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***Research Article** (Open Access)*

HAEMATOLOGICAL STUDIES ON THE EFFECTS OF L-GULUTAMINE IN COPPER ($CuSo_4$) EXPOSED FRESH WATER ZEBRA FISH (*BRACHYDANIO RERIO (Ham*)

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ABSTRACT
Worldwide, heavy metal contamination of aquatic ecosystems is a problem for the environment. Copper sulphate is a crucial micronutrient that is needed by all living things. Because copper is a heavy metal, excessive usage of it can have a variety of negative impacts on living things. However, when used sparingly, it can also aid with nourishment. The free intracellular amino acid glutamine is a naturally occurring substance that may have antioxidant properties. It contributed to raising the blood parameters level. The goal of the current study was to assess the toxicity of copper in blood cells and the effect of L-glutamine on recovering copper toxicity in freshwater zebrafish (B. danio rerio). By using probit analysis, the acute median lethal concentration (LC50) of *Branchydanio rerio* was determined to be 3.083 mg/l. Based on this LC50 value, twenty zebra fish were exposed to one sublethsal concentration of copper sulphate, which was 0.20 ppm. Fish blood samples should be collected using a microcentrifuge tube and a collecting tube prepared with a 2% EDTA solution. The fish should then be left in 0.10 mg L-glutamine for a duration of 96 hours. Fish blood samples should be collected using a microcentrifuge tube and a collecting tube prepared with a 2% EDTA solution. The collected blood samples should then be stored at 200^{o}C for additional examination. Haematological markers, such as RBC, WBC, Hb, Ht, MCV, and MCHC levels, are monitored during the extended course of treatment and compared to control. The study's findings unmistakably showed that copper sulphate can alter the branchy daniorario's haematological characteristics, which can thus be used as nonspecific biomarkers for heavy metal contamination in aquatic environments. Heavy metal use will be curtailed by this research.

Keywords: *Zebra fish, L-glutamine, copper sulphate, Haematological characteristics*

INTRODUCTION
The concentrations of heavy metals in natural streams have unavoidably increased due to intense activity in the agricultural and industrial sectors (Jordao *et al.,* 2002). Among the pollutants that are of concern to the environment, heavy metals are a prominent player (Singer *et al.,* 2005). Due to their environmental durability and capacity for accumulation by aquatic species, heavy metals are significant contaminants of the aquatic environment (Veena *et al.,* 1997). Fish are good bioindicators for metal pollution. As metals are well-known oxidative stressors, metal contamination of the aquatic environment may be reflected in fish oxidative damage and antioxidant defences (Livingstone, 2003). As human society has advanced technologically, metal pollution has increased. The activities that are thought to be the main sources of metal pollution include mining, industry, domestic garbage, and motor traffic. According to Luoma and Rainbow (2008), metals have the ability to accumulate in aquatic creatures such as fish and to linger in water and sediments. The many ways that metals are exposed also affect the toxicity of those metals. Metals are absorbed by fish through their gills, digestive systems, and skin (Kamunde *et al.,* 2002). Accordingly, they modify osmoregulation (Singh *et al.,* 2008) and blood parameters (Carvalho and Fernandes, 2006). Because monocytes and neutrophils are susceptible to heavy metals, immunosuppression has also been documented (Tavares-Dias *et al.,* 2002). According to toxicants, blood

assessment is a crucial instrument for assessing the health of fish (Singh *et al.,* 2008). Metals are major global pollutants in aquatic habitats, particularly heavy metals like copper.

Copper (Cu), an essential micronutrient for humans and animals, is an important and vital trace element needed for the multiple normal metabolic functions in various living organisms. Copper also has an essential role in the regular activity of connective tissue, iron metabolism, and several functions in the central nervous system. Plants and animals need copper to perform everyday tasks in different organs, e.g., hemoglobin synthesis. It is also a cofactor for various enzymes. Widespread application of copper sulfate ($CuSO_4$) in various sectors like building materials, agricultural sector, electronic industry, water pipes, transportation sector, intra-uterine contraceptive devices, and as an antifungal agent and growth promoter in poultry has also induced toxicological impacts in the exposed species. Copper sulfate is used worldwide as a fungicide in agriculture and as an algaecide in aquaculture. Unlike other synthetic and organic pesticides, Cu cannot be degraded and can easily be deposited in various tissues of the treated animals. Due to frequent application, Cu can damage the cells due to its potential to catalyze the generation of reactive oxygen species. Cu also causes toxic impacts in terms of poor feed intake, increased oxidative stress, reduced body mass, and biochemical and hematological changes (Naz S, Hussain R, 2023). According to Booth *et al.* (1988), fish that are exposed to environmental contaminants display a range of physiological reactions, including abnormalities in their blood balance. Fish haematological characteristic evaluation has grown in importance as a tool for comprehending both diseased and normal processes as well as the effects of toxins (Sudova *et al.,* 2009).

