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Research Article

Effect of Mercuric Chloride on Some Hematological, Biochemical Parameters in Silver Carp (*Hypophthalmichthys Molitrix*)

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Abstract

The mutual action between a toxicant and a biological system can be calculated using hematological, biochemical biomarkers. The immunological, hematological and biochemical indices of silver carp (*Hypophthalmichthys molitrix*) were investigated under low (10%LC50) and high (50%LC50) concentrations of mercury chloride for 96h. In both concentrations Ht, Hb, RBC, lymphocyte were significantly ($p<0/05$, $p<0/01$) lower than controls group whereas MCH, MCHC, WBC, Glucose, neutrophil, eosinophil were significantly ($p<0/05$, $p<0/01$) larger contrasted to the respective controls group. In low and high concentrations MCV, cortisols respectively were lower than control group. A significant decrease in high concentrations in cortisol level may be as cortisol suppression

due to high stresses. The consequential discoveries of this study are that mercury chloride concentrations (low and high) may cause some substitutions in the hematological, biochemical and immunological parameters of the studied fish, so estimation of these indices, could supply a useful indicator of mercury chloride of water bodies. It appears that MCH, eosinophil in low concentrations and lymphocyte in high concentrations is appropriate biomarkers of mercury chloride in silver carp.

Keywords: Aquatic ecosystems; Heavy metals; Toxicology; Pollutions.

Introduction

Heavy metals have been announced to exert a vast range of metabolic, physiological, ecological and behavioral influences on fish (Soengas et al., 1996). Mercury is non-biodegradable and non- advantageous heavy metals and their role in the cell is not understood (Bailey et al., 1999). 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 et al., 2004). The unfastened of manufacturing, domestic and urban wastes produced by human activities to aquatic ecosystems potentially induces different combinations of stresses to wildlife. The evaluation of environmental perturbations requires the explanation of stress effects throughout the pecking order of

biological organization, from molecular and cellular levels up to organism and people levels (Moore 2002). To do so, the expansion of distinctive biomarkers to examine the in vivo effects of contaminants is a preference necessity to show the action mechanisms of toxicants. As aquatic ecosystems are the most prominent final containers to industrial and urban waste ejections (Hoffman et al., 1995), an essential target in ecotoxicology is to assess the risks for fishes and human populations. Hematology furnishes an index of physiological position of fish and the use of blood picture of fish is an impressive tool for detection of alterations in practical state of organism (Rambhaskar and Rao 1987). Behavioral changes in animal are symptomatic of internal interferences in the body functions, such as disturbances in metabolic routes and ionic imbalance in blood serum (Lewis and Lewis 1971; Shah 2002).

Blood indices are more frequently utilized when clinical diagnosis of fish physiology are used to ascertain sublethal and chronic exposure of contaminants (Kim et al., 2008). Fish hematological indices are very susceptible to water contaminants, and its commutation due to the hematological and immunological parameters can be utilized as toxicity indices of xenobiotics (Sancho et al., 2000). however fish blood indices have been increasingly tested in marine environmental monitoring as important parameters of physiological changes in the presence of contaminant, the absence of basic knowledge about the hematological reaction to the main stressors of at risk species is the most important leakage for using these indices in environmental monitoring plans (Affonso et al., 2002). Examinations on the toxic effect of metals on fish are joined by

the analysis of exchanges in some haematological and biochemical blood indices (Hoyle et al., 2007).

The mutual action between a toxicant and a biological system can be calculated using hematological, biochemical biomarkers (Li et al., 2010). The change in hematological (RBC and WBC count, Hb, Hct, MCV, MCH and MCHC), biochemical (glucose and cortisol) biomarkers and immunological (lymphocyte, neutrophil and eosinophil) parameters are greatly used to evaluate the toxic stress, integrity of the immune system and tissue damage (Nemcsok and Benedeczky 1990; Talas and Gulhan 2009; Kavitha et al., 2010). The changes in these biomarkers are frequently susceptible to environmental or physiological changes and are simply quantifiable and supply an integrated measure of

the physiological changes in organisms (Remyla et al., 2008).

Materials and Methods

Juvenile samples of silver carp with mean weight 200gr provided by fisheries research laboratory. Fish were adapted to laboratory conditions for 7 days in a 400L tank with dechlorinated tap water. During adaption, all fish were fed with trading pellet twice a day.

fish were exposed to a low concentration of 10% LC50 (0/09 mg/l) mercury chloride and high concentration of 50% LC50 (0/27 mg/l) for a period of 24h, 48h and 96h static toxicity examination, presented in tank of 400L, each including 21 fish.

One group control was conserved in a fiberglass tank without accessing toxicant by storing 21 fish. For each experimental time were carried out three repeats. PH, dissolved oxygen, temperature and conductivity were monitored during the experiment (Hedayati et al., 2010).

Fish were without delay anesthetized with 200ppm clove power. Blood samples rapidly were taken from tail blood vessel by heparinized syringes. Appointments of the blood indices were carried out on fresh blood. Amounts of blood leukocytes and erythrocytes were carried out at 1:30 dilution by diluting heparinized blood with Giemsa stain. Cells were enumerated using a hemocytometer Neubauer below the light microscope (Stevens 1997)

According to Beutler et al., (2001) the leukocyte differential tab was made in peripheral blood smears stained with Merck Giemsa, giving the neutrophils extent of differential neutrophils and the mononuclear value of differential lymphocytes too eosinophil and monocyte.

After sampling Hematocrite values were shortly ascertained by putting new blood in glass capillary tubes and centrifuged in a microhematocrit centrifuge at 10000 rpm for 5 min (Hettich, Germany). With assist of a microhematocrit were accomplished Hematocrite readerships.

According to Lee et al., (1998) by measuring the formation of cyanmethemoglobin were found hemoglobin levels (Hb mg/l). Erythrocytes Indices (MCH or Mean Corpuscular Hemoglobin,

MCHC or Mean Cell Hemoglobin Concentration and MCV or Mean Corpuscular Volume) were computed from RBC, Ht, and Hb (Lee et al., 1998).

To determine meaningful disagreements to evaluate the effect of methyl mercury on blood indices was used an analysis of variance (ANOVA) with Duncan Post Hoc.

Pearson coefficients of correlation(r) were evaluated between mercury concentrations and blood indices to investigate associations between bioaccumulation and its effects. Multiple regressions were used to ascertain the kinship between mercury concentration and blood parameters. The data were analyzed by

means of statistics at p<0/05. Data are told as means ± standard deviation ($\overline{X} \pm SD$).

