Assessment of $AlCl_3$ on Haematological Study of Freshwater Fish *Cirrhinus mrigala*

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Rekha Rani
Sandeep

Abstract

Water is essential for the survival of living beings. Whenever heavy metal enters the water resource, it causes toxicity to the aquatic ecosystem. In present investigation, the impact of sublethal concentration of aluminium chloride on the haematological parameters of the freshwater fish Cirrhinus mrigala has been analysed. To observe toxicity, the primary calculated LC_{50} of aluminium chloride for Cirrhinus mrigala, that was 0.087ml/L after 24, 48, 72 and 96 hours of exposure. To evaluate the acute toxicity of aluminium chloride on haematological parameters, fish were exposed to a sublethal concentration of LC_{50}. i.e., 0.0087ml/L for 7, 14, 21 and 28 days. In case of the RBC, the number of red blood cells may have increased due to a compensatory response to hypoxia or anaemia caused by the toxicant. WBC is increased due to the exposure to aluminium chloride because of the immunological response in blood of the fish. PCV percentage is increased due to the increase of the RBC and the decrease in plasma cells after exposure to the toxicant. MCV decreased due to packed cell volume, and the hemolytic effect can lead to a decrease in the size of RBCs, reflected in a lowered MCV. An increase in mean corpuscular haemoglobin (MCH) in Cirrhinus mrigala in aluminium chloride exposure could lead to dehydration or a decrease in plasma volume in the fish. The results underscore the importance of monitoring water quality and

Rekha Rani
Aquatic Toxicology Lab, Dept. of Zoology, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh. Email: reet14@yahoo.co.in
Sandeep
Aquatic Toxicology Lab, Dept. of Zoology, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh.

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the potential risks posed by aluminium contamination in aquatic ecosystems as well as human health.

Keywords: *Water, Aluminium chloride, Toxicity, Cirrhinus mrigala, Haematology, Health.*

1. Introduction

Water is precious for all living organisms, playing crucial roles in world-scale ecosystems, such as organisms' various body functions, drinking and hydration, regulating temperature, agriculture, and other livelihood processes. The aquatic organism whose food, shelter, reproduction and other essential activities depend on the water. Heavy metals include metalloids, such as arsenic and aluminium that are able to induce toxicity at low levels of exposure [1]. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments [2].

Aluminium is the third most abundant element in the earth's crust, after oxygen and silicon [3]. Aluminium (Al^{+3}) is the most widely distributed metal in the environment [4], approximately 8.8% on the Earth's crust. Occurring naturally in the trivalent state (Al^{+3}) as silicates, oxides and hydroxides, but may combine with other elements such as chlorine, sulphur, fluorine, as well as form complexes with organic matter [5]. It is commonly found in combination with other elements such as chloride, hydroxide, phosphate, silicate and sulphate to form aluminium compounds such as $AlCl_3$, $Al(OH)_3$, $Al_2(SO_4)_2$, $Al_2(SO_4)_3$ and $AlPO_4$, respectively. These aluminium compounds are used in chemical manufacture, paints, aircraft industries, construction, food packaging, automobiles, cooking utensils, in metal alloys production, electric industry, cosmetics, welding, etc. Waste discharge from manufacturing units' releases aluminium into water bodies, soil, and the atmosphere. By these sources, aluminium chloride reaches into aquatic ecosystem. The continuous release of aluminium impairs water quality and becomes unsuitable for aquatic organisms due to its persistence, bioaccumulation, toxicity and bio-magnification in the food chain [6].

The dissolved concentration of aluminium in water is toxic to aquatic organisms. These aluminium ions in water can be hazardous to fish, invertebrates, other aquatic species and humans. Aluminium ions disturb ion control in fish gills, causing osmotic stress and reduced respiration, haematological alteration, etc. [7]. When aluminium is exposed heavily, or for a long time, toxicity may occur, including encephalopathy,

osteomalacia, proximal myopathy, an increase in blood infection risk, an increase in left ventricular mass and a decrease in myocardial function, microcytic anaemia with very high levels or sudden death [8]. Haematological alterations have been extensively utilized as indicators in assessing the well-being of fish subjected to toxic substances, both in controlled experiments and real-world observations [9].