Different amounts of CuSO4 had some effect on certain of the blood parameters under investigation. The primary haematological reaction of the Cyprinus carpio fingerlings in this investigation to the sublethal dose of $CuSO_4$ was a markedly reduced RBC, Hb, in comparison to the control group. Channa punctatus exposure to sublethal concentrations of $CuSO_4$ was shown to produce similar effects, including a decrease in Hb and RBC content. Following treatment with varying concentrations of $CuSO_4$, it was noted that the amount of RBC in C. macropomum decreased (Griffin *et al.,* 1999). Zebrafish (Danio rerio) are one of the best leading models to investigate developmental biology, cancer, toxicity, drug discovery, and molecular genetics. In the current work, zebrafish are used as experimental animals. Furthermore, the zebrafish is being used more and more as a genetic model organism for aquaculture species, in toxicogenomics, and to create illness models of zebrafish for use in human biomedicines. This little fish's easy upkeep, ability to reproduce, and transparent early growth make it a useful model organism for many study fields. Free intracellular amino acid glutamine is a naturally occurring substance that may have antioxidant properties. (Çevik, Mehmet Ug˘ur, 2016). The application of L-glutamine in sickle cell disease is predicated on its antioxidant activity, despite the fact that its exact mode of action is unknown. This illness affects the blood cells. Because l-glutamine has antioxidant qualities, it may lessen the harmful effects of DLM on brain tissue. (Çevik, Mehmet Ug˘ur, 2016) The most prevalent amino acid in the body, and the one that controls the production and breakdown of proteins, is glutamine. Additionally, it is crucial to controlling the acid-base balance. boosting the immune system and enhancing stress tolerance. (Novak F, Heyland DK, 2002 and Melis GC, 2004) L-glutamine is the body's precursor of glutathione (GSH), the most significant antioxidant that has been demonstrated to enhance recovery from critical illness in both clinical and experimental forms. (Wischmeyer PE, 2003) Glutamate, the precursor of GSH production, also has a role in antioxidant properties. (Gündüz E, 2015) While the role of oral l-glutamine treatment in DLM-induced hepatotoxicity is well understood, its protective effects against DLM toxicity in cerebral tissue are still unclear. Mehmet Ugnevik Çevik (2016) conducted a study to examine the impact of L-glutamine post-treatment on blood parameters pertaining to copper sulphate toxicity while also delving into the underlying mechanisms. The study was motivated by the antioxidant characteristics of L-glutamine. Due to its antioxidant qualities, l-glutamine may lessen the harmful effects of copper sulphate in fish blood.

METERIAL and METHODS

Fish from an aquarium were used to obtain a mature zebra fish. In this experiment, Danio rerio, approximately 3 - 3.5 cm in length and weighing roughly 4 - 4.5 grams, was employed. Because of its easy culture, short developmental period, well-documented general biology, and year-round reproduction, *B. danio rerio* was chosen as the model organism for this investigation. These qualities made *B. danio rerio* a good candidate for the toxicity test. The fish were kept in glass aquariums with dechlorinated water for at least 15 days before the experiment to help them get used to the laboratory environment. Characteristics of the water quality were identified. The following were the test water quality's mean values respectively 31°C, pH 7.8, 7.5 mg/l of dissolved oxygen, 125 mg/l of alkalinity as $CaCO_3$, 100 mg/l of total hardness, 6.0 ppm of nitrate, 0.02 ppm of nitrite, 1.1 ppm of iron, 140 mg/l of calcium, and 72 mg/l of magnesium. Commercial fish food was provided to the fish on a daily basis. The fish were kept in a photoperiod of 12 hours of light and 12 hours of darkness. Analytical-grade copper sulphate ($CuSO_4$) was dissolved in double distilled water to create a stock solution of copper sulphate. A typical static-renewal technique was used to test the acute toxicity of copper sulphate to B. danio rerio. The stock solution of copper sulphate in double-distilled water was used to create the desired concentrations of copper sulphate. Every 24 hours, a new solution with the same concentration was added to the test chambers to fully replenish the toxicant. Using the Finney (1971) probit analysis method, the 96-hour LC50 value of copper sulphate in *B. danio rerio* was determined to be 0.3038 ppm. Twenty fish were added to the solution after the LC_{50} 96-hour value for sublethal doses of copper sulphate was determined. Five duplicates for every sublethal exposure were kept. Throughout the course of the trial, the concentration of copper sulphate did not change, despite daily water changes in both the treatment and control groups. The fish were given commercial food, just like in the control group. There was a facility available to oxygenate the test solution. 0.20 ppm of $CuSO_4$ were subjected to B. Danio rerio Following 5, 10, and 15 days, and Fish blood samples should be collected using a centrifuge and a collecting tube prepared with a 2% EDTA solution and the balance fish were switched back to 0.10 mg L-glutamine for 96 hours. After 96 hours, the blood samples were collected in Eppendorf tubes using centrifuge that contained EDTA anticoagulant. The haematological parameters were estimated using these treated and control blood samples. The Dacie and Lewise (1984) approach came next.

Total RBC count: The haemocytometer pipette was used to gradually draw up blood samples until they reached the 0.5 mark (marked 0.5, 1.0, and 101). As with Mark 101, the diluting fluid was then swallowed. This resulted in 1 in 200 dilutions. The pipette was turned gently to initiate the mixing process while this was being done. The pie was thoroughly shaken for approximately a minute after being forcefully cut by its ends between the forefinger and thumb. After that, the finger was blown out of the capillary tube and taken out of the diluting solution. A single drop of the diluted blood was added to the slide after a few drops had been shaken out. Red blood corpuscles were counted using at least five sets of sixteen squares.

Calculation: Number of RBC /cu.mm

$$= \frac{Total\ NO\ \ of\ cell\ counted}{Total\ NO.of\ small\ square\ counted} \text{ x Dilotion}$$

Total count of WBC: The Neuberger counting chamber of the haemocytometer was used to count the total white blood cells. Using a WBC pipette, blood samples were taken up to the 0.5 mark. They were then diluted using Turk's fluid (Gentian Violet, glacial acid, 3 ml, and distilled water, 97 ml) until they reached mark 11. This resulted in 1 in 20 dilutions. The subsequent steps for the RBC counting were the same as those described earlier. Out of nine squares, four sets of sixteen were counted to determine the number of leucocytes. It is easier to count the entire set of sixteen squares at once rather than counting them in rows of four.

