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Title:	**Toxic Effects of Lead Chloride ($PbCl_2$) on Behavioral, Hematological, and Serum Biochemical Parameters in Labeo rohita**
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Toxic Effects of Lead Chloride ($PbCl_2$) on Behavioral, Hematological, and Serum Biochemical Parameters in *Labeo rohita*

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ABSTRACT

Background. Lead (Pb) is an extremely toxic metal in aqueous environments. Fish are highly susceptible to the lethal effects of lead exposure since it induces toxicity in fish, with oxidative stress causing neurotoxicity. Moreover, lead affects immune responses in exposed fish. This study aimed to determine the numerous lethal effects of lead exposure, comprising oxidative stress, immune responses, and neurotoxicity. It also aimed to identify its indicators to assess the degree of lead toxicity.

Method. Freshwater fish *Labeo rohita*, (number of fish = 60), with body weight (70–120 g), were divided into one control and three experimental groups namely T1, T2, and T3 (0.44mg/l, 0.89mg/l, and 1.34 mg/l), randomly. For comparative analysis of changes in hematological and biochemical parameters, samples were collected on 7th, 14th, and 21st day.

Results. There was a significant increment in WBC. While, RBCs count, hemoglobin (Hb), and hematocrit were significantly decreased in treated groups as compared to the control group. The mean corpuscular hemoglobin (MHC) and the mean corpuscular hemoglobin concentration (MCHC) indicated a non-significant reduction in lead treated groups as compared to the control. On the other hand, serum biochemical parameters comprising total proteins (TP), albumin, and globulin reduced significantly ($p<0.05$). Simultaneously, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, and glucose significantly ($p < 0.05$) increased in the treated groups compared to control.

Conclusion. The study showed that lead chloride exposure can adversely alternate hematological and biochemical parameters in *Labeo rohita*, even in minor concentrations.

Keywords: heavy metal, hematology, lead chloride, *Labeo rohita*, serum biochemistry

Highlights

- Different concentrations of lead chloride cause various behavioral alterations and clinical signs in *Labeo rohita*.
- Significant alterations in hematological parameters of *Labeo rohita* were observed in lead chloride ($PbCl_2$) treated groups.
- Lead chloride induced variations in biochemical markers in the fish's serum.

1. INTRODUCTION

Lead is a non-essential element that naturally occurs in the environment. Since ancient times, lead and lead based compounds have found wide applications. Lead was used in pipes, pigments, pottery, boat building, book printing, arms industry, and the manufacturing of windows. From the 16th to 19th century, lead poisoning remained a major problem. Although, during the 19th century, most of these uses of lead either declined or disappeared. Instead, new uses of lead were introduced due to its application in the improvement of the octane rating of gasoline (by adding tetraethyl lead), its use in glass containers for cooking, and the use of paint with lead compounds [1].

Numerous studies conducted in diverse biological systems point to the effects of lead as a genotoxicant. The most important ones are the study of DNA lesions, for example, structural and numerical chromosomal aberrations (CAs), micronucleus (MN) test, sister chromatid exchanges (SCE), and DNA strand breaks with the help of single cell gel electrophoresis (comet) assay, the use of which has been increasing mainly because of its sensitivity and simplicity [2]. Additionally, in human beings, numerous mutational mechanisms are associated with the development of specific types of tumors [3,4]. Furthermore, different types of tests have been developed to determine the frequency of mutations in somatic cells caused by mutagenic agents. Among the mutation assays, hypoxanthine-guanine phosphoribosyl-transferase (hprt) gene and T-cell receptor (TCR) are used more often [5].

In the aquatic environment, heavy metals get deposited onto the sediment which functions as an ecological reservoir [6]. As a result of variables such as pH, temperature, salinity, and others, there is always a risk that these metals will transfer upon transitioning from sediment to the water column above, rapidly becoming a part of the aqueous environment's food chain, impacting both aquatic animals and human populations dependent on them [7]. Presently, aquatic pollution has increased manifold because of the development of modern technologies that use heavy metals as raw materials. In recent decades, the accumulation of chemicals from agriculture and industry in the aquatic environment has appeared as a critical and universal concern [8]. Pollution caused by heavy metals has recently become a top worry for environmentalists, especially the pollution in aquatic ecology [9]. Aquatic ecosystems are made up of a wide variety of habitats, from very oligotrophic highland lakes and streams to chemoautotrophic black smokers on volcanic deep-sea ridges. Over the past few years, freshwater ecosystems have encountered significant challenges due to human activities including the discharge of industrial pollutants, agricultural practices, urban waste management issues, and the expanding urban landscape [10]. Rapid

industrialization produces a lot of waste, much of which is frequently dumped into adjacent bodies of water [11].

Water pollution is mostly caused by increased urbanization, industry, and rapid population growth beside aquatic bodies such as rivers, lakes, and reservoirs. This, in turn, leads to the degradation of the ecosystem [7]. Contamination with heavy metals is a significant issue in emerging countries with expanding cities, mostly as a result of unchecked pollution levels brought on by technological changes including industrial expansion and significant increase in traffic powered by petroleum fuels [12]. Both naturally occurring and human-caused environmental releases of heavy metals occur. These metals originate from multiple sources including industrial discharges, soil erosion, urban runoff, mining activities, wastewater discharge, crop pesticides, and due to the natural weathering of the Earth's crust [8].

Hematology serves as a vital biomarker in environmental monitoring, investigation, and assessment domains including toxicology, chemical risk evaluation, safety analysis, and environmental surveillance [13]. After toxicants such as heavy metals enter the fish body, blood forming tissues remain consistently exposed to their detrimental impacts [14]. Being a fragile connective tissue, blood is impacted by the environment and changes in its parameters act as first indicators of path physiological disorders caused by various toxicants. Thus, they are a good tool to observe the health of fish [15]. Fish health can be evaluated with the help of hematological and biochemical signs [16].