Results

In the present examination the 96 h LC 50 value of mercury chloride to (H. molitrix) was determined to be 0/55 mg/l.

Results of Survey Blood Factors in Low Concentrations (10%Lc50)

Fish exposed to mercury for 96h presented a significant change in hematological (Hb, Ht, MCH, MCHC, MCV, RBC), biochemical (glucose, cortisol) and immunological (neutrophil, lymphocyte,

eosinophil) parameters concentrations toward the control groups ($p<0/05$), whereas among significant indices MCV, RBC, Ht, Hb, Lymphocyte in fish exposed to mercury for 96h were significantly lower than control groups ($P< 0.05$) and WBC, MCH, MCHC, glucose, cortisol, neutrophil, eosinophil were significantly ($P< 0.05$) greater compared to the control groups (Fig 3-10) . In low concentrations, the correlation between mercury with all parameters was statistically tested by analyzing the data procured during the mercury exposed. MCH, MCHC, cortisol, glucose, neutrophil and eosinophils levels showed significant ($p<0/01$) positive correlations but RBC, lymphocyte ($p<0/01$) and Ht ($p<0/05$) exerted significant correlation with mercury exposure that altogether were negative in 10%Lc50 concentrations. To appointment the relationship between mercury concentrations with biochemical, hematological and

immunological activity accustomed to curve estimation regressions. Only the MCH and eosinophil levels showed significant linear regression ($p<0/05$) $Y = a \pm bX$ with mercury (Fig. 1).

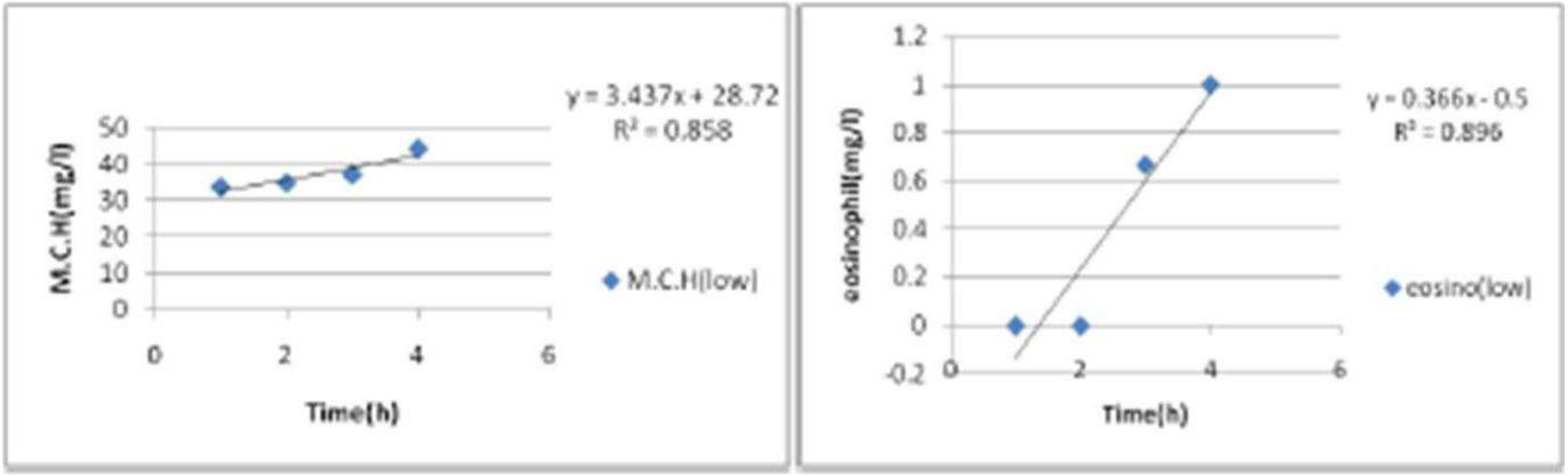

Fig .1: Regression Curve M.C.H, Eosinophil of Silver Carp during Exposure to Mercury Chloride in Low Concentrations

Results of Survey Blood Factors in High Concentrations (50%Lc50)

Change in all the blood factors was similar to low concentrations except MCV, cortisol (Fig. 3, 9).

MCV levels were significantly greater ($P< 0.05$) than controls groups and cortisol levels were significantly lowers ($P< 0.05$) toward the control groups.

MCH, glucose, eosinophil levels showed significant ($p<0/01$) positive correlations but RBC ($P<0/01$), Ht and lymphocyte ($p<0/05$) levels displayed significant negative correlations with mercury exposure.

Only eosinophils and lymphocyte introduced significant linear regression ($p<0/05$) $Y = a \pm bX$ with mercury (Fig. 2).

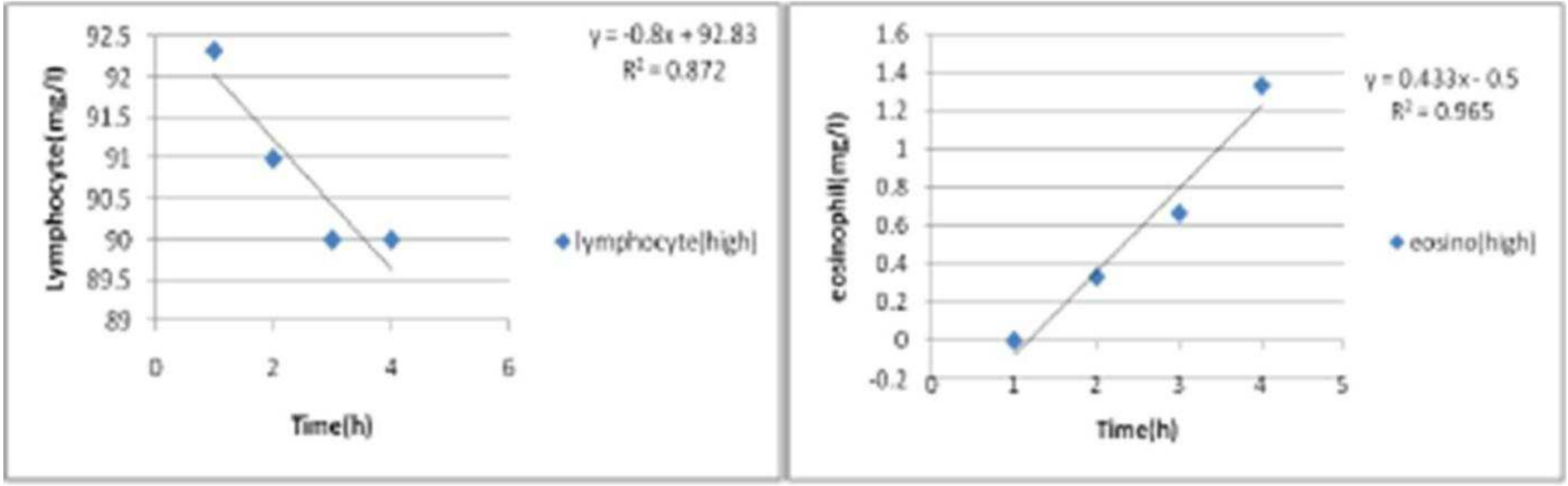

Fig .2: Regression Curve Lymphocyte, Eosinophil of Silver Carp during Exposure to Mercury Chloride in High Concentrations