Calculation: Number of WBC/Cu.mm

$$= \frac{Total\ No\ of\ leucocytes}{Total\ No\ of\ large\ squers\ counted} \text{ x Dilution}$$

Estimation of haemoglobin (Hb) content : Using a permanent colored glass comparison standard and a Haldane's haemoglobinometer (superior, Germany), the haemoglobin count of the blood was calculated and reported in gm/100 millilitres of blood.

Estimation of hematocrit value (Ht) or packed cell volume (PVC): By centrifuging blood in heparinized hematocrit tubes (Germany) at 7,000 rpm/min for many minutes, the hematocrit value of the blood was calculated. After centrifugation, the packed cell volume, or haemoglobin percent, was computed from the extracted blood volume.

Estimation of mean corpuscular haemoglobin (MCH): Using a formula, the values of haemoglobin content and erythrocyte count were combined to calculate the mean corpuscular haemoglobin (MCH) count, which was then expressed as pictograms.

MCH is calculated as follows:

$$\text{MCH} = \frac{Haemoglobin\ (\frac{gm}{100ml})}{erythrocyte\ count\ (\frac{million\ cells}{cu.mm\ blood}} \text{ x } 10$$

Determination of mean corpuscular haemoglobin concentration (MCHC): Using a formula, the mean corpuscular haemoglobin concentration (MCHC) was estimated and expressed as a percentage based on the haemoglobin value and the hematocrit percentages.

$$\text{MHCH} = \frac{Haemoglobin\ (\frac{gm}{100ml}}{haematocritmpercentage} \text{ x Dilution}$$

The changes in the hematological parameters of Branchydanio rerio exposed to sublethal concentration of copper sulphate and L-glutamine exposure

RESULTS AND DISCUSSION

Total RBC count: Table 1 shows the red blood cells of Branchydanio rerio subjected to L-glutamine, a sublethal concentration, and a control. The mean value of the erythrocyte counts in the healthy control group was 0.72 mm^{-3}. For the 5, 10, and 15 days that they were exposed to sublethal concentrations of copper sulphate, the fishes' mean RBC values were 0.56, 0.45, and 0.35, mm^{-3}. The total count of RBCs was observed to be significantly reduced after treatment with copper sulphate. The declining percentages were -22.22, -37.5 and -51.38 points. respectively. Fish exposed to L-glutamine had an average red blood cell count of 0.71 mm^{-3}. This value somewhat agrees with the control value. The percentage of decrees is -1.38. At the $P<0.05$ level, both the increased and decreased RBC counts for all exposure periods were statistically significant (**Table 1; Fig. 1.1**).

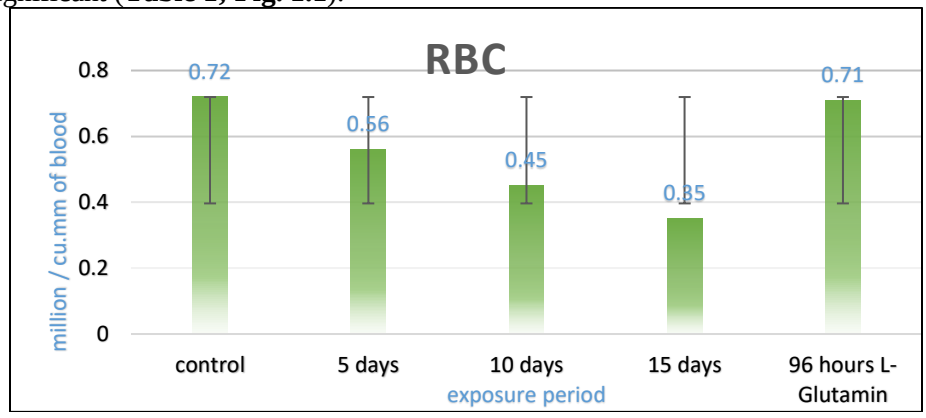

Figure 1.1: Changes of the red blood cells (RBC) of Branchydanio rerio exposed to sublethal concentration of copper sulphate and L-Glutamine exposure

Table 1: Total count of RBC's, WBC's, Heamoglobin, Hematocrit, MCV, MCH and MCHC in the control, copper sulphate and L- gulutamine treated *B. danio rerios*

Parameter	Exposure period in days					p-value
	Control	5days	10days	15days	96 hours L-glutamine	
RBC ($x10^6$/Cu mm^3)						
%COC	0.72±0.0141	0.56 ±0.1649-22.22	0.45±0.2428-37.5	0.35±0.1870-51.38	0.71±0.1726-1.38	0.0035*
WBC($x10^3$/Cu mm^3)						
%COC	8.36±0.1624	7.59 ±0.4242-9.21	7.1 ±0.3741-15.07	6.61±0.0959-20.93	8.11 ±0.2622-2.99	4.4353*
Hemoglobin g/dl						
%COC	5.56±0.3922	5.11 ±0.4694-3.09	4.52 ±0.2798-18.70	3.97 ±0.3741-28.59	5.51 ±0.5700-0.89	2.0324*
Hematocrit (%)						
%COC	15.2±0.1414	14.6 ±0.2366-3.94	13.36±0.514-12.10	12.13±0.4396-20.19	15 ±0.5656-1.31	2.2836*
MCV(μm^3)						
%COC	9.60±0.2607	9.11 ±0.5487-5.10	8.39 ±0.5346-12.81	7.53 ±0.3143-21.56	9.54 ±0.2731-0.62	6.8736*
MCH(Pg)						
%COC	40.20±0.5099	38.81±0.4552-3.45	38.40±0.2601-4.47	37.28±0.6227-7.26	39.90 ±0.4472-0.74	3.4758*
MCHC (%)						
%COC	20.30±0.5099	20.1±0.4551-0.98	20.1 ± 0.2607-0.98	19.13±0.6227 -5.76	19.94 ±0.4472-1.77	1.0498*