Serum biochemistry encompasses the measurements of various components including serum proteins, albumin, triglycerides, HDL-C, cholesterol, and total cholesterol, as well as enzymes such as ALP and ALT [17]. Previous research revealed that when fish were subjected to various exposure treatments, the levels of total protein (TP) significantly decreased ($p>0.05$). Fish subjected to increasing amounts of metals and metal oxides also exhibited a significant increment in total lipid levels, as compared to the control group. In response to extended exposure and rising concentrations of metals or metallic oxides, the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) simultaneously showed a substantial rise [18].

This study aimed to understand how lead chloride ($PbCl_2$) affects *Labeo rohita* in different concentrations by examining both the hematological (blood collection) and biochemical parameters (serum). *Labeo rohita* is commonly consumed by human beings.

2. MATERIALS AND METHODS

The study involved conducting an experiment on *Labeo rohita* of approximately the same age (90-120 days old), weight (70-120 g), and length (15-20 cm), with sex ratio 1:2 (male:female). The fish were collected from Fisheries Complex Bahawalpur in October 2022 to account for potential losses (in term of the death of fish) during transportation and due to environmental factors. Live specimens (n= 60) were collected using specialized nets. Following the collection phase, fish samples were delicately positioned within plastic bags which were filled with water sourced from the pond. Subsequently, these bags were transported to the laboratory of Zoology Department at Government Sadiq College Women University, Bahawalpur. To ensure optimal hygiene, all glassware

and containers were meticulously cleansed and subsequently rinsed with deionized water. Before the start of the experiment, the glass aquaria containers, each with a capacity of 60 liters, were systematically filled with tap water that had been dechlorinated and adjusted to attain the desired pH and hardness levels. Prior to the experimental phase, 60-liter aquaria were filled with dechlorinated tap water while maintaining constant aeration by using compressed air pumps (Silver Lake super pump SL-2800). The light period at 12:12 h light; while dark cycle was maintained throughout the day using fluorescent light tubes (Deebow Aquarium Light) (D-53 4W 20-30cm, Pakistan). Before the beginning of the experiment, a total of 49 fish were allocated to four (04) separate aquaria. Out of 60 fish, 7 were kept in control group for an acclimatization phase lasting 7 days during which they were subjected to a natural light period of 12 hours of light and 12 hours of darkness. The remaining fish were divided into three (03) treatment groups. During the experimental period, Group T_0 consisted of fish maintained under standard conditions with control water. After the acclimatization phase, fish from treated groups, that is, T1, T2, and T3 were exposed to different concentrations of lead chloride. The treatment duration was of 21 days and divided into three (03) sampling intervals on the 7^{th}, 14^{th}, and 21^{st}.

At the conclusion of the trial, a sampling process was conducted to assess hematology and biochemical aspects in *Labeo rohita*. Various solutions at different desired doses were prepared for 60-liter capacity aquaria. These concentrations, specifically 0.44 mg/l (T1), 0.89 mg/l (T2), and 1.34 mg/l (T3), were derived from the referenced LC_{50} value of 74.35 mg/l^{-1}. The preparation process involved dissolving the requisite quantity of lead chloride in distilled water. Subsequently, these solutions were administrated to the 60-liter aquaria on alternate days. Throughout the study period, physicochemical parameters of the water were measured. Key parameters including electrical conductivity (Model S-611L by Peak Instruments US), pH (Model STARTER 300 by OHAUS Corporation, USA), and water temperature (with a glass thermometer) were assessed. Traditional manual methods in the laboratory were employed for measuring total dissolved solids (TDS, Model- BANTE-510, BANTE Instruments, USA) and dissolved oxygen (DO, Model- DO200A, YSI, USA). Additionally, quantification of total hardness, sodium, and potassium followed the methodologies outlined in the A. P. H. A guideline [19].

Clinical and behavioral symptoms in fish exposed to different concentrations of lead chloride included loss of equilibrium, bulging eyes, mucus secretion from the mouth and gills, rapid operculum movement, air gulping, coordination loss, and erratic swimming as well as swimming in isolation. Within the control group, the fish demonstrated no clinical symptoms. The pH, water temperature, and electrical conductivity of each aquarium were measured with the help of a pH tester (HI98107 pHep) and EC tester (HI98304 DiST 4). The temperature of each of the pond water was measured by using an electric thermometer. Each aquarium's dissolved oxygen, salinity, and total dissolved solids were tested by using a salinity tester (MarineLine/HI98319), a TDS tester (HI98302 DiST 2), and a dissolve oxygen (DO) meter (DO meter Hanna 2400). All physicochemical variables were tested on a daily basis during the trial period (21 days).

Blood samples were collected from the caudal vein of the fish at the 7th, 14th, and 21st day. Initially, blood was carefully transferred into tubes which contained the anticoagulant ethylenediaminetetraacetic acid (EDTA) to facilitate hematological analysis. Simultaneously, another portion of the blood was transferred into vacutainers. These blood samples were centrifuged (MinlCentrifuge, Model: mC12). The serum was separated from the blood cells. The serum was isolated using a suction device and stored in tubes for subsequent laboratory analysis [8].

An automated haematology analyzer was used to determine haematological parameters including red blood cells (RBC) count, white blood cells (WBC) count, hemoglobin (Hb) concentration, hematocrit, and mean corpuscular volume (MCV). Common biochemical assays, biochemical markers comprising ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), blood glucose levels, total protein, and albumin were examined. Hematological parameters were assessed using a digital analyzer from a local laboratory. These parameters encompass a range of quantifiable components present in blood, serving as crucial indicators for evaluating an individual's overall well-being and diagnosing a diverse array of medical conditions. These measurements offer valuable insights into the functioning of the hematopoietic system which encompasses the creation and maintenance of blood cells. Serum enzymes were determined spectrophotometrically using commercially available kits. The data thus obtained from hematological-biochemical parameters was represented as means ± standard error (SE).

