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*RESEARCH ARTICLE*

# MERCURIC CHLORIDE($HGCL_2$) INDUCED HEMATOLOGICAL CHANGES IN FRESH WATER FISH LABEO ROHITA(HAMILTON).

**Srinivasa Naik Banavathu and *Jagadish Naik Mude.**
Department of Zoology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.

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*Mercuric chloride,Toxicity, Labeo rohita, Haematology*

## *Abstract*

*Hematological indices are gaining general acceptance as valuable tools in monitoring various aspects the health of fish exposed to contaminants. In this work, some effects of m*ercuric chloride on hematological parameters by the fresh water fish labeo rohita. The acute toxicity value (96 h $LC_{50}$) of mercuric chloride was found to be 0.25 mgl-1 calculated by Finney's probit method. Fishes were exposed to sublethal concentration of 1/10th 96 h LC50 (0.025 mgl-1) for the period of 5,10 and 15days.The present study was revealed significant *decrease (P<0.05) for* RBC count, HB levels, andpacked cell volume (PCV) *than control group. In contrast, there was a significant increase in* WBC count, Glucose, alanine transaminase(ALT), mean corpuscular volume (MCV), and total leucocyte count(TLC) *in the mercuric chloride-treated group. But the total protein(TP), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were significant with control group. So, estimation of these indices, could provide a useful indicator of pollution of water bodies. The present finding clearly indicating of mercuric chloride toxicity is strong influence in aquatic environment on the hematological parameters in the fresh water fish Labeo rohita.*



## Introduction:-

Heavy metals constitute a core group of aquatic pollutants and additional concentrations of these metals accumulate in the aquatic ecosystems because of land-based activities (Vutukuru S., 2003). Heavy metals are persistent contaminants in the environment that come to the fore front of dangerous substances such as mercury, cadmium, lead, copper and zinc which causing a serious health hazard in humans and animals (Abbas H et al., 2002). Mercuric chloride (HgCl2) is one of the environmental pollutants that are currently used as a catalyst or reagent in various chemical reactions, and to a lesser extent as a disinfectant or pesticide it is also a developmental toxicant in experimental animals following both oral and inhalation exposure (US EPA., 1984). Mercury is nonbiodegradable and non- advantageous heavy metals and their role in the cell is not understood (Bailey S. E et al.,1999).The soil with methyl mercury contamination or water with mercury methyl chemical species is easily absorbed by aquatic organisms, because of its strong affinity of sulfhydryl biopolymers such as proteins (Jayaprakash, K. 2009). Human destructive influence on the aquatic environment in the form of sub-lethal pollution usually results in chronic stress conditions that have negative influence on aquatic life (P. Palanisamy., 2011). Haematological variables remain as veritable tools in determining the sub-lethal concentration of pollutants such as heavy metals in fish (Witeska M.,

**Corresponding Author:-Jagadish Naik Mude.**
Address:-Department of Zoology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.

2003). Haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources in the tropics. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Iwama GK et al.,1976).Haematological abnormalities have also been studied in various toxicant- Haematological variables remain as veritable tools in determining the sublethal concentration of pollutants such as heavy exposed fish: Chana punctatus to cadmium (KaruppasamyRet al.,2005). Physiological stress indicators such as sum hematological and blood parameters could be useful to evaluate effects of contaminants such as heavy metal in fish (Oliveira et al.,2006). Usually, RBC system of fish reacts to heavy metal intoxication with anemia but sometimes, particularly after short exposures, blood parameters (Hct, RBC, mean corpuscular volume, Hb may be increased (Vosyliene MZ.,1996). The mutual action between a toxicant and a biological system can be calculated using hematological, biochemical biomarkers (Li, Z. H et al.,2010). Mercury cycles in the environment as a result of normal and anthropogenic bustles. Anyway, the amount of mercury liberated via human activities severely increased since the commencement of the industrial age (Wang, Q et al.,2004).The present study on the effect of mercury chloride exposure during the haematological parameters of the fishes are light on the effect, so it was decided to attempt the present study.