The fishing sector contributes significantly to the socioeconomic development of the countries. India is the second-largest producer of fish worldwide, producing 6.57 million metric tons. 55% are freshwater fish, with carps making up more than 87% of the total in India. India contributes 4.5% of the world's fish production as per Fisheries Research and Development in India, 2006. Fish is a main source of protein that contains approximately 16 g in per 100 g. Fish have approximately 18-20% of proteins. Vitamin D present in fish liver and oil is necessary for bone growth, and fish oil helps in brain growth and development of humans because it is essential for absorption and metabolism of calcium [10]. It is composed of 8 important types of amino acids, such as lysine, methionine, and cysteine. The chances of a heart attack decrease with fish consumption. Fish ingestion prevents age related deterioration and depression and also improve sleep quality and old age vision.

Cirrhinus mrigala (White carp) is an edible freshwater fish, worldwide distributed, best study model due to adopted in lab, and tolerate a broad range of water quality and frequent use as a bioindicator in toxicity studies. Blood is an essential fluid of the body and helps to assess the impurity of the tissues. The fish species is known for its sensitivity to contaminants and its economic and ecological importance [11]. Haematological alterations function as indicators to evaluate the impurity of the tissues of fish subjected to toxicants [12]. The purpose of this study is to find the sublethal effect of aluminium through aluminium chloride in haematology, and other tissues of the organisms such as fish, humans, invertebrates, etc.

2. Materials and Methods

2.1 Fish Collection and Sampling

For experimental purposes, 60 male and female freshwater fish *Cirrhinus mrigala* of average size (15 to 25cm) with average weight (150 to 240 grams) were collected with the help of local fishermen employing

seine nets from the Johilla River, Amarkantak. Carp were transported to the aquatic toxicology laboratory in the Department of Zoology at IGNTU, Amarkantak. Fishes were dipped in 1% $KMnO_4$ upon arrival at the laboratory for disinfection.

Six well-ventilated aquariums (75 x 35 x 37.5cm) with capacity of around 100L in the laboratory were cleaned before one day arrival of the fish, using 1% $KMnO_4$ solution to remove any kind of disinfection. Then each aquarium was filled with 50L of tap water. The water temperature was measured at 27±2°C by using the thermometer, and the pH measured ranged from 7 to 7.2 by using litmus paper. A digital hook weighing machine (GLUN) was then used to weight the fish. After weighing the fish, ten fish were put in each of the glass aquariums.

The fish *Cirrhinus mrigala* was identified by seeing morphometric characters and Fin formula of *Cirrhinus mrigala* -D.15(3/12); P.18; V.9; A.8(2/6); C.19; L1.42; L.tr.7 [13] (where, D- Dorsal fin, P- Pectoral fin, V-Ventral fin, A-Anal fin, C-Caudal fin, L- lateral line, L.tr.-Transverse scale).

2.2 Acclimatization and Maintenance

The fishes were acclimatized for 7 days in the laboratory before the experiment. Throughout the acclimatization period, the fish were fed twice daily with approximately 4-5 grains per fish. Artificial food pellets (TAIYO) were used to feed the fish. Water was changed throughout the acclimatization phase as per the requirement. Faecal matter was routinely removed from the aquarium by using a siphon pipe, to prevent fouling smell, died fish removed, if any. Extreme care was taken to make sure that no external influences contaminated the water.

2.3 Test Compound

Aluminium chloride was selected as the toxicant for this study due to its potential to induce metabolic effects. 500 gm of anhydrous aluminium chloride, manufactured by Central Drug House (CDH), was obtained from Jabalpur for the experiment. Emergency Response Guidebook (ERG) 2020 stated that $AlCl_3$ is highly corrosive and toxic. It is non-explosive, non-flammable, and anhydrous, soluble in hydrogen chloride, ethanol, and carbon tetrachloride, but in benzene it is slightly soluble. When it is exposed to water, it forms an aqueous solution, it caused water decomposition, and the aqueous solution is acidic in nature.

2.4 Experimental Design

The experiment was divided into two parts with ethical permission from the IAEC Reg. No. 2004/GO/ReBi/S/18/CCSEA, according to the CPCSEA guidelines for fish given by the Ministry of Fisheries, Govt. of India.

2.4.1 Experiment 1

For the determination of the $LC_{50,}$ log dose/probit regression line analysis was used [14]. 24 fish were taken, and the target aluminium chloride concentration was assessed to assess haematological alteration in fish. Four aquariums were labelled as I, II, III, and IV, which were filled with 25 Litres of tap water to determine the lethal concentration of aluminium chloride in each aquarium. Six carp were taken randomly from the stock. An electronic weighing balance machine (Shimadzu ATX224) was used to measure aluminium chloride dosages as 0.025, 0.05, 0.1, and 0.2 mg [15]. Feeding was stopped for 24 hours prior to exposure of toxicant under investigation. The percentage mortality and survival of fish in the treated groups were measured at 24 hrs, 48-hour, 72, and 96 hrs intervals. In order to prevent infection in the aquarium, the number of dead fish in each tank was promptly removed using a fish net as soon as, fish death identified by the operculum movement stopped.