*The values are mean ± S.D OF six individual observation. % COC-percent change over the control. *indicates significance of Anova (P<0.05).*

Total WBC count: The blood of the control fish in **Table 1** had a mean value of 8.36 mm^3x10^3, according to the results of the total count of white blood cells. The fish exposed to sublethal amounts had mean WBC counts of 7.59, 7.1, and 6.61 mm3x103 after receiving a 0.02 ppm treatment of copper sulphate for 5, 10, and 15 days. The percentages that have declined are -9.21, -15.07, and -20.93. The fish exposed to L-glutamine had a mean WBC value of 8.11 and a reduced percentage of -2.99. When compared to the control, the aforementioned values demonstrated a significant decrease and rise (P< 0.05). (**Figure 1.2**)

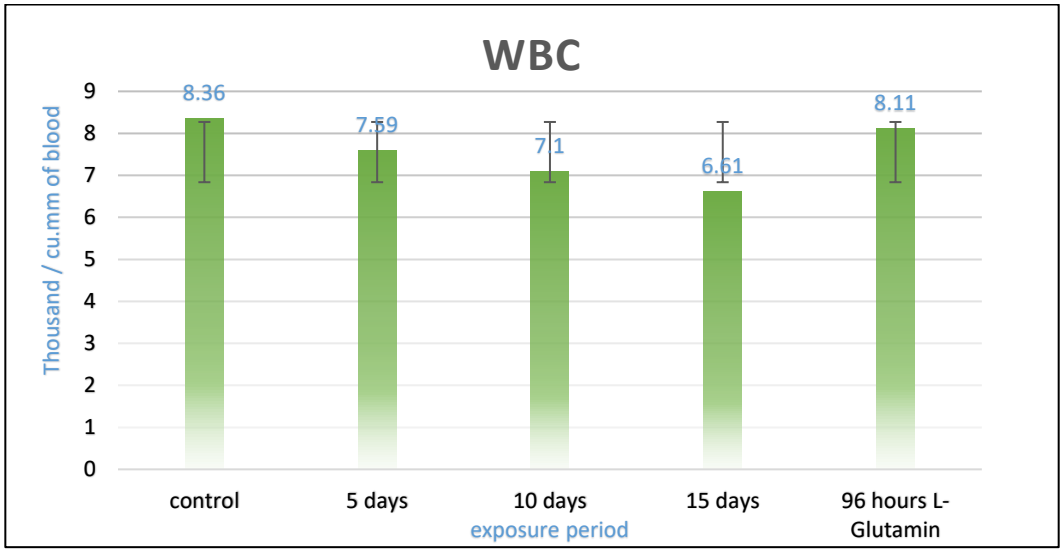

Figure 1.2: changes of the White blood cells (WBC) of Branchydanio rerio exposed to sublethal concentration of copper sulphate and L-Glutamine exposure

Estimation of Haemoglobin: The haemoglobin in the control fish in **Table 1** had a mean value of 5.56 g/dL. After being subjected to sublethal quantities of copper sulphate for five, ten, and fifteen days, the fish's haemoglobin mean levels were 5.11, 4.52, and, 3.97 g/dL at 0.02 ppm. Three percentage points were reduced: -8.09, -18.70, and -28.59. Fish exposed to L-glutamine had a mean haemoglobin count of 5.51 g/dl, with a lowered percentage of -0.89. When compared to the control, the treatment values exhibited a substantial rise and decrease (P<0.05). (**Figure 1.3**)

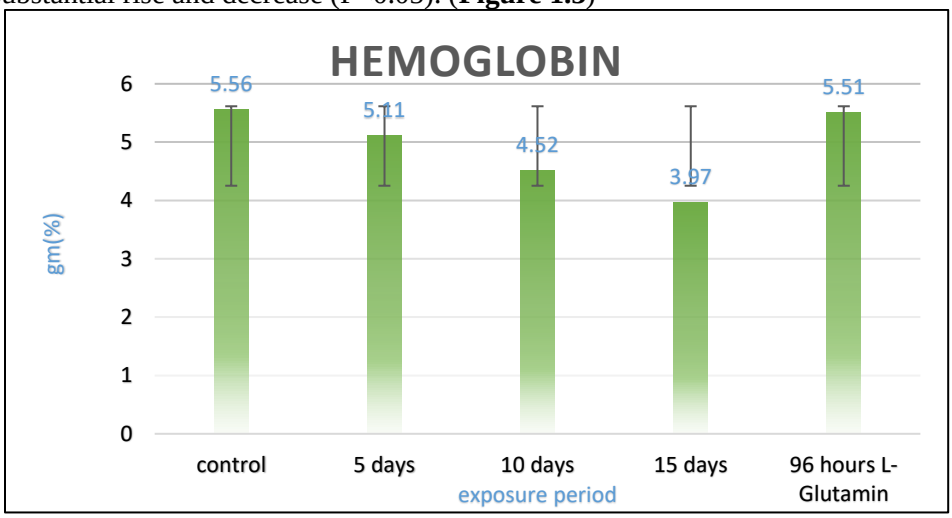

Figure 1.3: Changes of the Hemoglobin (Hb) of Branchydanio rerio exposed to sublethal concentration of copper sulphate and L-Glutamine exposure