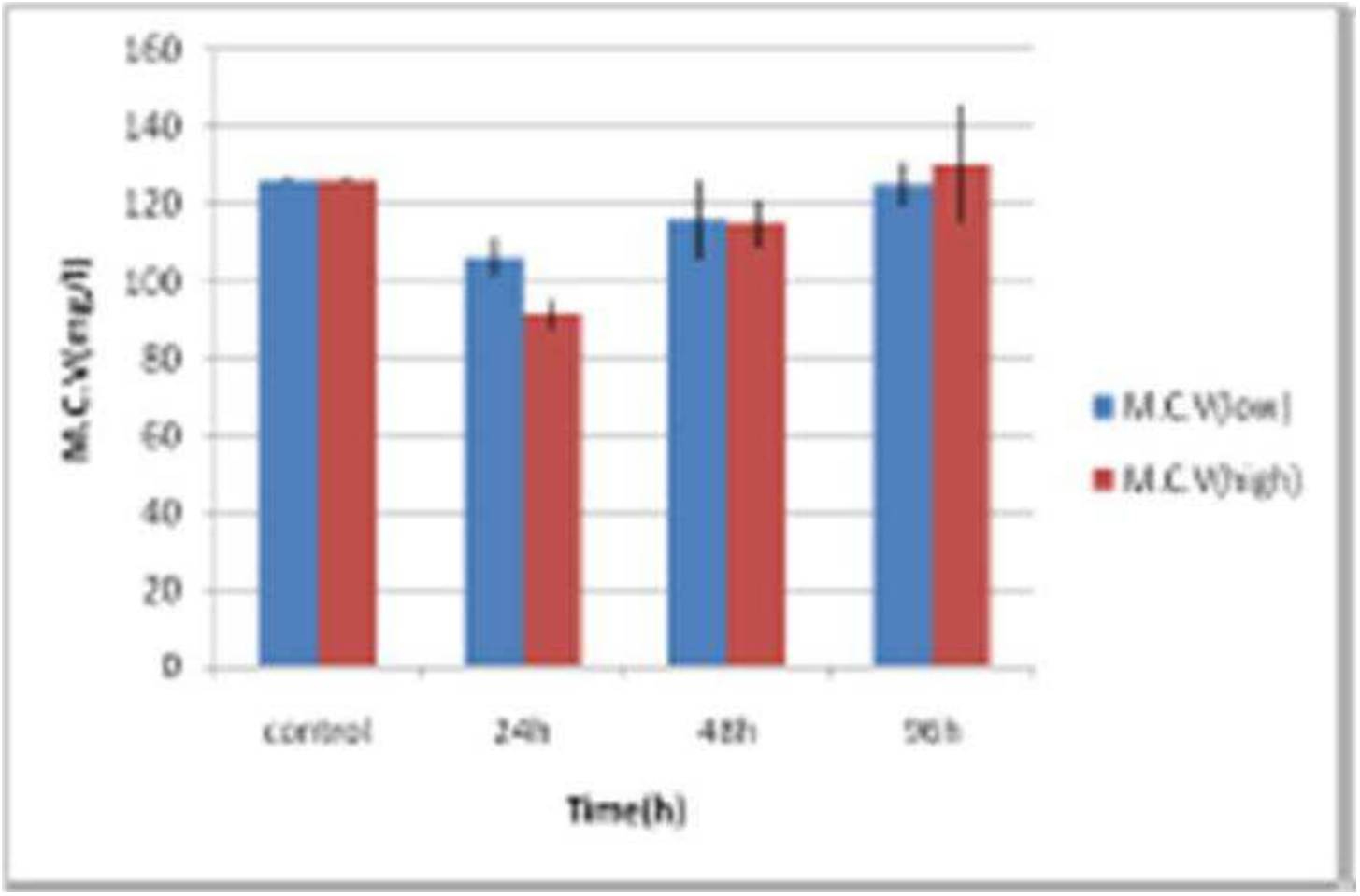

Fig .3: MCV Change of Silver Carp during Exposure to Mercury Chloride in Low and High Concentrations