2.4.2 Experiment 2

To observe the haematological alteration of *Cirrhinus mrigala* after exposure to aluminium chloride, a 28-day experiment with 30 fish was carried out in April 2024. For the study, five glass aquariums with the labels control I and tested, II, III, IV, and V were set up. Six fish were chosen randomly from the stock and put into each aquarium. The four groups were exposed to aluminium chloride, and the control group was kept in its natural condition of water. To standardize conditions, feeding was stopped one day before the experiment. Each tested aquarium fish was exposed to the sublethal concentration of calculated LC_{50} (1/10th) of aluminium chloride [16].

2.5 Blood sample collection

Fish from the control and treated groups were taken out of their individual aquariums at 7^{th}, 14^{th}, 21^{st}, and 28^{th} day intervals. Blood samples were collected by using standard method [17]. Then the fish was simply sacrificed by cervical dislocation with a slight strike on its head. The blood samples of the control and treated groups were collected directly from the

gills using a sterile 5ml disposable plastic syringe (BD Emerald™). 3ml of blood was obtained by gently aspirating the needle, and the drawn blood was dispensed into an anticoagulant EDTA tube. In the EDTA tube, blood was gently tapped by the first finger of the right hand to mix it well. Haematological analysis was performed by methods as-

Erythrocyte count by Rusia and Sood method, 1992 [18], Leucocyte count by Rusia and Sood method 1992 using a haemocytometer. Haemoglobin estimation by oxyhaemoglobin method Szigeti, 1940 [19], Hematocrit by Nelson and Morris method 1989 [20], Red blood cell indices by Wintrobe method1929 [21], Mean cell volume (MCV), Mean cell haemoglobin (MCH), Mean cellular haemoglobin concentration (MCHC).

2.6 Statistical calculations

Different statistical calculations are used. The student't' test applied in order to determine the significance of variations in haematological parameters.

3. Result and Discussion

3.1 Lethal concentration (LC_{50})

The study administered aluminium chloride to freshwater fish *Cirrhinus mrigala* at various concentrations for 24, 48, 72, and 96 hours to assess its relative toxicity through lethal concentration (LC_{50}) determination. The LC_{50} value of aluminium chloride for *Cirrhinus mrigala* was calculated i.e.0.087 ml/L, and for the present study sublethal dose, 1/10th of the $LC_{50,}$ was taken 0.0087ml/L.

The toxicity of aluminium chloride exhibits variability among species and even within strains of the same species. The lethal concentration of aluminium in various fish species was determined to be 1.53 mg/ L in *Rasbora sumatrana* [22] and 0.25mg /L for *Labeo rohita* [23]. The lethal concentration (LC_{50}) of aluminium was found 0.5mg/L [24]. For other heavy metals like copper, the LC_{50} were noted as 1.25ppm for *Catla catla* [25], while for mercury, 0.24 ppm by [26]. The lower LC_{50} value observed in the present investigation may be attributed to factors such as fish weight, age, water quality, and the purity of the test compound.

3.2 Behavioural changes

After 7 days of acclimatization the fish behave normally. The fish exposed to aluminium chloride showed multiple abnormal behaviour by the toxicity of the aluminium chloride, which respond such as aggressive,

highly sensitive to stimulation produced, irregular movement and swimming, due to the irregular fin movement, congregating at the tank bottom, more frequent resting and loss of balance. Decrease the feeding activity during the first week of toxicant exposure, and fish showed surface-seeking behaviour, which is responsible for the gill modification. The gills were observed during the blood sample collection for the haematological analysis. A decrease in swimming activity was observed [27]. The changes in gills reduce the breathing capacity and decrease the oxygen level in body. Same behavioural changes were also observed by Senger et al. [28] in *Danio rerio* after exposure to aluminium chloride. Erratic swimming, jerky body movements, rolling the body, convulsions, loss of equilibrium and mucous secretion over the body of *Clarias batrachus* at 1.5mg/L cadmium sulphate exposure by Pundir [29]. Throughout the acute exposure period, the behavioural changes and morphological abnormalities of both the healthy/control fish and those exposed to different concentrations of copper were consistently observed and assessed for any alterations in behaviour. Air swallows, surprise reaction, disbalance swimming, in fish were also investigated during the study [30].