Amount of Hematocrit (Ht): Branchydanio rerio treated to sublethal concentrations of copper sulphate in **Table 1** showed decreased hematocrit levels during the whole exposure period. In the control group, the hematocrit was 15.2%. The readings for copper sulphate were 14.6, 13.36, and, 12.13% for the exposure durations of 5, 10, and 15 days, respectively. The percentages of the decrees were -3.94, -12.10, and -20.19. The fish exposed to L-glutamine had a mean value of 15%; this is a lowered percentage of -1.31. At the P<0.05 level, the decreased and elevated hematocrit readings during all exposure periods were statistically significant (**Figure 1.4**).

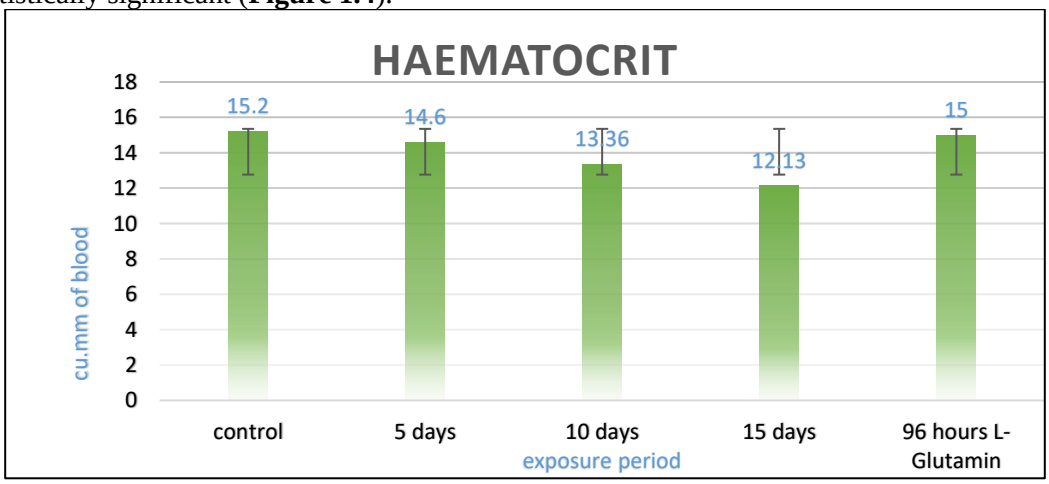

Figure 1.4: Changes of the Hematocrit (Ht) of Branchydanio rerio exposed to sublethal concentration of copper sulphate and L-Glutamine exposure

Mean cell volume (MCV): Table 1 shows the mean cell value of Branchydanio rerio subjected to sublethal concentrations of copper sulphate, L-glutamine for 96 hours, and control. In fish treated with copper sulphate, the MCV was 9.60 µm3 in the control group and 9.11, 8.39, and 7.53 µm3 for the exposure periods of 5, 10, and 15 days, respectively. The percentages of the decrees were -5.10, -12.81, and –21.56. Fish exposed to L-glutamine had a mean value of 9.54 µm3, and their lowered percentage was -0.62. At every exposure interval, the MCV increases and decreases were statistically significant at the P<0.05 level (**Figure 1.5**).

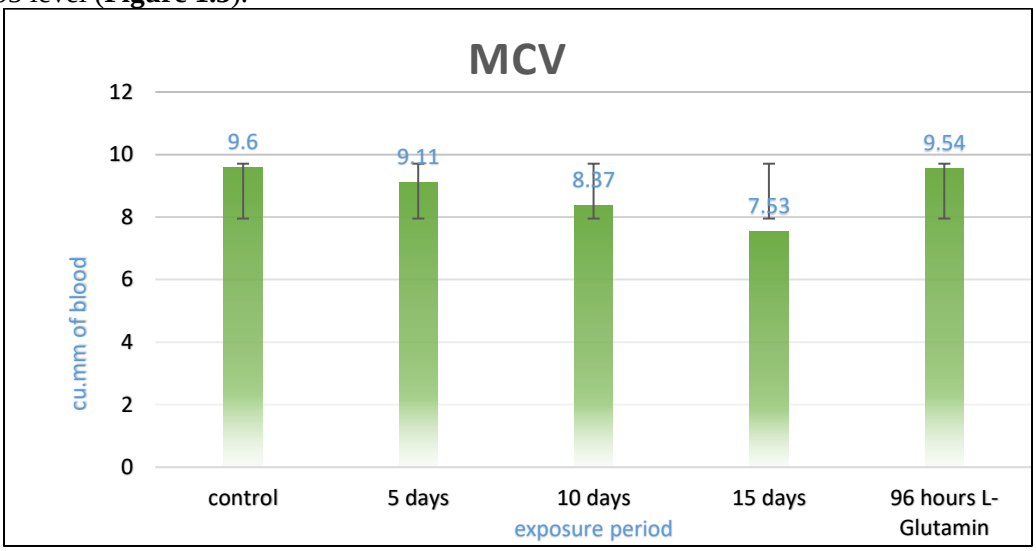

Figure 1.5: Changes of the Mean Cell Volume (MCV) of Branchydanio rerio exposed to sublethal concentration of copper sulphate and L-Glutamine exposure

Mean cell haemoglobin (MCH): The control fish in **Table 1** had a mean cell haemoglobin of 40.60 ppg. The MCH in the fish treated with copper sulphate was 38.81, 38.40, and, 37.28 Pg for exposure durations of 5, 10, and 15 days, respectively. The percentages that decreased were -3.45, -4.47 and, -7.26. Fish exposed to L-glutamine had an MCH mean value of 39.90 pg and a lowered percentage of -0.74. The decreed MCH were statistically significant at the P<0.05 level for all the exposure durations (**Figure 1.6**).