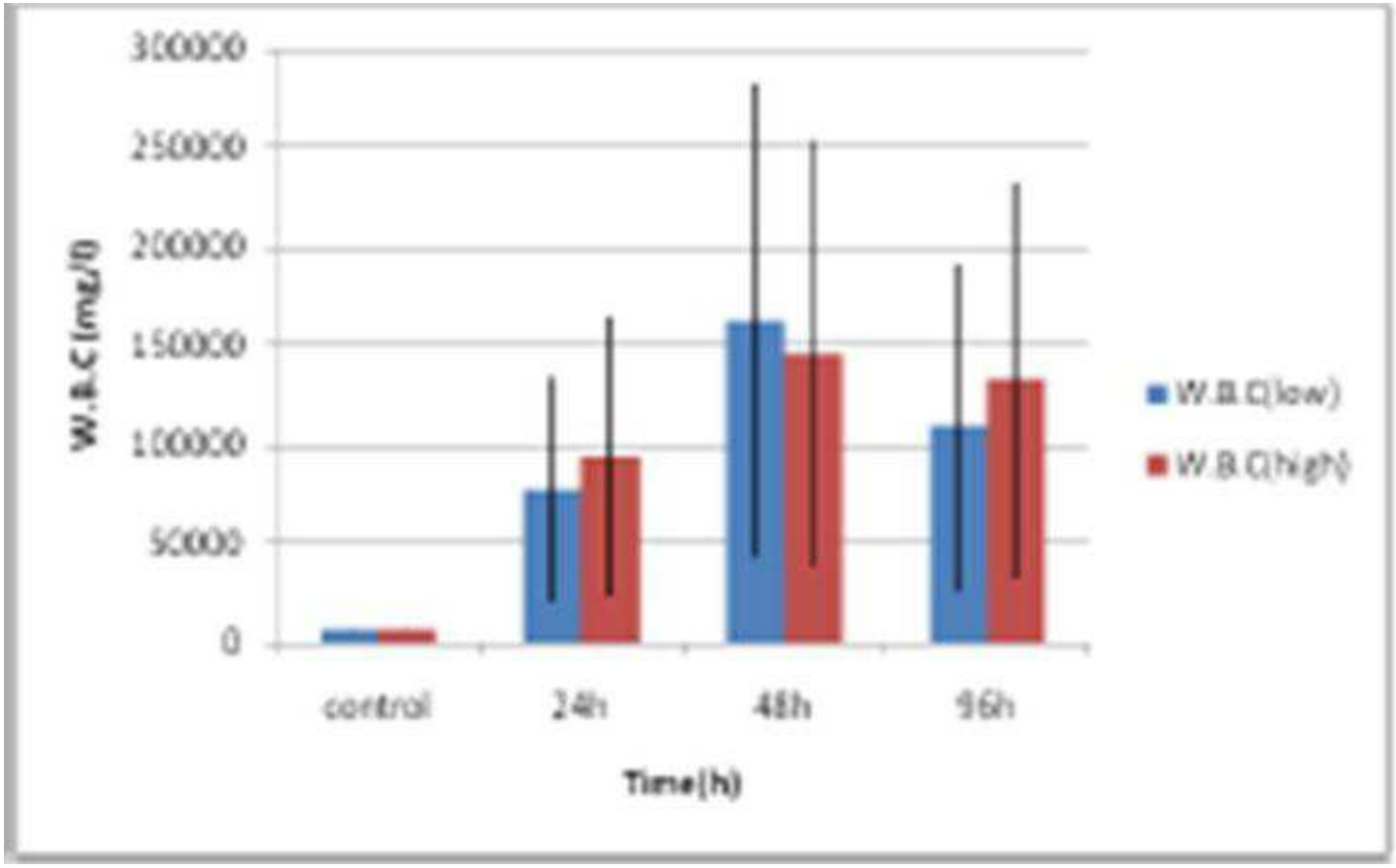

Fig .4: WBC Change of Silver Carp during Exposure to Mercury Chloride in Low and High Concentrations

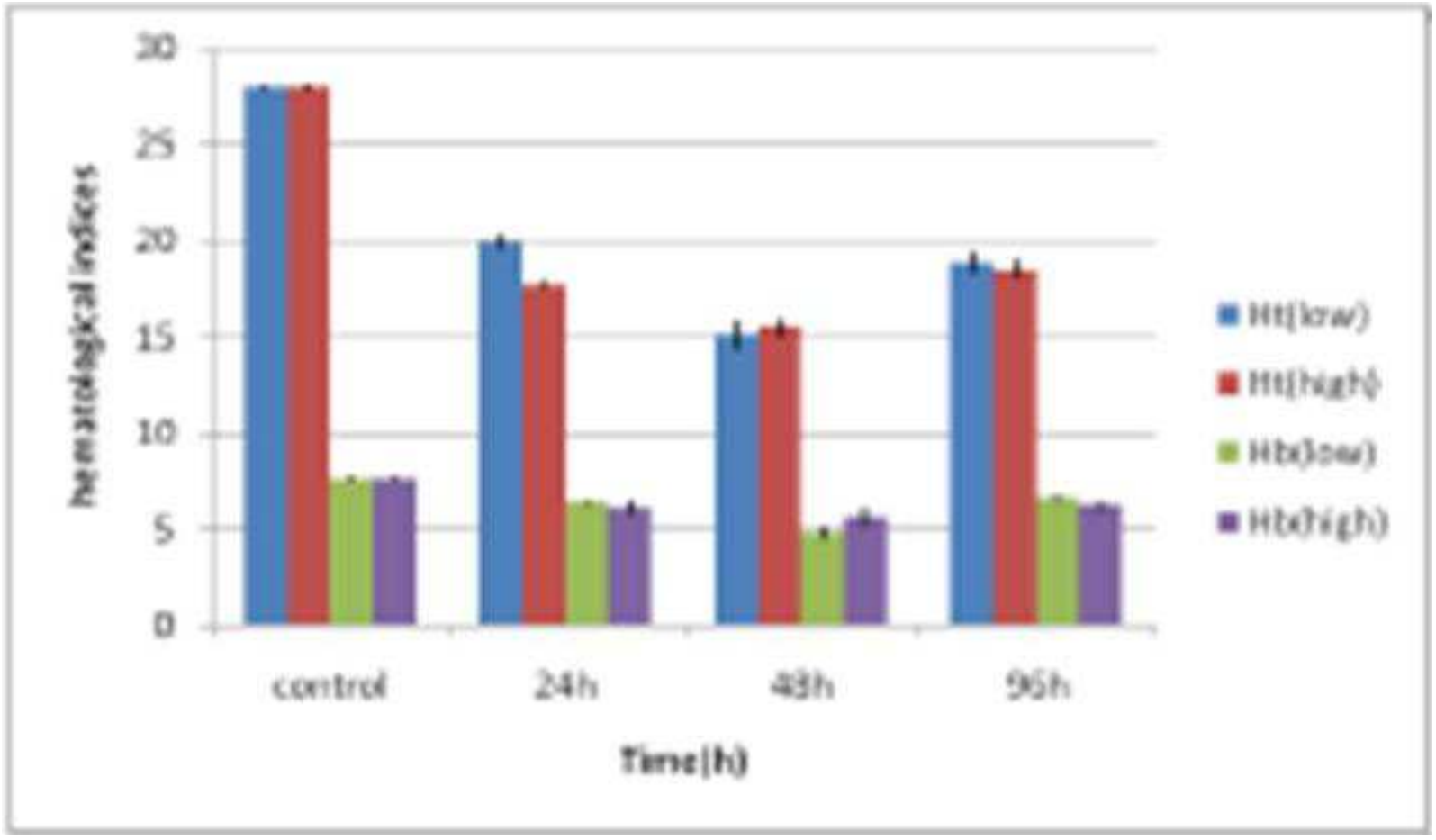

Fig .5: Ht, Hb Change of Silver Carp during Exposure to Mercury Chloride in Low and High Concentrations