## Material and Methods:-

**Animal:-**

The fresh water fish, Labeo rohita (Rohu) (8-10 cm length and 28 ± 0.6 g weights) was used for the toxicity tests were fishes are selected forty healthy fish from the stock and divided into four groups. These fishes were collected from local fish ponds at Nujividu village, Guntur district, Andhra Pradesh, India (latitude: 16.1808576.N, longitude: 80.6646.E). Fish were brought to the wet laboratory and acclimatized for the period of 10-15 days prior to conducting experimentation. The dechlorinated tap water was used throughout the course of the experiments and the physico-chemical parameters of water determined according to American Public Health Association (APHA.,2005).The values are as follows: temperature, 28 ± 2°C; pH, 7.12; total hardness, 170 mgl-1 (as CaCO3); total suspended solid (TSS), 4 mgl-1; turbidity, 7.5 Silica units and dissolved oxygen concentration, 5-6 mgl-1.The LC50 value was estimated in the laboratory conditions as per the method of Finney's probit analysis (1971) starting with minimum range for acute toxicity trials. The acute toxicity for 96 h LC50 was found to be 0.25 mg/l and 1/10th of 96 h LC50 was 0.025mgl-1.

**Chemical:-**

The inorganic Mercuric Chloride (HgCl2) authorised stockists for 'fisher scientific' (CAS number 7487-94-7), was used as the test chemical.

**Experimental Design:-**

Fishes were divided into equal 4 groups each group contain 10 fish. Each group fishes were transferred separately in KMnO4 washes plastic tubs of 20lit volume.

Group-I fish were maintained as control without any treatment.

Group-II, III, IV were exposed to sublethal concentration of 0.025mg/l mercuric chloride (1/10th of its 96h LC50 concentration) for 5, 10 and 15 days respectively. Sublethal concentration of mercuric Chloride (HgCl2) test chemical was freshly prepared in distilled water before mixing in aquatic water of tubs. Mouth of tubs was tied up by mosquito nets to prevent the escape of the fishes. The tub containing fish was aerated with rich oxygen. The hygienic conditions were maintained by renewing water after every 24hrs and, fish were daily fed with rice bran and fish pellets. All the tubs were kept under same laboratory conditions and under observation.

The fish specimens were anesthetized with methane sulfonate (MS- 222, Sigma Chemical Co, USA). 1 mL of blood was obtained by caudal vein puncture, with heparin as an anticoagulant and transferred into small vials kept in ice-cold condition and placed in glass tubes containing EDTA (Sigma Chemical Co, USA), while the fish were sedated.

Plasma was obtained by centrifugation of blood at 3000rpm for 15 min and non–haemolysed plasma was stored in a deep freezer for further biochemical analyses. From the blood sample in the glass tube containing EDTA, RBC, WBC and hemoglobin were determined as follows: according to method of Hesse (1960) and Wintrob (1967) used improved Nebulae's Haemocytometer. To determine RBC and WBC, Care was taken to avoid trapping of air bubbles. The RBC lying inside the five small squares were counted under high power (40X) of light micro scope. The following formula was used to calculate the number of RBC per mm3 (µL) of the blood sample:

Number of RBC/mm3 = (N × dilution)/ area counted × depth of fluid.

The WBC lying inside the four large squares were counted under high power (40X) of light micro scope. The following formula was used to calculate the number of WBC per mm3 (µL) of the blood sample:

WBC = (N × dilution)/ area counted × depth of fluid.

Some blood dropped on glass slides prepared and were done on blood films stained with Giemsa. Replicate counts were made for each blood sample. Hemoglobin concentration was determined by the cyanmethaemoglobin procedure (Boehr inger Mannh eim kit, 124729). All colorimetric determinations were performed using a spectrophotometer (Perkin-E lmer Coleman) at 415 nm.

To determine PCV/ HCT, duplicate fresh blood samples were collected into heparinized micro hematocrit tubes sealed with plasticine at one end and centrifuged for 5 min (Hermle Z 383 K) at 3000 rpm. The mean values of PCV (%) were measured with a microhematocrit reader. Hematological indices were calculated from the equations given by (Ovie et al. 2007).

MCV (fL) = PCV (%) × 10 / RBC (106/L)

MCHC = Hemoglobin (g/dL) × 100 / PCV (%)

MCH (pg) = Hemoglobin (g/dL) × 10 RBC / (106 /L)

Plasma glucose was determined using assay kits supplied by Human Diagnostics Worldwide according to Trinder (1969). Total protein content was determined according to the method of Henry (1964). Total lipid content was determinate calorimetrically according to Joseph et al. (1972). The activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to Reitman and Frankel (1957).

The nominal and measured concentrations were compared for significant difference using student test using SPSS software the values considered significance at p -value < 0.05.