3.3 Haematological alteration

Normal range of blood haematological parameters of carp *Cirrhinus mrigala* is reported here. Haematocrit ranged from 29.0 to 44.0%. The range of haemoglobin was 7.06 to 11.86 g/100 ml of blood, and the total number of erythrocytes varied from 1.7 to 3.0 million/mm^3 of the blood, both haemoglobin and erythrocyte concentrations being higher in males than in females. The clotting time in males (41.0 seconds) was lower than in females (63.5 seconds). Haematological values varied with the size and weight [31].

Fish is a major source of protein and other nutrients .Therefore, the American Heart Association recommends fish twice a week for human adults. Fish possess the capacity to accumulate heavy metals, and the accumulated heavy metals in fish can have harmful effects on the environment and ecosystem health, and can also impact human health through the food chain [32]. In the present study, the evaluation of the haematological changes in the freshwater fish *Cirrhinus mrigala* exposed to a sublethal concentration of 1/10th LC_{50}-0.0087 ml/L of aluminium chloride for 7, 14, 21, and 28 days. The findings indicate highly significant

haematological alterations in different parameters **[Figure 1-6].** The blood carries various components such as RBC, WBC, platelets, plasma and haemoglobin. In the case of RBC, the number of red blood cells may have increased, which may be due to a compensatory response to hypoxia or anaemia caused by the toxicant. There was a highly significant increase ($P < 0.001$) in the mean number of RBC count after 28 days. This can stimulate the production of larger and more haemoglobin-rich red blood cells to improve oxygen transport efficiency in response to the impaired oxygen-carrying capacity. Reduction of oxygen binding capacity occurs due to the irregular shape of the RBC. The WBC count increased by the effect of aluminium chloride at different time intervals. There was a highly significant increase ($P < 0.001$) in the mean number of WBC count after 28 days. WBC increased due to the exposure to aluminium chloride because of the immunological response in the blood of the fish. WBCs defend against the infection and disease in the body, it usually caused by infection or inflammation in the body. The increase in concentration of haemoglobin was observed because of an increase in red blood cell count. The increase in haemoglobin levels in fish after aluminium chloride exposure may be a physiological response to compensate for reduced oxygen transport efficiency caused by aluminium-induced damage to gill tissues, leading to hypoxia and stimulating erythropoiesis. A very highly significant ($p<0.001$) increase in the mean of the haemoglobin was observed. In the case of haematocrit, the percentage increased after exposure to aluminium chloride. The sizes of the WBC blood cells decreased due to the frequent multiplication of the white cells. The enlargement of the nucleus of nucleated red blood cells in the fish *Cirrhinus mrigala* was observed. PCV percentage is increased due to the increase of the RBC and the plasma cells after exposure to the toxicant. The RBC indices alterations were also observed due to the effect of heavy metals. MCV decreased frequently due to the aluminium chloride exposure to *Cirrhinus mrigala* because of packed cells volume alteration in the blood. Significant ($p<0.05$) was observed in the MCV of the *Cirrhinus mrigala*. MCH slightly increased by the exposure to aluminium chloride in the body of the fish according to the intervals of time.

An increase in the number of red blood cells and white blood cells, and also an increase in the haematocrit value and haemoglobin content under the effect of aluminium chloride at the different periods were noted.