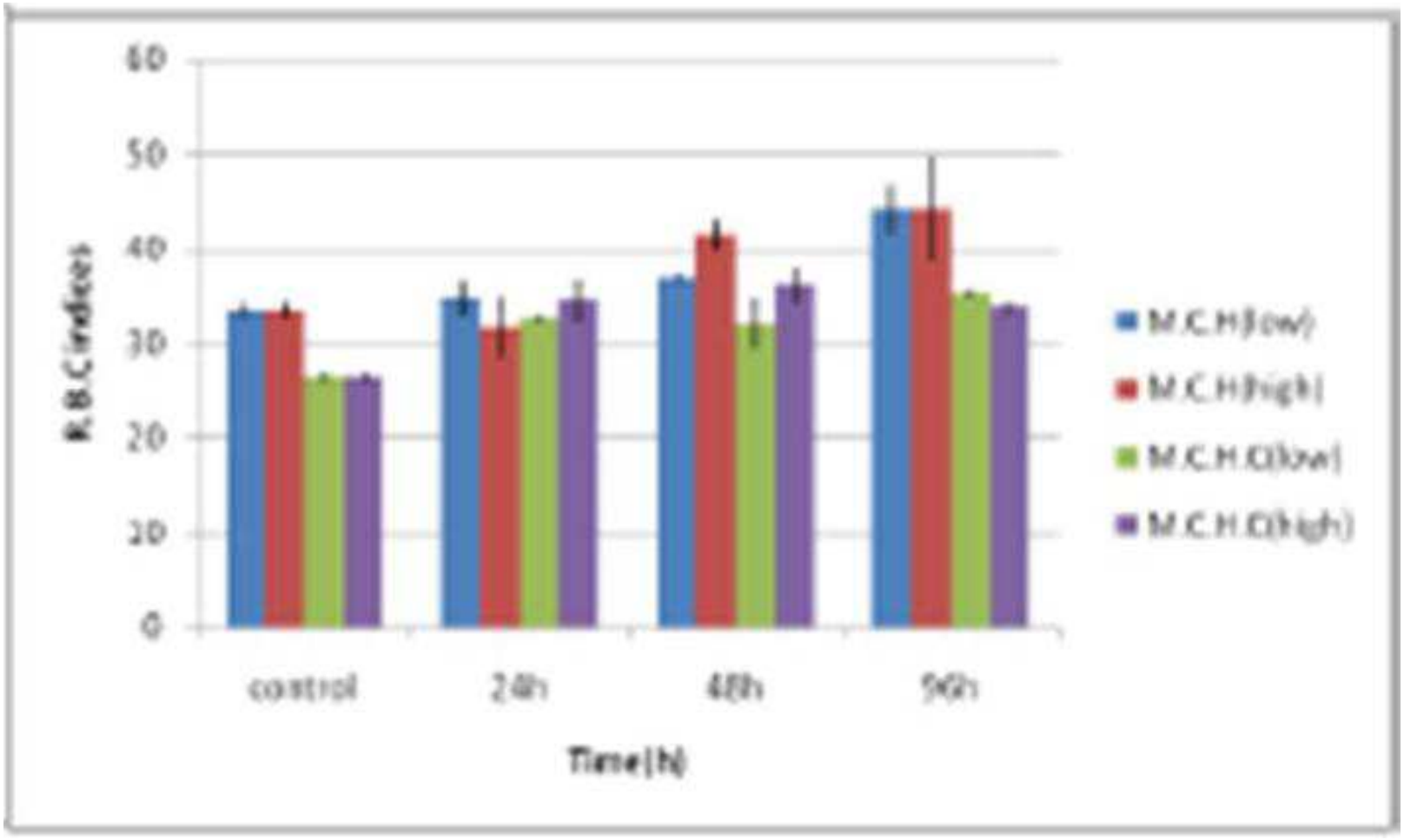

Fig .6: MCH, MCHC Change of Silver Carp during Exposure to Mercury Chloride in Low and High Concentrations

