially disrupting population dynamics within their ecosystem. [34, 35]. Prolonged exposure to heavy metals can exhaust the antioxidant reserves in fish, leaving them more susceptible to oxidative stress and its associated health impacts [36].

The hemato-biochemical indicators of *W. attu* exposed to Cd over different time intervals are given in Table 1. In the control group, most parameters remained relatively stable, with no significant changes over time. However, TP exhibited a noteworthy and statistically significant ($P<0.05$) increase only from day 1 to days 20 and 40. Conversely, the Cd-exposed group displayed substantial changes in hemato-biochemical parameters. On day 1, the RBC count, Hb, Hct, and TP levels in the Cd-exposed group were significantly ($P<0.05$) higher compared to days 20 and 40. However, with increasing exposure duration, these parameters showed a decreasing trend. These findings align with Hedayati and Darabitabar's [37] study on *Rutilus rutilus* exposed to Pb sublethal levels and Fazio et al. [29] observations on *Mystus seenghala* exposed to sublethal Cd. Heavy metals exhibit toxicity toward hematopoietic tissues, which are crucial for red blood cell production, potentially disrupting their normal function and impairing blood cell formation [38]. Variations in HB levels directly impact the oxygen-carrying capacity of the blood, while changes in HCT levels due to heavy metal exposure can affect the balance between RBC production, survival, and destruction [39].

The cortisol and glucose levels in the Cd-exposed group were significantly higher on day 40. Their levels were found to increase progressively with the duration of exposure. These findings resonate with those of Atli et al. [31], who observed similar spikes in cortisol and glucose in *Oreochromis niloticus* exposed to Cd and Pb, and with Nha Khanh et al. [40], who reported comparable changes in *Anabas testudineus* subjected to Cd and Pb over varying durations. Heavy metal exposure induces stress in fish, leading to the release of cortisol, a stress hormone synthesized by the adrenal glands [41-43].

Conclusions

In conclusion, this study demonstrated that sublethal Cd exposure on day 40 caused marked physiological stress in *W. attu*, reflected by rapid and temporary shifts in stress-related parameters. The kidneys exhibited the highest Cd accumulation, owing to their crucial roles in filtration, reabsorption, and excretion, followed by the gills. These results are pivotal for assessing pollution stress in aquatic habitats and their residents. The data acquired can significantly inform the development of policies and measures to curtail the discharge of harmful chemicals and heavy metals into freshwater ecosystems.

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Conflict of Interest

The authors declare no conflict of interest.

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