3. RESULTS

3.1. Clinical and Behavioral Signs due to Lead Chloride Exposure

Table 1 depicts the results of clinical signsnd behavioral alterations in *Labeo rohita* after treatment to different concentrations of lead chloride. The exhibited signs of air gulping, bulging eyes, erratic swimming, coordination loss, increased swimming, mucus secretion from mouth and gills, loss of equilibrium, rapid operculum movement, and swimming in isolation across treatment groups T1, T2, and T3 revealed a clear and consistent pattern of increasing severity in response to higher chemical concentrations. As chemical concentration increased, the severity of these symptoms increased, indicating a noteworthy impact on the overall health status and well-being of the fish and emphasizing the need for careful consideration of chemical exposure and its potential consequences for aquatic life. To summarize, detailed clinical observations suggested that fish in T2 and T3 groups showed more severe clinical manifestations than fish in T1 group. These results showed the significance of lead chloride dosage in influencing the health and behaviors of living organisms. No mortality was observed in treatment groups (T1, T2, and T3) during the trial.

Table 1. Intensity of Various Clinical Signs Showed by *Labeo rohita* Exposed to Different Concentrations of Lead Chloride ($PbCl_2$)

Clinical Ailments	Control Group	Groups/Treatments		
		T1 0.44 mg/l	T2 0.89 mg/l	T3 1.34 mg/l
Loss of equilibrium	-	+ – +	– - +	+ + -

Clinical Ailments	Control Group	Groups/Treatments		
		T1 0.44 mg/l	T2 0.89 mg/l	T3 1.34 mg/l
Mucus secretion from mouth and gills	-	+ – +	– - +	+ + -
Air gulping	-	+ – +	– - +	+ + + -
Rapid operculum movement	-	+ +	– - + +	+ + + -
Bulging eyes	-	+ +	+ - +	+ + -
Coordination loss	-	+ – +	– - +	+ + -
Erratic swimming	-	+ +	+ ++	+ + + -
Swimming in isolation	-	++	+ _ +	+ + -
Average	Absence	Mild	Moderate	Severe

Absence (-), Mild (25%) (+), Moderate (50%) (++), Severe (75%) (+++), Very severe (100%) (++++)

3.2. Physiochemical Parameters of Water

Table 2 illustrates the variations in different parameters across distinct treatment groups labeled as control, T1, T2, and T3, each associated with varying concentrations (0.44 mg/l, 0.89 mg/l, and 1.34 mg/l). The parameters include pH, temperature (°C), dissolved oxygen (mg/l), electrical conductivity (µScm-1), and total dissolved solids (mg/l). During the experiment, the control group served as the reference point against which the changes in the treatment groups were noted. The table shows that as the concentration levels increase from control to T3, a general trend of decreasing values across the parameters becomes noticeable. For instance, the temperature reduces progressively from 387.12 ± 0.23 (control) to 36.92 ± 0.12 (T3), indicating a decline in temperature as the concentration level rises. Similarly, pH demonstrates a slight decrease from 8.55 ± 0.03 in control to 7.21 ± 0.01 in T3. Likewise, electrical conductivity, dissolved oxygen, and total dissolved solids also depict a declining pattern, suggesting a correlation between increased concentration levels and reduced values across these parameters. Overall, the table highlights a trend where an increase in concentration is associated with a decrease in the measured parameters, indicating a potential relationship between varying concentrations and the changes observed in specified characteristics within treatment groups (Table 2).

Table 2. Physiochemical Parameters of Water

Parameters	Groups/Treatments			
	Control	T1(0.44mg/l)	T2(0.89mg/l)	T3(1.34mg/l)
Temperature (°C)	38.12 ± 0.23	37.71 ± 0.42	37.32 ± 0.31	36.92 ± 0.12
pH	8.55 ± 0.03	8.55 ± 0.03	8.44 ± 0.12	7.21 ± 0.01
Electrical conductivity(µScm^{-1})	469.5 ± 0.01	464.2 ± 0.05	458.9 ± 0.02	452.7 ± 0.07

Parameters	Groups/Treatments			
	Control	T1(0.44mg/l)	T2(0.89mg/l)	T3(1.34mg/l)
Dissolved oxygen (mg/l)	29.75 ± 0.02	28.48 ± 0.12	69.21 ± 0.08	27.94 ± 0.05
Total dissolved solids (mg/l)	295.32±1.12	292.21 ± 1.10	289.16 ± 0.88	286.24 ± 1.11

3.3. Hematological Parameters

To determine the effect of lead chloride on the blood profile of the fish (*Labeo rohita)*, hematological parameters were evaluated. The results obtained are given below in Table 3.

On the 7th day, the control group exhibited a white blood cell (WBC) concentration of (182.97±3.19). Whereas, for the treatment groups T1, T2, and T3, the WBC concentrations were (236.45±3.15), (252.73±2.11), and (271.19±2.05), respectively. On the 14th day, the control group displayed the WBC level of (182.07±8.90), while the respective levels for the treatment groups were (213.79±9.18), (206.24±3.38), and (231.88±9.75). On the 21st day, the WBC concentration for the control group was (186.35±7.62). While, for the treatment groups, the respective concentrations were (217.02±8.15), (208.76±4.29), and (233.91±9.12). Significant changes ($p < 0.05$) in WBC levels were observed in both short-term and long-term experiments involving the lead chloride-exposed groups, as indicated in Table 3. These findings are further elucidated in the accompanying Figure 1.

On the 7th day, the RBC concentration level for the control group was (8.88±0.06). While, for the treatment groups T1, T2, and T3, the levels were (8.58±0.002), (8.65±0.03), and (8.44±0.02), respectively. On the 14th day, the RBC concentration level for the control group was (8.89±0.02). Whereas, for the treatment groups, the levels were (8.56±0.025), (8.43±0.02), and (8.11±0.061), respectively. On the 21st day, the RBC concentration level was (8.92±0.03) for the control group and (8.59±0.027), (8.46±0.025), and (8.14±0.057) for the treatment groups, respectively. Notably, there were significant changes in WBC counts in both short-term and long-term experiments involving lead chloride-exposed groups, as presented in Table 3. These findings are also elaborated in the accompanying Figure 1.