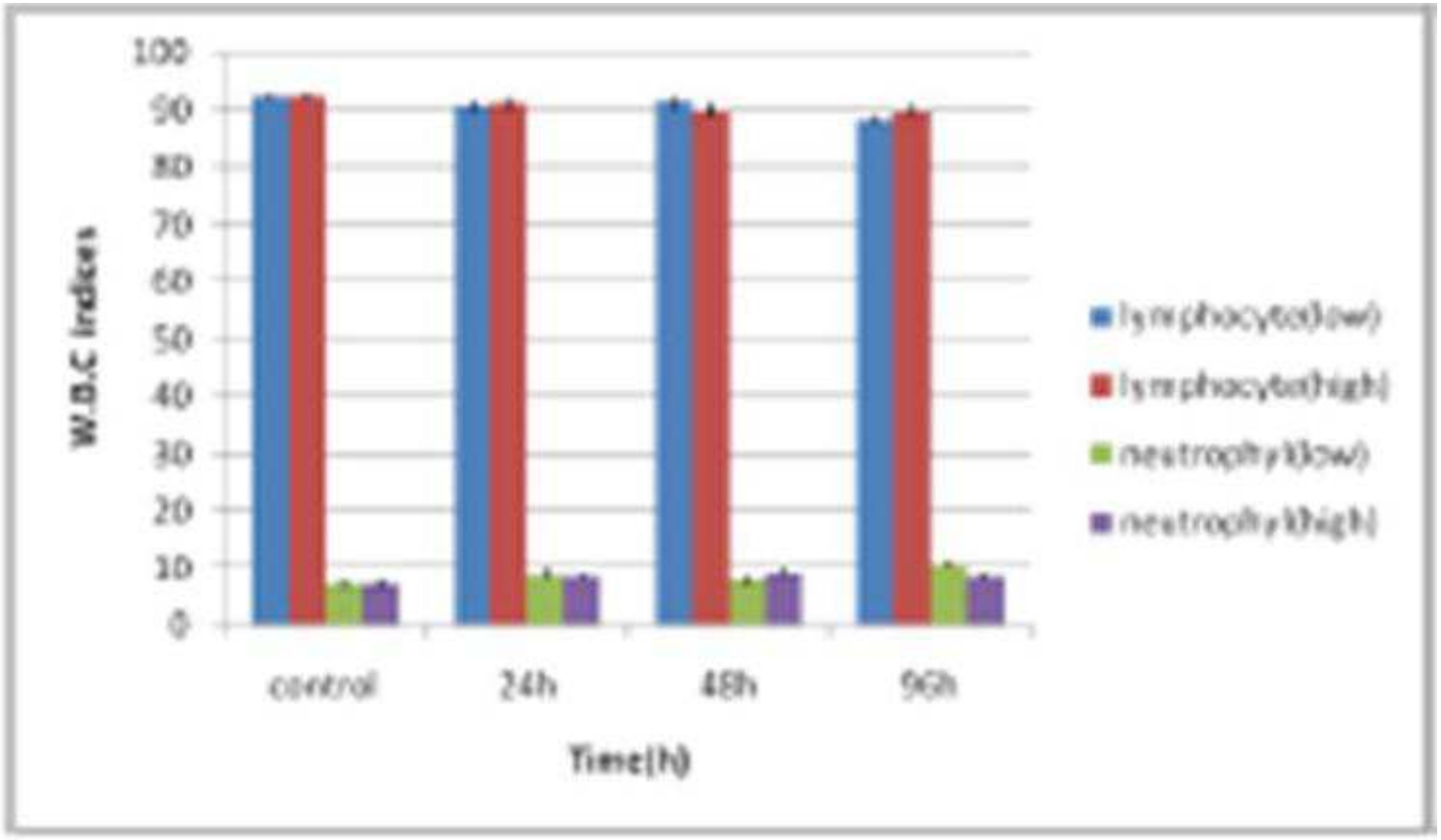

Fig .7: Lymphocyte, Neutrophil Changes of Silver Carp during Exposure to Mercury Chloride in Low and High Concentrations

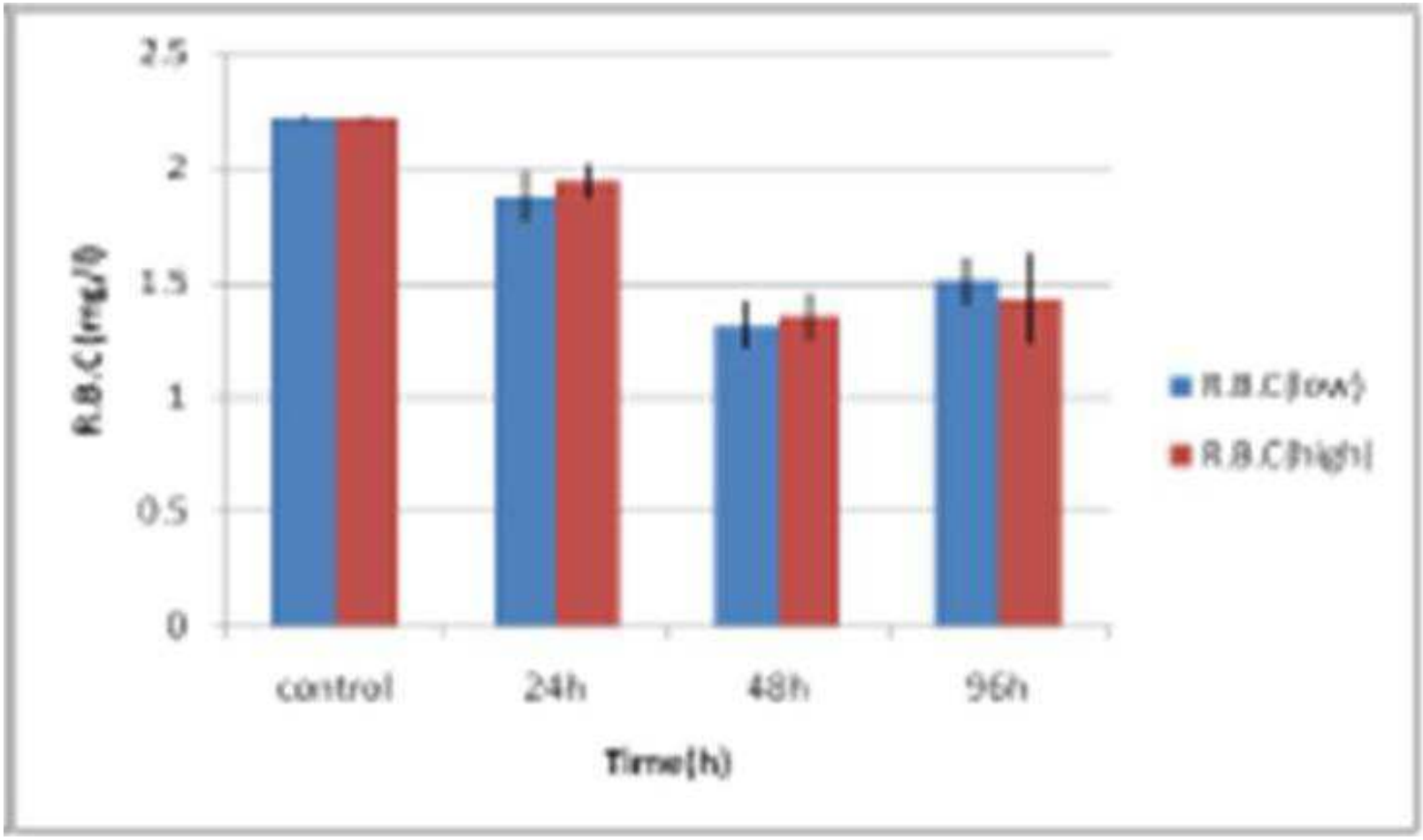

Fig .8: RBC Changes of Silver Carp during Exposure to Mercury Chloride in Low and High Concentrations