In the freshwater fish *Tilapia zilli* increased haematocrit and haemoglobin value were noted [33]. An increase in the haematocrit value and haemoglobin content under the effect of aluminium chloride was observed. The doubling of haematocrit value was observed due to the effect of aluminium on rainbow trout [34]. In the present study increase in the number of RBCs was found due to aluminium chloride effects. Similar findings were observed in the fish *Cirrhinus mrigala* after exposure to aluminium chloride [35]. Increase in the range of mean No. of RBC count with increasing the exposure period and concentration, there was a significant increase ($P < 0.001$) in the mean numbers of RBC count after 48 hours of exposure. Significant ($P < 0.05$) increase was seen in the number of red blood cells (RBCs) count after 96 hours of exposure [36]. In the experiment, an increase in RBCs at 21 hours was observed after exposure to aluminium chloride. Similarly, in the dogfish, red blood cell counts increased when fish were exposed to cadmium for periods ranging from 24 to 96 hours [37]. The current study observed an increase in haematocrit. The increase in haematocrit values was observed in the fish rainbow trout [38]. An increase in the blood HCT has often been shown to be a good indicator of aluminium toxicity [39]. The MCV gives an indication of the condition or size of the red blood cells and reflects an abnormal or normal cell division during erythropoiesis also observed. In the present study, increased WBC was observed due to the aluminium chloride, which caused infections in the body and immune development against the toxicants. High levels of WBC counts indicate damage due to infection of body tissues, severe physical stress and as well. The increased WBC was observed in the fish *Tillapia Zilli* [39]. In most cases, abnormal red cell morphology is noted [40]. The same result was noted in the fish *Cirrhinus mrigala*. Significant increase in haematocrit values could be observed in the blood of fish exposed to zinc sulphate during different periods of exposure [41]. The data on MCV, MCH and MCHC showed a significant decrease in all exposed concentrations of aluminium phosphide assessed in the fish *Clarias gariepinus* Juvenile [42]. Haematocrit and haemoglobin are directly influenced by the fluctuation of RBC observed [43]. The decreased MCV was observed in the fish *Cirrhinus mrigala,* and increased MCH and MCHC were observed. Tissues reflect the physiologic state of an animal because they are the products of intermediate metabolism, also evaluated [44]. Haematological indices like haemoglobin (Hb) content, total red blood

cell count (RBC) and total white blood cell (WBC) may be altered after exposure to heavy metals evaluated [45]. A number of blood parameters, such as total RBC count, total WBC count and haemoglobin (Hb) content, have been used as indicators of metal pollution in the aquatic environment(46). Haematological profile as Hb, RBC and WBC, in aluminium-treated haematological profile in *Catla catla* was significantly altered in the size of blood components in different parameters [46]. Blood parameters of fish are suitable biomarkers for evaluating the potential risk of chemicals [47]. Sub-chronic dietary exposure to Cd causes a significant reduction in haemoglobin and hematocrit. The current study found an increase in haemoglobin and hematocrit in animals exposed to the test compound, aluminium chloride. Increase in RBC count and Haematocrit value of the two studied species may be attributed to impairment of gas exchange by the gills and a release of erythrocytes from the spleen to compensate for impaired oxygen uptake, which resulted from disturbed gill function [48]. Copper led to higher WBCs but lower RBCs, Hb, Ht, Hb, glucose and total protein of *Nile tilapia* plasma [49]. The anaemia condition in fish resulted from an unusually low number of red blood cells observed [50]. WBC is the primary line of immune defence. One of the most elementary ways to assess the immune system is to explore changes in the white blood cell count and its types [51]. The exposure to copper induces blood alterations, characterised by an increase in the Hb and RBC concentration [52].

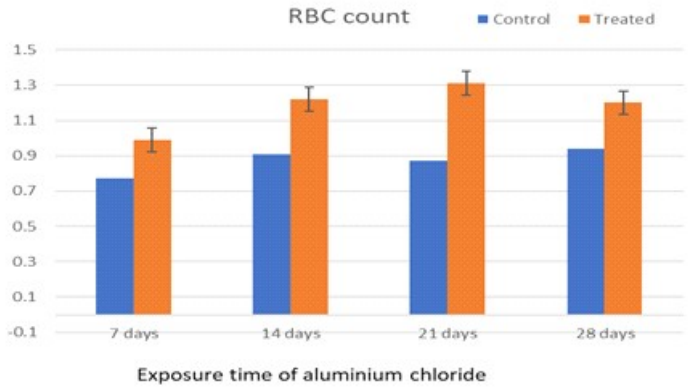

Figure 1: RBC count in blood *of Cirrhinus mrigala* in control and treated groups.

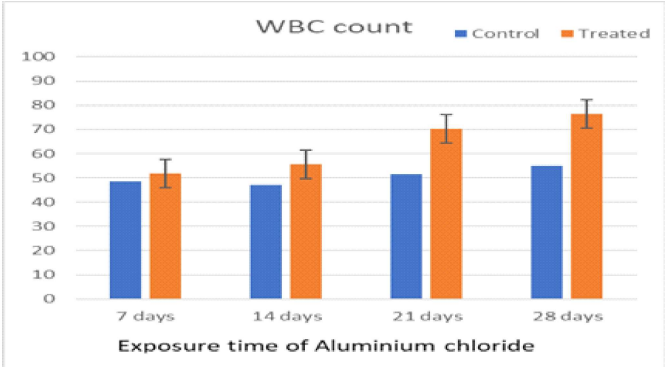

Figure 2: WBC count in the blood of *Cirrhinus mrigala* in control and treated groups.