On the 7th day, the hemoglobin (Hb) concentration level for the control group was (8.37±0.51). While, Hb concentration levels for the treatment groups T1, T2, and T3 were (7.17±0.21), (6.22±0.49), and (6.78±0.31), respectively. On the 14th day, the Hb level was (8.83±0.35) for the control group and (7.68±0.37), (7.23±0.23), and (5.99±0.42) for the treatment groups, respectively. On the 21st day, the Hb concentration level was (8.99±0.42) for the control group and (7.84±0.39), (7.38±0.27), and (5.99±0.45) for the treatment groups, respectively. Notably, there were significant changes in Hb levels in both short-term and long-term experiments involving lead chloride-exposed groups, as presented in Table 4. These findings are also detailed in the accompanying Figure 1.

On the 7th day, the hematocrit (Hct) level for the control group was (27.83±2.63). Whereas, for the treatment groups T1, T2, and T3, the levels were (28.25±2.30), (22.44±2.55), and (30.02±1.93), respectively. On the 14th day, the Hct level was (36.71±1.65) for the control group and (24.47±1.55), (25.82±1.05), and (30.02±0.68) for the treatment groups, respectively. On the 21st day, Hct level for the control group was (37.81±1.73). While, the levels were (25.12±1.55), (26.48±1.05), and (28.62±0.78) for the treatment groups, respectively. Significantly, there were changes in Hct levels observed in both short-term and long-term experiments involving lead chloride-exposed groups, as indicated in Table 3. These results are also detailed in the accompanying Figure 1.

On the 7th day, mean corpuscular volume (MCV) for the control group was (166.47±11.54). Whereas, for the treatment groups T1, T2, and T3, the levels were (170.67±9.85), (153.84±20.91), and (206.29±11.91) in femtoliters (fl), respectively. On the 14th day, the level of MCV was (214.4±9.29) for control. Whereas, for the treatment groups the levels were (173.80±12.85), (186.49±10.95), (271.98±7.62), respectively. On the 21st day, the level of MCV was (218.9±9.87) for control. Whereas, for the treatments groups the levels were (1.77.88±12.31), (191.76±11.04), and (275.35±8.27), respectively. Statistically, there was no significant change in MCV both in short-term and long-term experiments, as depicted in Table 3. All of these results were also explained in Figure 1.

On the 7th day, the concentration level of mean corpuscular hemoglobin (MCH) for the control group was (46.43±1.59). While, for the treatment groups T1, T2, and T3, the levels were (46.99±0.86), (39.95±3.21), and (47.82±2.06), respectively. On the 14th day, MCH level for the control group was (48.83±1.68). Whereas, for the treatment groups, the levels were (53.88±2.38), (54.72±2.44), and (58.03±7.52), respectively. These levels showed a gradual increase but they were statistically insignificant. Furthermore, MCH concentrations for the control group were (60.85±1.92), (60.66±2.58), and (60.39±7.86) on the 7th, 14th, and 21st day respectively, as indicated in Table 3. All of these findings are also depicted in the accompanying Figure 1.

On the 7th day, the level of mean corpuscular hemoglobin concentration (MCHC) for the control group was (29.77±1.20). Whereas, for the treatment groups T1, T2, and T3, the levels were (26.25±1.02), (28.12±5.67), and (24.57±0.74), respectively. On the 14th day, the MCHC level was (23.79±1.87) for the control group, While, these levels were (31.05±3.60), (28.25±1.35), and (21.19±1.95) for the treatment groups, respectively. On the 21st day, the MCHC level for the control group was (24.71.95). Whereas, for the treatment groups, the levels were (32.17±3.75), (28.75±1.52), and (22.27±2.12), respectively. Importantly, no significant changes were observed in MCHC in both short-term and long-term experiments involving treatment groups, as indicated in Table 3. All of these results are further elucidated in the accompanying Figure 1.

On the 7th day, the platelet count for the control group was (73.77±4.16). While, for the treatment groups T1, T2, and T3, the counts were (85.11±2.52), (113.07±5.03), and (128.46±2.98), respectively. On the 14th day, the platelet count for the control group was (801±4). Whereas, for the treatment groups, the counts were (85.20-

±6.51), (176.08±7.28), and (103.27±3.06), respectively. There was a gradual increase observed in the treatment group T1, which became significant in treatment groups T1 and T2. On the 21st day, platelet count for the control group was (831±4.25). While, for the treatment groups, the counts were (88.71±6.68), (176.92±7.91), and (103.05±3.28) respectively, as indicated in Table 3. All of these results are also elucidated in the accompanying Figure 1.

Table 3. Variations in Hematological Profile of *Labeo rohita* Exposed to Various Doses of Lead Chloride ($PbCl_2$) during Treatment