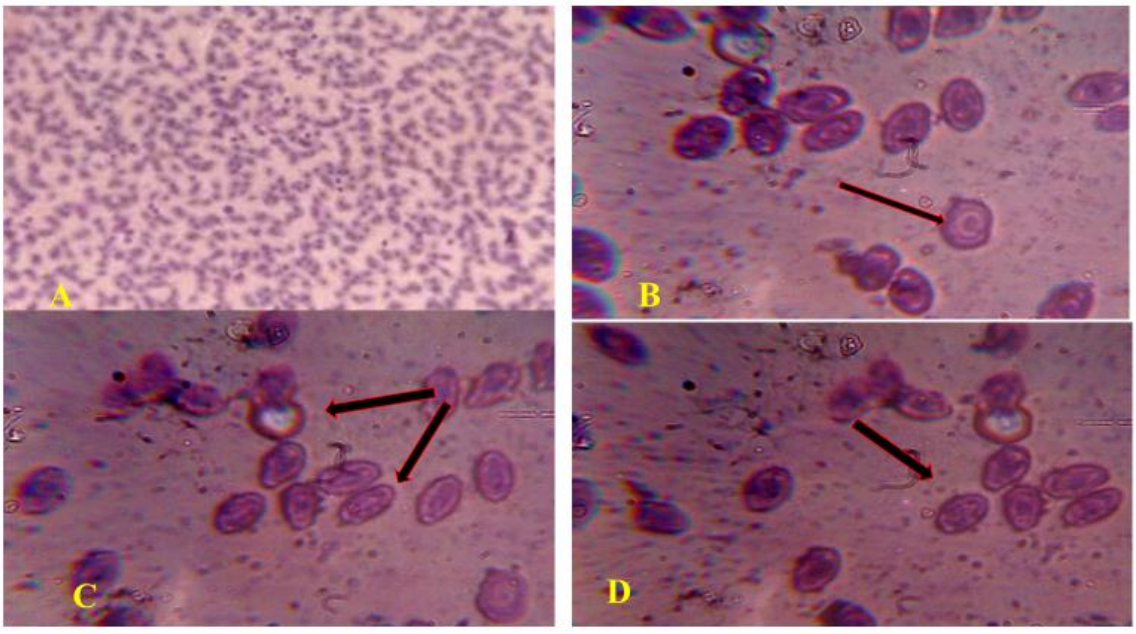


**Fig.1. A:-** Showing Blood smear of Labeo rohita (Healthy) (40X), **B**) showing blood smear abnormal shapes and enlarged RBC with treated mercuric chloride($Hgcl_2$) to fish after 5 days of exposure, **C**) Showing blood smear Swelled erythrocytes and rupture nucleus after 10days, **D**) Showing blood smear distorted erythrocytes with distorted nucleus and lysed erythrocytes after 15days exposure.

## Result:-

The results of exposure fish, Labeo rohita to sub lethal concentration of mercuric chloride are shown in table 1, figer2,3. The toxic of metal caused the significantly decrease was observed in totalRBC count, HB levels, and PCV ($p < 0.05$) of fish after treatment with metal for 5 and 15 day's exposure from the control group. In the mean level of (control/15days) from 1.79±0.06to1.30±0.11($10^6$/$mm^3$),7.49±0.72to5.38±0.55gm/100mland27.35±2.37to5.38±0.55(%) respectively (**Table-1, Fig-2**). A slight decrease was seen in RBC count, HB levels, and PCV during the 10 days of treatment when comparing control group.

The significant increase was observed in total WBC count, Glucose, ALT, MCV, and TLC of fish after treatment with mercuric chloride metal for 5and15 days' exposure period from the control group. In the mean level of (control/l5days) from 27.99±1.64to32.30±1.20 thousands/$mm^3$,58.88±1.08to70.54±1.98mg/l,28.13±1.61to47.54±1.60IU/l,154.07±7.74to169.57±6.61FL and11.37±0.89to17.26±0.83g/l respectively (**Table-1, Fig-3**).