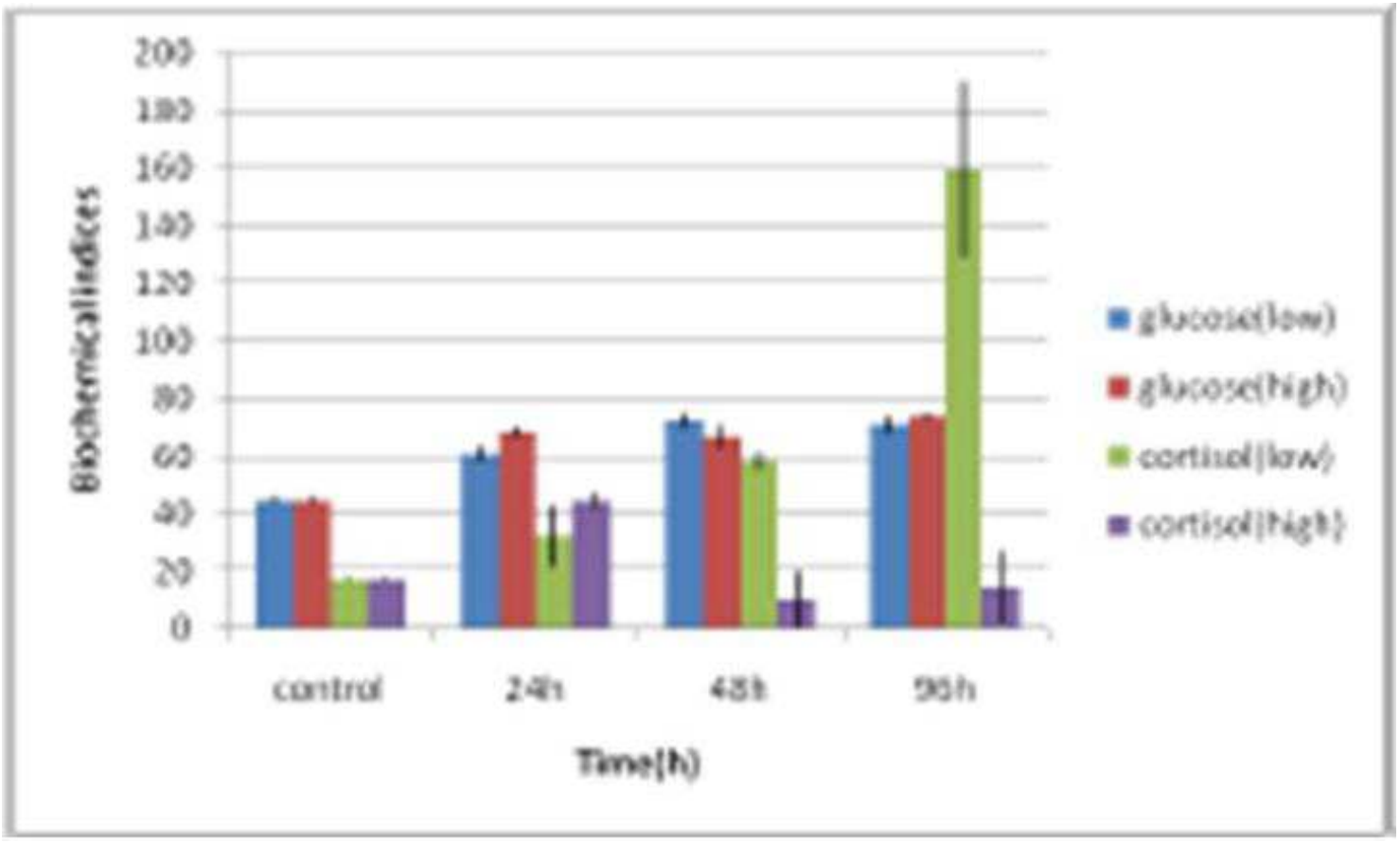

Fig .9: Glucose, Cortisol Changes of Silver Carp during Exposure to Mercury Chloride in Low and High Concentrations

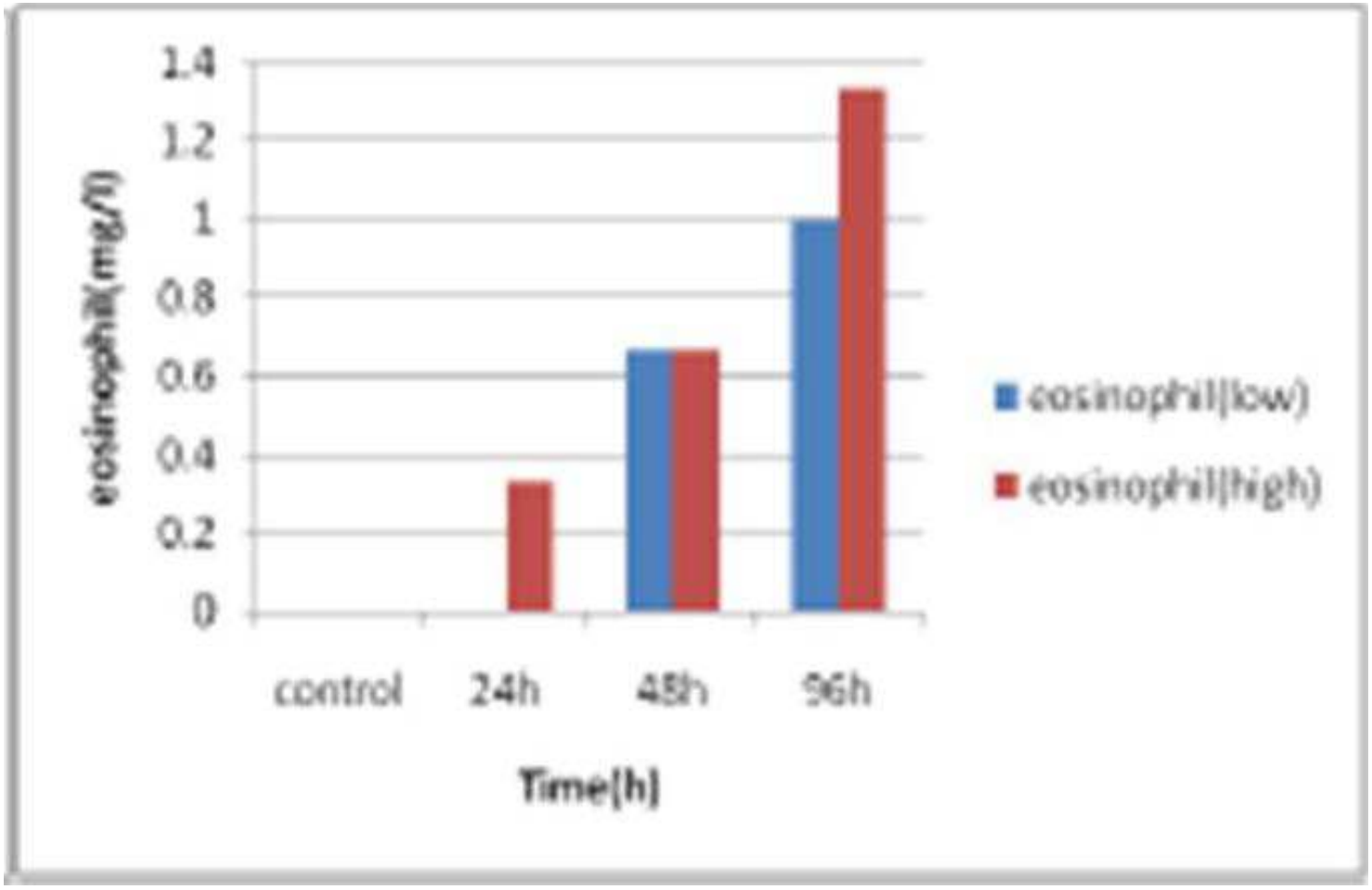

Fig .10: Eosinophil Changes of Silver Carp during Exposure to Mercury Chloride in Low and High Concentrations

Discussion

Metallic mixtures remaining in wastes may collect in various organs of animals or reason huge alteration in plant and fish biomass manufacture (Barman and Lal 1994). The toxicity data in this perusal showed that mercury chloride is toxic to silver carp (*H. molitrix*). In this report a series of hematological, biochemical and Immunological parameters were examined in silver carp after exposure to 10% and 50% LC50 doses of mercury chloride.