Parameters	Days	Control	T1	T2	T3
White blood cells (WBC)	7	182.97±3.19	236.45±3.15	252.73±2.11	271.19±2.05
	14	182.07±8.90	213.79±9.18	206.24±3.38	231.88±9.75*
	21	186.35±7.62	217.02±8.15	208.76±4.29	233.91±9.12*
Red blood cells (RBC)	7	8.88±0.06	8.58±0.02	8.65±0.03	8.44±0.02
	14	8.89±0.02	8.56±0.025	8.43±0.02*	8.11±0.061*
	21	8.92±0.03	8.59±0.027	8.46±0.025	8.14±0.057
Hemoglobin (HGB)	7	8.37±0.51	7.17±0.21	6.22±0.49	6.78±0.29
	14	8.83±0.35	7.68±0.37	7.23±0.23	5.99±0.42*
	21	8.99±0.42	7.84±0.39	7.38±0.27	5.08±0.45*
Hematocrit (Hct)	7	27.83±2.63	28.25±2.30	22.44±2.55	30.02±1.93
	14	36.71±1.65	24.47±1.45	25.82±0.95*	28.14±0.63*
	21	37.81±1.73	25.12±1.55	26.48±1.05	28.62±0.78
Mean corpuscular volume (MCV)	7	166.47±11.54	170.67±9.85	153.84±20.91	206.29±11.91
	14	214.4±9.29	173.12±12.85	186.49±10.50*	271.98±7.62*
	21	218.9±9.87	177.88±12.31	191.76±11.04	275.35±8.27
Mean corpuscular hemoglobin (MCH)	7	46.43±1.59	46.99±0.81	39.95±3.21	47.82±2.06
	14	48.83±1.68	53.88±2.38	54.72±2.44*	58.03±7.52*
	21	60.01±1.92	60.85±2.58	60.66±2.62	60.39±7.86*
Mean corpuscular hemoglobin concentration (MCHC)	7	29.77±1.20	26.25±1.02	28.12±5.67	24.57±0.74
	14	23.79±1.87	31.05±3.60	28.25±1.35*	21.19±1.95
	21	24.71±1.95	32.17±3.75	28.75±1.52	22.27±2.12
Platelet (PLT)	7	73.77±4.16	85.11±2.45	123.07±4.90	128.46±2.98
	14	801±4	85.20±6.32	176.08±7.28	103.27±3.06*
	21	831±4.25	88.71±6.68	176.92±7.91	103.05±3.28

Values (Mean +SE) with *asterisks in each row differ significantly ($p < 0.05$) from untreated fish

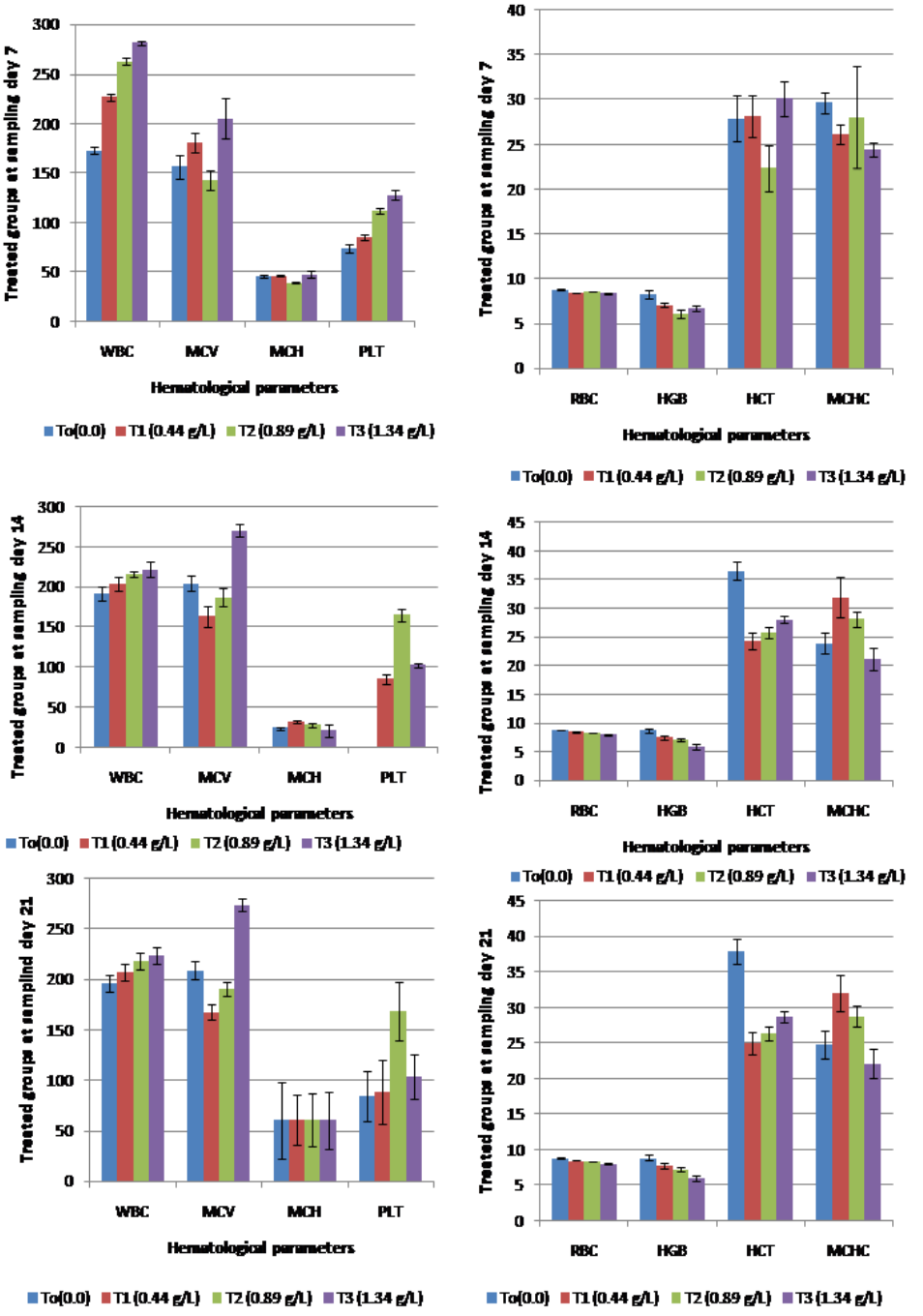

Figure 1. Hematological Parameters of Lead Chloride ($PbCl_2$) Exposed *Labeo rohita*

3.4. Serum Biochemical Parameters

Biochemical parameters were examined to evaluate the impact of lead chloride on *Labeo rohita*. Alkaline transaminase (ALT) exhibited no significant differences between the short-term and long-term experimental groups. The ALT levels on the 7th day were (32.58±3.25) for the control group and (27.84±2.91), (22.18±3.30), and (13.85±2.50) for the treated groups T1, T2, and T3, respectively. Similarly, the ALT concentration levels on the 14th day were (35.35±1.60) for the control group and (32.80±1.28), (24.60±1.48), (17.20±1.18) for the treated groups, respectively. Likewise, on the 21st day, the ALT levels for the control group were (36.65±1.70) and (33.70±1.50), (25.40±1.70), and (18.70±1.50) for the treated groups, respectively. Notably, a gradual reduction was observed in the three different treatments. This reduction was statistically significant, as presented in Table 4. All of these results are further elucidated in the accompanying Figure 2.