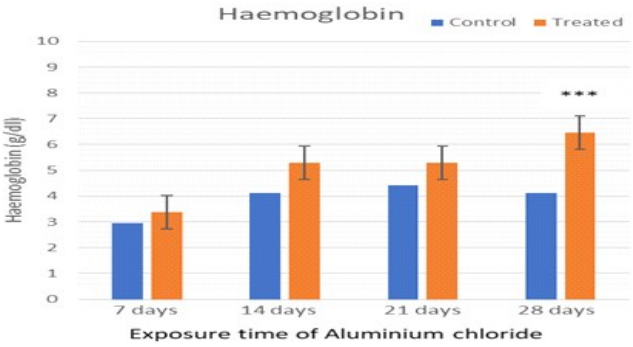

Figure 3: Hb level in the blood of *Cirrhinus mrigala* in control and treated groups.

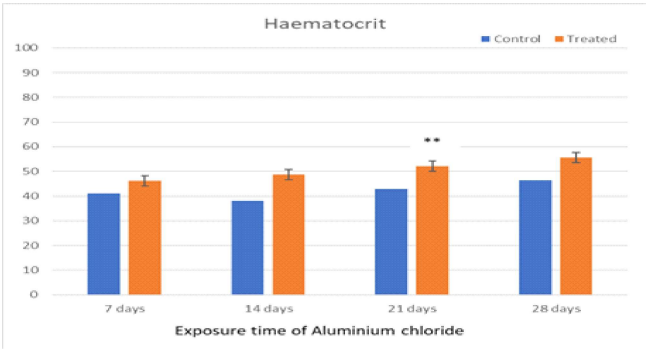

Figure 4: Haematocrit % level in the blood of *Cirrhinus mrigala* in control and treated

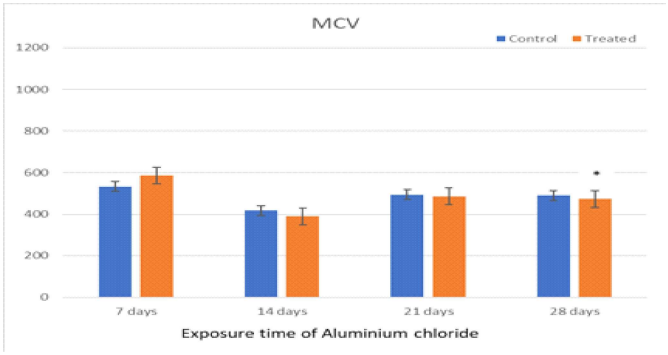

Figure 5: MCV level in the blood of *Cirrhinus mrigala* in the control and treated groups.

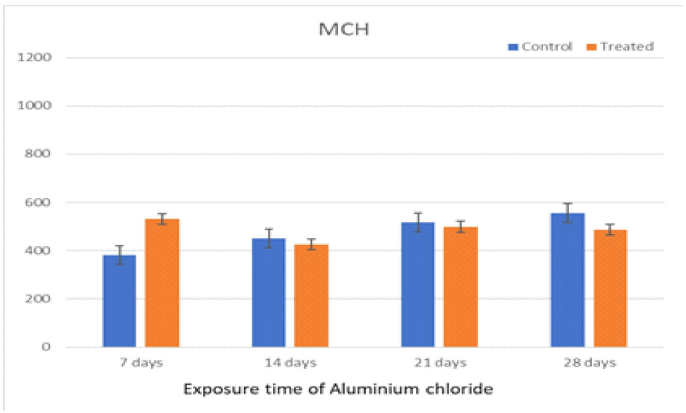

Figure 6: MCH level in the blood of *Cirrhinus mrigala* in the control and treated groups.

4. Conclusion

The present study has evaluated that even low quality of aluminium chloride can cause hazardous alteration in the fish *Cirrhinus mrigala,* including behavioural changes and haematological alterations. These changes indicate an adaptive response to the toxicant exposure, likely reflecting a compensatory mechanism to maintain oxygen transport and immune function under stress conditions. The presence of trace amounts of aluminium chloride in water can harm aquatic organisms and disrupt the entire freshwater ecosystem. Humans depend on these water sources for various needs, which can lead to hazardous effects on human health due to continuous exposure to aluminium chloride. The results underscore

the importance of monitoring water quality and the potential risks posed by aluminium contamination in aquatic ecosystems. This study contributes valuable information for environmental monitoring and fish health assessment, emphasizing the need for stringent regulations to control aluminium pollution in freshwater habitats.

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