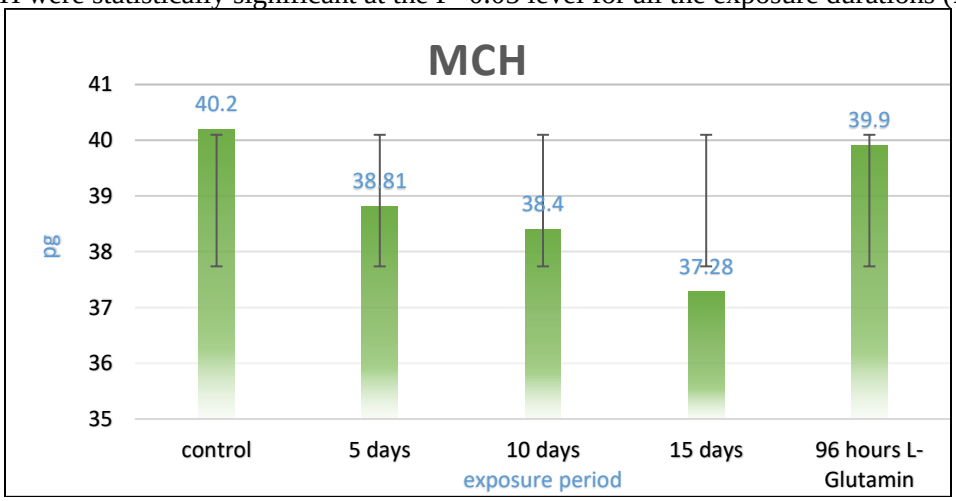

Figure 1.6: Changes of the Mean Cell Hemoglobin (MCH) of Branchydanio rerio exposed to sublethal concentration of copper sulphate and L-Glutamine exposure

Mean cell haemoglobin concentration (MCHC): Table 1 presents the mean cell haemoglobin content of Branchydanio rerio subjected to sublethal concentrations of copper sulphate, L-glutamine for 96 hours, and a control group. The control group's MCHC was 20.30 percent. The MCHC for fish treated with copper sulphate were 20.1, 20.1, and 19.13 for the 5, 10, and 15-day exposures. The percentages of the decrees were -0.98, -0.98, and -5.76. Fish exposed to L-glutamine displayed a mean value of 19.94 with a negative percentage of -1.77. **Figure 1.7** show that the declining MCHC at all exposure times was statistically significant at the P<0.05 level.

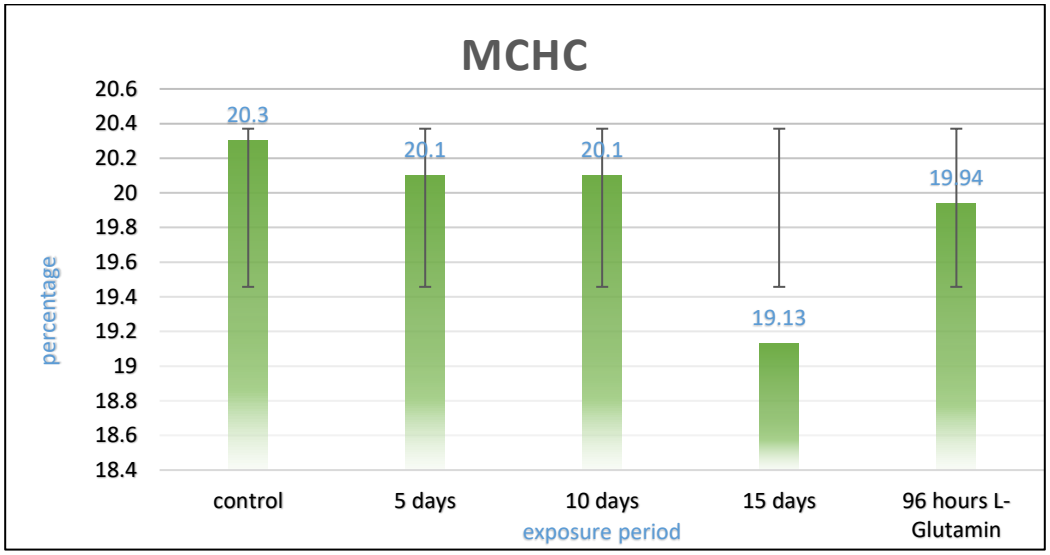

Figure 1.7: Changes of the Mean Cell Haemoglobin concentration (MCHC) of Branchydanio rerio exposed to sublethal concentration of copper sulphate and L-Glutamine exposure

Copper is a significant trace element necessary for the normal growth and metabolism ofliving organisms. However, this element may become very dangerous if used beyond its limit, turning into continuous metal compounds with the ability to accumulate in water and cause imbalance to the biological system. Aquaculture activities can also be affected due to the increase in environmental pollution. Bradl and Heike (2005) have provided explanations for why copper concentrations higher than 20 microgrammes per gramme (µg/g) may be harmful. Humans have used copper for at least 6,000 years. Because of its malleability, ductility, and electrical conductivity, it is used in alloys, tools, coinage, jewellery, food and beverage containers, electrical wiring, and electroplating. Copper's great toxicity to aquatic creatures is demonstrated by its usage in the destruction of fungus, algae, and mollusks. In actuality, one of the metals that is most harmful to aquatic environments and species is copper. This is one of the main justifications for the significance of environmentally conscious mining techniques. Compared to mammals, fish are 10–100 times more vulnerable to the harmful effects of copper. Numerous writers have proven this, such as Hodson (1979). This deviates from the normal rule that aquatic animals are more susceptible to the harmful effects of metals than aquatic plants (Okocha and Adedeji, 2012).