Alkaline phosphatase (ALP) exhibited no significant differences in the short-term experimental groups but showed significant changes on the 14th day in comparison. The concentration levels of ALP on the 7th day were (58.90±4.12) for the control group and (42.38±3.38), (32.64±4.40), and (35.28±3.03) for the treated groups T1, T2, and T3, respectively. Similarly, the concentration levels of ALP on the 14th day were (57.70±2.42) for the control group and (42.70±1.92), (34.50±3.90), and (36.80±0.53) for the treated groups, respectively. Likewise, on the 21st day, ALP concentrationlevels were (59.30±2.40) for the control group and (44.20± 2.20), (35.80±2.90), and (38.10±0.90) for the treated groups, respectively (Table 5). All of these results are further elucidated in the accompanying Figure 2.

Total protein (TP) exhibited significant differences between the short-term and long-term experimental groups. The concentration levels of TP on the 7th day were (96.59±0.18) for the control group and (96.17±0.22), (96.98.88±0.18), and (96.97±0.28) for the treated groups T1, T2, and T3, respectively. Similarly, the concentration levels of TP on the 14th day were (97.65±0.12) for the control group and (97.18±0.15), (97.82±0.10), and (97.85±0.13) for the treated groups, respectively. Likewise, on the 21st day, the concentration levels of TP were (98.70±0.13) for the control group and (98.20±0.16), (98.84±0.11), and (98.88±0.11) for the treated groups, respectively. Notably, a gradual decline was observed in the three different treatments.This decline was statistically significant, as indicated in Table 4. All of these results are further elucidated in the accompanying Figure 2.

Globulin levels exhibited a significant gradual decrease in both short-term and long-term experimental groups. This decline became more pronounced with higher levels of toxicity. The concentration levels of globulin on the 7th day were (7.50±0.05) for the control group and (7.35±0.20), (7.28±0.08), and (7.69±0.19) for the treated groups T1, T2, and T3, respectively. Similarly, the concentration levels of globulin on the 21st day were (7.40±0.06) for the control group and (7.25±0.19), (7.09±0.05), and (7.55±0.09) for the treated groups, respectively. Likewise, on the 21st day, globulin (g/dl) levels were (7.55±0.05) for the control group and (7.40±0.22), (7.22±0.06), and (7.69±0.09) for the treated groups, respectively. These trends are presented in

Table 5. All of these results are further elucidated in the accompanying Figure 2.

Albumin levels showed a significant decrease in both short-term and long-term experimental groups, with variations observed at different levels of toxicity (T1, T2, and T3). The concentration levels of albumin on the 7th day were (6.17±0.18) for the control group and (6.73±0.05), (6.78±0.09), and (6.35±0.10) for the treated groups T1, T2, and T3, respectively. Similarly, the concentration levels of albumin on the 14th day were (6.82±0.10) for the control group and (6.75±0.08), (6.80±0.13), and (6.47±0.05) for the treated groups, respectively. Likewise, on the 21st day, albumin levels were (6.85±0.11) for the control group and (6.77±0.06), (6.83±0.14), and (6.50 ±0.08) for the treated groups, respectively. These findings are presented in Table 4. All of these results are further elucidated in the accompanying Figure 2.

Bilirubin levels exhibited significant differences between short-term and long-term experimental groups. The concentration levels of bilirubin on the 7th day were (5.12±0.20) for the control group and (5.71±0.14), (5.56±0.20), and (5.55±0.20) for the treated groups T1, T2, and T3, respectively. Similarly, the concentration levels of bilirubin on the 14th day were (5.20±0.09) for the control group and (5.86±0.08), (5.58±0.10), and (5.58±0.12) for the treated groups, respectively. Likewise, on the 21st day, bilirubin levels were (5.22±0.09) for the control group and (5.88±0.11), (5.60±0.13), and (5.60±0.13) for the treated groups, respectively. Notably, it was observed that bilirubin reached its minimum level in the T3 treatment group, as detailed in Table 4. All of these results are further elucidated in the accompanying Figure 2.

Cholesterol levels displayed significant differences between short-term and long-term experimental groups, particularly in treatment groups T1, T2, and T3. Cholesterol levels on the 7th day were (163.50±4.50) for the control group and (163.80±4.50), (181.00±4.80), and (258.40±5.80) for the treated groups T1, T2, and T3, respectively. Similarly, cholesterol levels on the 14th day were (160.50±2.90) for the control group and (165.62±3.10), (183.15±43.90), and (227.50±4.80) for the treated groups, respectively. Likewise, on the 21st day, cholesterol levels for the control group were (162.80±2.70) and (167.90±3.00), (186.10±3.50), and (228.80±4.40) for the treated groups, respectively. Notably, it was observed that cholesterol level was the highest in T1 and the lowest in T2 treatment group, which changed significantly. These findings are detailed in Table 4. All of these results are further elucidated in the accompanying Figure 2.

Triglycerides (TG) levels exhibited significant differences in both short-term and long-term experimental groups, with notable increases in T1 and T2 treatments as compared to other groups. On the 7th day, TG levels were (118.80±4.20) for the control group and (98.66±4.20), (124.50.56±5.00), and (141.12±4.70) for the treated groups T1, T2, and T3, respectively. Similarly, on the 14th day, TG concentration levels were (119.30±3.30) for the control group and (103.90±3.02), (128.80±3.35), and (143.80±2.40) for the treated groups, respectively. Likewise, on the 21st day, TG levels were (120.00±2.90) for the control groupand (105.60±3.00), (130.50± 3.30), and (144.60±2.50) for the treated groups, respectively. These findings are presented in Table 4. All of these results are further elucidated in the accompanying Figure 2.