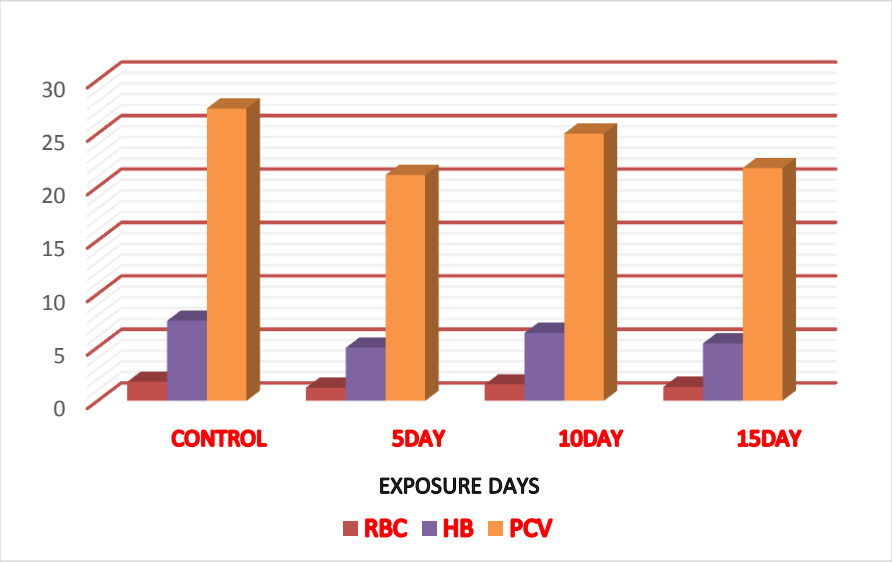


**Figure 2:-** Total RBC, HB and PCV changes in Labeo Rohita during Exposure to Mercuric Chloride .

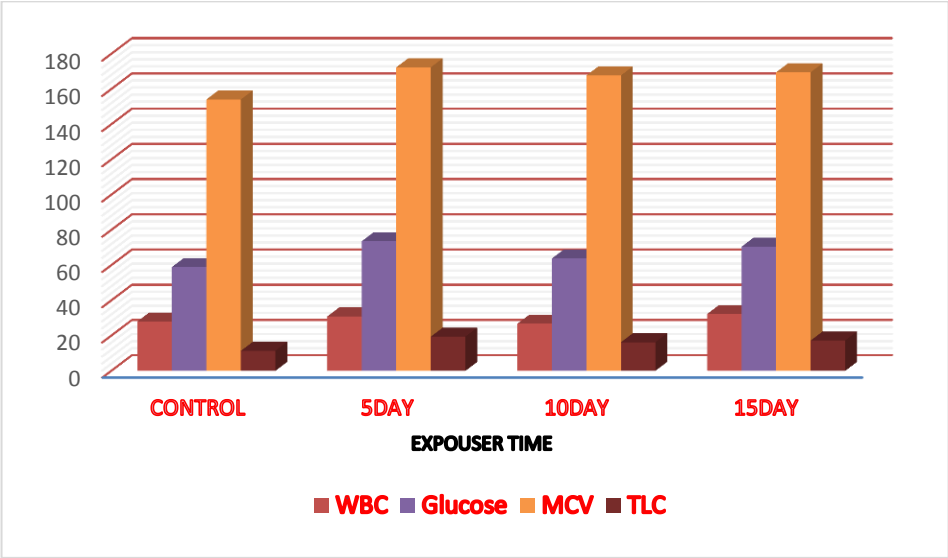


**Figure 3:-** Total WBC, MCV and TLC changes in Labeo Rohita during Exposure to Mercuric Chloride

However moderately increase mean levels in same blood parameters of after 10dyas exposure period when compare to the control group. But at the same time the total protein(TP), mean corpuscular hemoglobin concentration (MCHC) and mean cell haemoglobin (MCH) values were decrease non-significantly. In this report, a series of hematological parameters were examined for studying the toxicity induced for 5,10 and 15days exposure to sublethal concentrations of mercuric chloride in labeo rohita.

**Table.1:-**Variations in the hematological parameters following exposure to one tenth of the 96h$LC_{50}$ concentration of mercuric chloride in freshwater teleost fish labeo rohita.

| **Parameters** | **Control** Mean ± SD | **Exposure period(days)** | | | **P-valve 15 days'** |
|---|---|---|---|---|---|
| | | **5 Days** Mean ± SD | **10 Days** Mean ± SD | **15 Days** Mean ± SD | |
| **RBC Count ($10^6$/$mm^3$)** | 1.79 ± 0.06 | 1.23 ± 0.07 | 1.53±0.03 | 1.30 ± 0.11 | 0.0072 P<0.05* |
| **HB (gm/100ml)** | 7.49+0.72 | 4.97 ± 0.33 | 6.35 ± 0.73 | 5.38 ± 0.55 | 0.027 P <0.05* |
| **PCV (%)** | 27.35 ± 2.37 | 21.13+1.22 | 25.65 ± 1.84 | 21.78 ± 1.37 | 0.039 P<0.05* |
| **WBC ($10^3$/$mm^3$)** | 27.99 ± 1.64 | 30.90+1.52 | 27.99 ± 1.64 | 30.90+1.52 | 0.035 P>0.05# |
| **Glucose(mg/l)** | 58.88+1.08 | 73.63+1.32 | 63.78+0.78 | 70.54+1.98 | 0.0030 P>0.05# |
| **ALT(IU/l)** | 28.13+1.61 | 54.17+2.05 | 42.27+1.94 | 47.54+1.60 | 0.0007 P>0.05# |
| **TLC(g/l)** | 11.37+0.89 | 19.46+1.02 | 11.37+0.89 | 19.46+1.02 | 0.0036 P>0.05# |
| TP(g/100ml) | 2.98+0.29 | 1.98+0.30 | 2.33+0.22 | 2.01+0.07 | 0.032 S |
| MCH (pg) | 41.83+3.88 | 41.10+1.10 | 38.73+1.97 | 41.40+2.95 | 0.05 S |
| MCHC (%) | 27.20+2.91 | 24.00+2.53 | 23.25+2.97 | 24.46+2.39 | 0.03 S |