As copper's concentration in water rises, aquatic organisms may become exceedingly harmful to them. Copper is a necessary trace metal for cellular functioning. Numerous human activities (industries, farms, and harbours) have significantly boosted the intake of this metal in estuaries and marine habitats worldwide, making them important natural sources of copper (Flegal, 1993 and D'Adamo, 2008). Natural waters contain very few free copper ions at equilibrium because the majority of copper is bound to inorganic ions or organic materials. $CuSO_4$ has been extensively utilised in fish culture ponds to manage algae and certain diseases, hence elevating the water's copper content. Because $CuSO_4$ is highly

poisonous to fish, the doses needed to inhibit disease agents or algae must be lower than the fish toxicity thresholds.

Numerous studies have been conducted on the impact of $CuSO_4$ on fish, and the results have shown that certain species are more vulnerable to copper than others. This suggests that prior to employing $CuSO_4$, one was aware of a particular farmed fish species' vulnerability. Haematological measures are crucial for environmental monitoring, toxicological research, and as markers of illness and stress in the environment. Numerous studies have shown how pollutant presence and environmental factors can alter blood variables (Azarin *et al.*, 2012). Since blood is a sign of an animal's physiological state, fish exposed to environmental pollutants display a range of physiological reactions, including abnormalities in blood balance, ion regulation, oxygen absorption, and health assessment. Although they are not frequently used in the diagnosis of fish diseases, haematological analysis and assessment of blood plasma are helpful in tracking the physiological status of fish and as markers of the condition of the aquatic environment. When applying clinical diagnosis of fish physiology to ascertain the impact of contaminants at sublethal concentrations, blood parameters are frequently evaluated. Because neutrophils and monocytes are susceptible to heavy metals, high amounts of $CuSO_4$ can also cause immunosuppression and change blood parameters.

Fish are an essential source of food and a vital part of the ecosystem, so it's critical to evaluate the effects of Cu in fish and to look for signs of heavy metal toxicity stress in the blood of Cyprinus carpio fingerlings exposed to sublethal concentrations of $CuSO_4$ over an extended period of time (Azarin *et al.*, 2012). The fact that fewer red blood cells were seen in C. macropomum following treatment could be explained by a compensatory effect in response to oxygen transport capacity (Mazon *et al.*, 2002). The red blood cell count in Channa punctatus was lowered by 0.36 mg/L of copper sulphate (Griffin *et al.*, 1999). This feature can be explained by the fact that the haematological organs produce fewer erythrocytes as a result of the circulating cells being destroyed (Singh *et al.*, 2008). However, Williams & Wootten (1981) reported that rainbow trout (Oncorhynchus mykiss) treated with $CuSO_4$ for 24 hours had a higher hematocrit, supporting the findings with the maximum concentration (8.75 mg L-1). The hypoxic condition-induced increase in erythrocyte volume in P. mesopotamicus, as documented by Tavares-Dias *et al.* (2002). (Mazon *et al.*, (2002) attribute these changes to copper-induced injury to the hematopoietic organs and gills. Fish require copper; however, when using it for prophylaxis or therapy, caution must be exercised. Fish may have either short-term or long-term effects when the concentration of copper is higher than what is tolerated. The toxic effects of the toxicant may be predicted by measuring the haematological alterations in the blood of fish exposed to the toxin. Fish blood parameters are known to alter as a result of copper stress. The majority of blood cell alterations manifest as problems with fluid volume and ionic status (Tavares-Dias *et al.*, 2011).

Pamila *et al.* (1991) suggest that the decrease in haemoglobin concentration in fish exposed to a toxicant may also be caused by the toxic substance's inhibitory action on the enzyme system that produces haemoglobin. According to Joshi *et al.* (2002), poor intestinal absorption of iron caused by exposure to heavy metals also resulted in a decrease in RBC and Hb content. It's possible that the anaemia caused the RBC and Hb concentrations to decrease. Anaemia under copper-induced stress may also result from disruptions to Hb production and damage to blood cells. Fish that are anaemic have either an abnormally low red blood cell count or an excessive amount of haemoglobin in their red blood cells. Anaemia is a first sign of both acute and long-term heavy metal poisoning. The significance of these alterations can be comprehended in terms of fish dying from heavy metal pollution as a result of lower oxygen intake. On the other hand, following 21 days of $CuSO_4$, brook trout (Salvelinus fontinalis) showed higher haemoglobin levels and erythrocyte counts. Additionally, found in Prochilodus lineatus and Cyprinus carpio (Witeska *et al.*, 2005), common carp. Increases in the concentrations of haemoglobin and red blood cells are two of the blood changes brought on by copper exposure. These unfavourable outcomes may possibly be attributable to the various treatment durations and concentrations, the physicochemical

characteristics of the water, and the $CuSO_4$ sensitivity of monogenean species. Channa punctatus exposure to sublethal concentrations of $CuSO_4$ was shown to produce similar effects, including a decrease in Hb and RBC content. Following treatment with varying concentrations of $CuSO_4$, it was noted that the amount of RBC in C. macropomum was reduced (Griffin *et al.*, 1999). It was discovered that fish exposed to 0.04 and 0.004 mg/L $CuSO_4$ had a significantly higher WBC concentration than the control groups. Examining variations in the WBC count and its kinds is one of the simplest methods to evaluate the immune system (Moraes *et al.*, 2007).