The level of high-density lipoprotein cholesterol (HDL-C) exhibited significant reduction in both short-term and long-term experimental groups, particularly in T1 and T2. On the 7th day, HDL-C levels were (72.10±3.90) for the control group and (52.10±3.90), (39.13±4.70), and (63.90±4.20) for the treated groups T1, T2, and T3, respectively. Similarly, on the 14th day, HDL-C concentration levels were (71.80±2.0) for the control group and (51.70±1.90), (40.40±2.30), and (63.20±1.70) for the treated groups, respectively. Likewise, on the 21st day, HDL-C levels were (73.60±2.00) for the control groupand (53.60±1.90), (32.20±2.40), and (65.50±1.80) for the treated groups, respectively. These findings are detailed in Table 4. All of these results are further elucidated in the accompanying Figure 2.

Table 4. Variations in Serum Biochemistry Parameters Profile of *Labeo rohita* Exposed to Various Doses of Lead Chloride ($PbCl_2$) during Treatments

Treatments	Days	Control	T1	T2	T3
Alanine Aminotransferase (ALT)	7	32.58±3.25	27.84±2.91	22.18±3.30	13.85±2.50
	14	35.35±1.60	32.80±1.40	24.60±1.60*	17.20±1.40
	21	36.65±1.70	33.70±1.50	25.40±1.70	18.70±1.50
Alkaline Phosphatase (ALP)	7	58.80±4.12	42.38±3.38	32.64±4.40	35.28±3.03
	14	57.70±2.30	42.70±2.00	34.50±3.00	36.80±0.80*
	21	59.30±2.40	44.20±2.20	35.80±2.90	38.10±0.90*
Total protein (T.P)	7	96.59±0.18	96.17±0.22	96.98±0.18	96.97±0.28
	14	97.65±0.12	97.18±0.15	97.82±0.10	97.85±0.10
	21	98.70±0.13	98.20±0.16	98.84±0.11*	98.88±0.11*
Globulin	7	7.50±0.05	7.35±0.20	7.28±0.08	7.69±0.19
	14	7.52±0.04	7.38±0.20	7.20±0.05	7.67±0.08*
	21	7.55±0.05	7.40±0.22	7.22±0.06	7.69±0.09
Albumin	7	6.17±0.18	6.73±0.05	6.78±0.09	6.35±0.10
	14	6.82±0.10	6.75±0.05	6.80±0.13*	6.47±0.07*
	21	6.85±0.11	6.77±0.06	6.83±0.14	6.50±0.08*
Bilirubin	7	5.12±0.20	5.71±0.14	5.56±0.19	5.55±0.20
	14	5.20±0.08	5.86±0.10	5.58±0.12	5.58±0.12*
	21	5.22±0.09	5.88±0.11	5.60±0.13	5.60±0.13*
Cholesterol	7	163.50±4.50	163.80±4.50	181.00±4.80	258.40±5.80
	14	160.50±2.50	165.60±2.90	183.80±3.40*	227.50±4.20*
	21	162.80±2.70	167.90±3.00	186.10±3.50	228.80±4.40
Triglycerides (TG)	7	118.80±4.20	98.66±4.20	124.50±5.00	141.12±4.70
	14	119.30±2.80	103.90±2.90	128.80±3.20*	143.80±2.40*
	21	120.00±2.90	105.60±3.00	130.50±3.30	144.60±2.50
High-Density Lipoprotein Cholesterol (HDL-C)	7	72.10±3.90	52.10±3.90	39.13±4.70	63.90±4.20
	14	71.80±1.90	51.70±1.80	40.40±2.30*	63.20±1.70*
	21	73.60±2.00	53.50±1.90	42.20±2.40	65.50±1.80

Values (Mean +SE) with *asterisks in each row differ significantly ($p < 0.05$) from untreated fish