Values are Mean ± SEM, n =5, * = Significant decrease at p <0.05, # = Significantly increase at p>0.05, S=Significantly.

## Discussion:-

Heavy metals induced a significant decrease in the RBC Count, HB, PCV level of the fresh water fish labeo rohita during the exposure period of 5,10 and 15 days (**Fig.2**). The results are in good understanding with previous works that reported a significant decrease in RBC's hemoglobin and packed cell volume of fresh water fish exposed to heavy metals (Vutkuru, S. Shalaby, A 2005,2001). Our results are supported by preceding research work that various heavy metals and toxins enter the aquatic system exerted a specific toxic effect on fish blood and tissues (Mousa, M. A et al.,2003). According to Nanda and Behera (1996) also reported decrease in total RBC, Hb% and PCV in the fish, Heteropneustes fossilis after nickel sulphate treatment for 15 days (Nanda, P et al.,1996).Our results are comparable to the previous reports of noticed anaemia with erythropenia has also been reported earlier in fishes after exposure to sublethal doses of other metals; mercury, lead and zinc (Panigrahi et al.,1983; Ubogu et al.,2008). Shah and Altindag also observed decrease in haemoglobin, RBC Count and PCV values in Tinca tinca exposed to Mercury and lead salts. Decrease in RBC count, haemoglobin and PCV value were also noticed in Nile tialapa exposed to the pesticide edifenphos (S.L.Shah et al., 2004). The significant decrease in haemoglobin concentration of fishes under toxic stress cold be either due to increased rate of destruction of haemoglobin or due to decrease rete of synthesis of haemoglobin (P.M.Reddy et al., 1989).

The blood of labeo rohita showed significant increase in WBC Count, Glucose, MCV level of the fresh water fish common carp during the exposure period of 5,10 and 15 days (**Fig. 3**). Previous investigation proved that heavy metals increase the glucose content in blood, because of intensive glycogenolysis and the synthesis of glucose from

extra hepatic tissue proteins and aminoacids (Almeida.J et al.,2001). Blood is highly susceptible to internal and external environment fluctuations because it is the vehicle for the transport of such pollutants. Metals are transported in the blood stream by binding to specific plasma proteins (Joseph. B et al.,2010). The prolonged reduction were observed in haemoglobin content could be deleterious to the oxygen transport and any blood dyscrasia and degeneration of RBC could be endorsed as pathological condition in fishes exposed to toxicant, other toxicant (ammoniumsulfate, cypermethrin) including heavy metals Zinc,lead,Copper,mercury also induces decreased erythrocyte count in several fishes (Buckley et.al., 1976). Found a significant decreased in total erythrocyte count, haemoglobin content, hematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, C. punctatus after exposure to sublethal dose of Cd (29 mg Cd/L) (Karuppasamy et al., 2005.

Wepener et al., (1992) were studied the effect of hexavalent chromium on the haematology of Tilapia sparrmani and similar results, which are well in agreement with the present results. The increase in number of WBCs may play an important role in immunological defence systems during exposure to toxicants like heavy metals and appears to be associated with increased circulatory levels of granulocytes, which are known to respond for phagocytosis (Briton, C. J and Kori-Siakpere O.,2008,1963). These parameters could be effectively used as potential biomarkers of heavy metal toxicity to the freshwater fish in the field of environmental biomonitoring

## Concluson:-

The present study was concluding the acute exposure to mercuric chloride proved to be highly toxic to Labeo rohita and induced cumulative deleterious effects at various vital functional sites like metabolic rate, hematological indices profiles. Though significant changes are observed both at the end of 5 and 15days exposure periods. The industries and dumping of e-waste are the major contributing factors for the release of mercury pollution in the environment. To be maintain proper dissemination and awareness among industrial entrepreneurs with strict rules and regulations can only mitigate this burden. Hence this study can be used as a tool for creating awareness among the local farmers and compare the sensitivity of various species of aquatic animals and potency of effluent using LC50 values and to derive safe concentration so that the use of the highly toxic heavy metal can be minimized.

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