Azarin *et al*. (2012) reported that fish subjected to elevated copper concentrations had comparable results. The first line of immunological defence is made up of WBC. Examining variations in the number and variety of white blood cells is one of the simplest methods to evaluate the immune system (Tavares-Dias and Moraes, 2007). Consequently, it seems that immune system regulation is connected to the immune system's reaction to copper sulphate. The leukocyte properties of C. macropomum that were studied in this investigation were identical to those reported by Tavares-Dias *et al*. (1999) for the same species. On the other hand, toxic granulations were discovered on a few neutrophils and eosinophils in this location, indicating a high production of its contents. According to Witeska and Wakulska (2007), phagocytes, which include neutrophils and monocytes, are susceptible to heavy metal poisoning. Increased $CuSO_4$ concentrations in this assay, which used young C. macropomum animals, resulted in leucopenia, which is characterized by a decrease in lymphocyte, neutrophil, and PAS-GL counts.

According to this theory, exposure to copper impairs phagocytic activity and migration in the kidney, liver, and gills in O. mossambicus in a state of neutropenia and monocytopenia (Nussey *et al.*, 1995c). On the other hand, in C. punctatus, leukocytosis was associated with an increase in lymphocyte and eosinophil counts and a subsequent drop in monocyte and basophil counts (Singh *et al.*, 2008). In tissue injured by copper, leukocytosis has been linked to an increase in leukocytes that shield the organism against infections (Mazon *et al.*, 2002). Given that 4.37 mg/L of $CuSO_4$ demonstrated 99.3% efficacy in treating monogenoideans A. spathulatus in juvenile C. macropomum, doses of 5.0–6.0 mg/L may be adequate to eradicate these parasites under the trial conditions employed here. Sublethal CuSO4 exposure for a brief period of time alters physiological processes that impact osmotic imbalance. Moreover, fish with greater $CuSO_4$ concentrations have significant immunosuppression, which may render the organism more susceptible to illness.

In order to ascertain the impact of outside stressors and illness conditions in fish, haematological variables have been widely employed in recent years when clinical diagnosis of fish physiology has been applied (Benerjec and Kamar, 1988). This is because of their interactions with energy (metabolite levels), respiratory mechanics (haematological levels), and defence mechanisms (leucocyte levels), according to Gill and Pant (1981). Haematological indicators offer a comprehensive assessment of fish health. When toxicants are introduced into a fish habitat, they stun the fish and/or cause stress to the fish and other creatures in the area (Olatayo, 2008). When a toxicant is added to an aquatic system, the concentration of dissolved oxygen may drop, causing breathing difficulties and suffocation (Warren, 1977). According to Stickney's 1977 report, a deficiency in dissolved oxygen is a contributing factor to fish species' mortality. Fish exposed to acute toxicant concentrations exhibit skin darkening, respiratory discomfort, and irregular swimming (Ayuba and Ofojekwu, 2002).

An amino acid is glutamine, amino acid molecules are involved in numerous bodily functions. Their primary function is to function as the building blocks of proteins. They also carry out other tasks, such as transferring chemicals throughout the blood, and glutamine aids the immune system in warding off dangerous bacteria and viruses. According to Cimen (2008), the primary roles of erythrocytes in respiration are the transportation of O_2 and the mediation of CO2 generation. In juvenile carp blood, L-glutamine raises Ht, RBC, Hb, MCV, and MCH, according to X.Q. Zhou's 2017 study. Hb is the oxygen transporter protein found in erythrocytes. It can bind oxygen to create oxygenated Hb, which releases red blood cells and HbC in carp blood. It's possible that Gln encourages fish erythrocyte maturation, which

explains the increases in HbC. Branchydanio rerio's primary haematological reaction to the sublethal dose of $CuSO_4$ in this investigation was a markedly reduced RBC, WBC, Hb, Ht, MCV, MCH, and MCHC than in the control group. Fish's hematopoietic system is primarily found in the kidney's interstitium, in contrast to humans. Therefore, structural changes in the renal interstitium may be the source of the hematopoietic system's inefficiency, which would explain a decrease in the haematological parameters. Furthermore, the alterations in haematological parameters may be explained as a compensatory mechanism enhancing oxygen transport ability to sustain gas transfer and a modification in the water-blood barrier for gas exchange in gill lamellae.

CONCLUSION

As a result, it has been determined that haematological parameters are the most sensitive indicators of copper toxicity, particularly at sublethal concentrations. Additionally, alterations in haematological variables following copper exposure suggest ionoregulatory or respiratory disturbances, which suggest increased energy expenditure to maintain homeostasis rather than other physiological processes such as weight gain or growth. Branchydanio rerio exposed to copper sulphate exhibited ionoregulatory interference in their blood parameters, but they also displayed compensatory responses that allowed the fish to survive. These changes had a substantial impact on the fish's haematology. When zebra fish are exposed to L-glutamine, their blood parameters such as RBC, WBC, Hb, Ht, MCV, MCH, and MCHC values increase relative to fish that have been exposed to copper. This finding suggests that L-glutamine may counteract the effects of copper sulphate. The table 1 shows changes of blood parameter values based on copper and L- glutamine exposer compared to control.

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