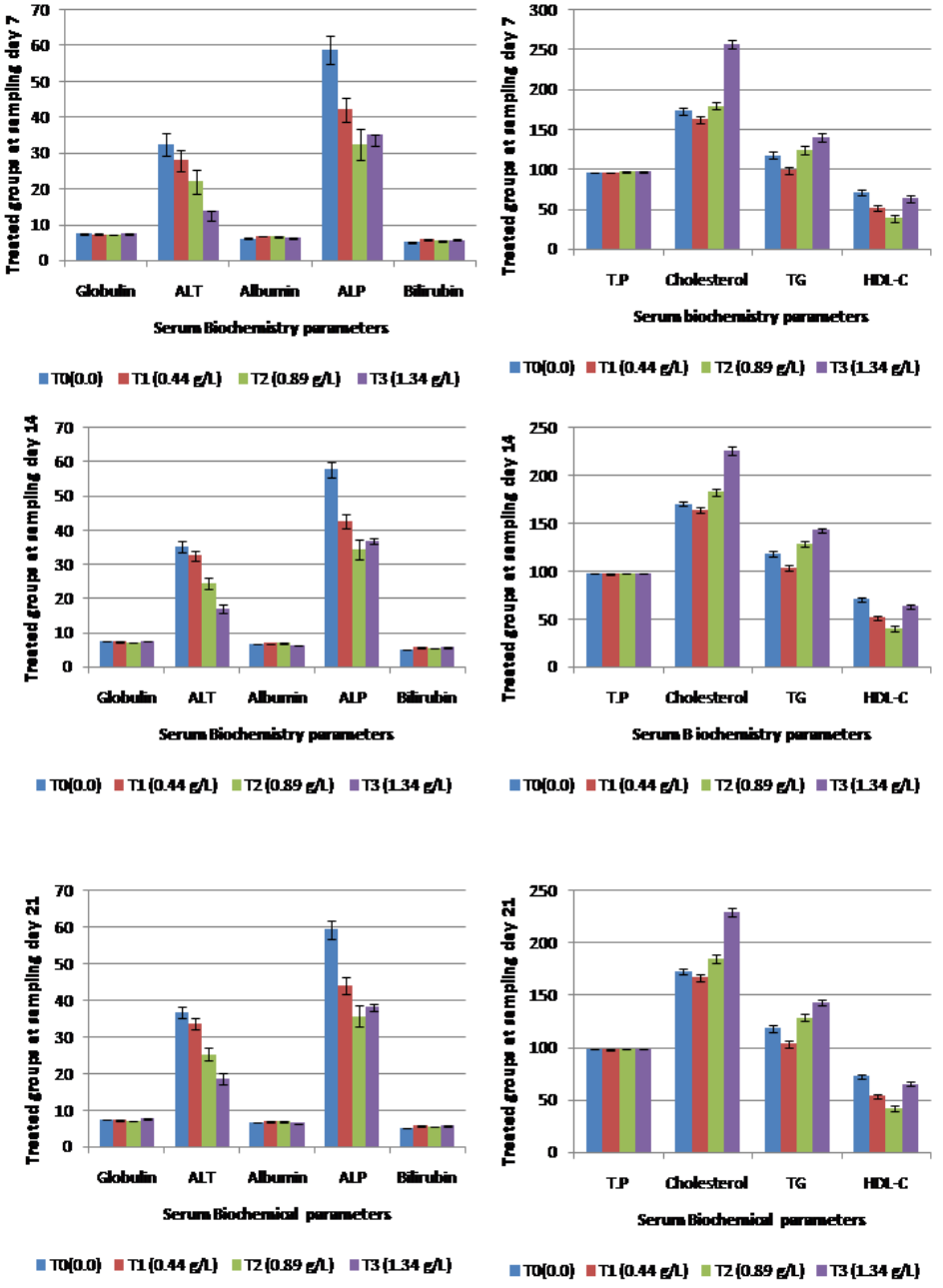

Figure 2. Variation of Serum Biochemical Parameters in *Labeo rohita* Exposed to Various Concentrations of Lead Chloride ($PbCl_2$)

4. DISCUSSION

Contrary to the findings of this study, some studies have reported different findings concerning the effect of lead chloride on fish, such as a study carried out on *Labeo rohita* exposed to lead chloride. The study found no significant differences in different parameters as compared to the control group [20]. These contrasting outcomes may be due to exposure duration, variations in species sensitivity, and experimental conditions. Different species of fish may show variable levels of susceptibility to lead chloride and other factors, such as pH, water temperature, and dissolved oxygen levels. The inhibition effect of lead chloride on hematological parameters is probable due to the disturbance of hormonal balance in aquatic animals. However, it is significant to consider variations on the basis of species sensitivity, as well as experimental conditions, when comparing the results across different studies.

Lead toxicity has been studied for many years. Still, contradiction is found in data related to the carcinogenic, mutagenic, and clastogenic properties of lead and lead containing compounds. The IARC categorized lead as a potential human carcinogen based on adequate evidences obtained from experimental animals about the carcinogenicity of lead, although only insufficient evidence is available about lead carcinogenicity in human beings. Inorganic lead compounds are categorized as possible human carcinogens. One of the most significant environmental problems is heavy metal pollution [21]. Certainly, these metals often exhibit toxicity even at low levels, remain non-biodegradable, and commonly show resistance to traditional removal techniques. Consequently, they have the potential to significantly lower drinking water quality. Exposure to these metals can cause a number of major human health problems including cancer, neurological disorders, kidney pathology, and respiratory problems. For instance, chromium, being a carcinogen, can cause skin lesions and respiratory problems [22].

There is intimate connection between fish and their environment encompassing aspects such as their physical maturation, growth, and reproductive processes [23]. Hence, it is important to comprehend how environmental elements affect the health of fish. This research aligns with similar work previously conducted by others [24] on *Labeo rohita*. In this research, significant alterations were observed in the hematological and biochemical parameters of fish, consistent with previous reports. The potential release or leaching of chromium chloride into aquatic ecosystems raises specific concerns, especially in light of research demonstrating its high toxicity to several different freshwater species [25-28].

All previously reported findings that support the current study proved that exposure to lead chloride results in significant changes in hematological parameters. Specifically, there is a significant increment in the number of WBCs and a significant decline in RBCs, Hb, hematocrit, and platelet counts. Additionally, it was determined that lead chloride exposure leads to increased levels of all serological parameters, except for total proteins. Furthermore, serum enzyme levels rise in response to toxic compounds, such as lead chloride [13].

In the current investigation, the levels of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) did not show significant differences among the groups. This

variation may be due to the selection of a different species of fish in the current experiment as compared to the previous ones. On the 14th day, significant changes were observed in the levels of MCV, MCH, and MCHC. These significant parameter changes may be attributed to the increasing concentration of lead chloride. The results match with the previous findings reported by [29, 30] for the 14th day. These results also align with the findings previously reported by [31]. In the current study, with the exception of ALP, total protein, and bilirubin, all other biochemical parameters showed no significant changes on the 7th and 14th day of exposure to the various concentrations of lead chloride.

4.1. Conclusion

The results indicated that lead chloride exposure had significant effects on most hematological parameters in *Labeo rohita*. This suggests that the fish blood parameters were significantly altered due to the exposure. Additionally, various biochemical markers in the fish's serum were examined. These included high-density lipoprotein cholesterol (HDL-C), total protein (TP), bilirubin, albumin, alkaline transferase (ALP), alkaline phosphatase (ALP), cholesterol, and triglycerides. The results underscore the importance of evaluating the effects of environmental contaminants such as lead chloride on aquatic organisms. The variations observed in hematological and biochemical studies provide valuable understanding of potential sublethal impacts on fish health and ecosystem integrity. Continued research in this area would contribute to a better insight into the risks associated with contaminant exposure and would also support strategies for sustainable aquatic resource management.

CONFLICT OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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