Hematotoxins alter qualitative and quantitative attributes of blood cells to produce toxic signs. when some of these other blood constituents are present or structural anomalies occurring in blood components meddle with normal functioning occur Hematotoxicity. Qualitative commutations in

blood cell components can consequence in contamination (Landis and M 2004).

Although adequate in quantity, the quality of these “small, less colored” red blood cells impedes them from conveying the normal extent of oxygen (Landis and M 2004). Five examinations are usually used to evaluate the quantitative and qualitative features of blood.

The white cell count, red cell count and platelet count are quantitative evaluations of blood constituents. Hemoglobin (Hb) and hematocrit (HCT) are indicators of the oxygen-conveying valence of blood.

The hematological parameters like Hb, Hct, RBC and WBC counts and other hematological indices like MCV, MCH and MCHC, could be delicate to sure types of contaminants because of its close relation with the exterior environment and often used to discover the physiological status of animals (van Vuren 1986; Adhikari et al., 2004). Most of the toxic compounds in the aquatic environment go into fish body inward gill and polluted food materials.

To appraise the sublethal toxicity of chemicals, the hematological and biochemical parameters of aquatic organisms may too utilize as possible biomarkers (Tellez-Banuelos et al., 2009; Saravanan et al., 2011).

The decreases in Hemoglobin concentration represents that the fish's power to supply adequate oxygen to the tissues is limited considerably and this will result in decline of physical stir (Nussey et al., 1995). The most common hematology discoveries in toxicology investigate is reductions in RBC count, hemoglobin concentration and Hematocrite that are like our detecting in present research. A decline in hemoglobin and Hematocrite levels can reveal an anemic situation (Kumar et al., 1999).

Panigrahi and Misra (1987) saw reductions in hemoglobin outturn and red blood cell count of the fish Anabas scandens treated with mercury. Reduction in Hct, Red blood cell (RBC) count and Hb was studied in fish Tinca, tinca exposed to lead and mercuric chloride (Shah and Altindag 2004).

The decline in RBC counts in the current study might have resulted from inhibition of RBC manufacture by the mercury chloride. Likewise, Li et al., (2011) announced a reduction of total content of RBC in the blood of rainbow trout (*Oncorhynchus mykiss*) when exposed to verapamil (VPR), a cardiovascular medicine. Mostly, the reduction in RBC counts in fish may imply the anemic condition of the fish under stress situations (Li et al., 2011). Furthermore, amplitude of toxicants in the gill area may injury the structure of gill resulting hemolysis and toxicant induced damaged osmoregulation may lead to a diminution in RBC counts (Kavitha et al., 2010; Saravanan et al., 2011). When erythrocytes have low hemoglobin extent reflects Microcytic hypochromic anemia.

The declined number of RBC in fish caused by toxicant exposure has been announced by Allin and Wilson (2000) and Chowdhury et al., (2004).

The increase in MCV in high concentrations can too result from an expansion of unripe RBC (Carvalho and Fernandes 2006). In the present study the important increase of MCV (in high concentrations) and MCH during low and high concentrations might be caused by the above said reason. The expansion of MCV studied in individuals of *H.malabaricus* exposed to MeHg may be illustrated by the existence of a larger amount of older or larger red blood cells as delineated by Hardig and Hoglund (1983). In the later phases the high percentage of unripe red blood cells in the circulation might be the cause for MCV decrease in low concentrations. The significant

expansion of MCHC value might be resulted from sphaerocytosis as mentioned by Sobecka (2001).

The expansion in MCV (in high concentrations) and MCH value show the anemia was of a macrocytic type as submitted by Talas and Gulhan (2009).

WBCs are involved in the adjustment of immunological work in many organisms and the saw increase in WBC count in mercury chloride treated fish shows a generalized immune reply and a defensive response to mercury chloride (Witeska 2004; Saravanan et al., 2011). Rousing effect of the toxicant on immune system and liberate of lymphocytes from lymphomyeloid tissue as a protection mechanism

may also redound to expansion in WBC count in fish (Ates et al., 2008).

Toxicants in the aquatic environment may cause influence at cellular or molecular level which results in significant changes in the biochemical parameters of the organisms (Kavitha et al., 2010). Between the several biochemical parameters, blood glucose level in toxicant treated animals has been greatly utilized as an indicator of environmental stress (Nemcsok and Boross 1982).

The blood glucose level has been utilized as an indicator of environmental stress and returned the changes in carbohydrate metabolism below hypoxia and stress conditions. Tseng (2004) announced that chronic exposure of

arsenic or its methylated metabolites caused Diabetes mellitus in rats and this condition may be accountable for hyperglycemia. Therefore an elevation of blood glucose level in the current study might be caused by gluconeogenesis to supply energy for the increased metabolic claims instituted by mercury chloride stress.

In the present study, a significant increase in cortisol level was observed in fish exposed to low concentrations.

Other stressors activate the hypothalamus-pituitary-interrenal (HPI) axis, resulting in a cortisol liberate that causes secondary stress answers.

A significant decrease in high concentrations in cortisol level may be as Cortisol suppression due to high stresses. However, the need for further research is felt in this area.

Our consequences show expansion in differential neutrophil and decrease in lymphocyte. It has been approved that the monocytes and neutrophils raised while lymphocytes decrease during different stressors in cultured fish Oreochromis aureus Silveira-Coffignya et al., (2004). It is thought that neutrophils and monocytes have phagocytic stir, which might elucidate their increased proportion during infectious conditions. Darwish et al., (2001) too discovered an increase in neutrophil amounts in channel catfish exposed to high doses of potassium permanganate. Regarding eosinophilia, Abdel-Ghafar and El-Khayat showed that the most common

cause of peripheral eosinophilia in patients of the third world was parasites.

The consequential discoveries of this study are that mercury chloride concentrations (low and high) may cause some substitutions in the hematological, biochemical and immunological parameters of the studied fish, so estimation of these indices, could supply a useful indicator of mercury chloride of water bodies. It appears that MCH, eosinophil in low concentrations and Lymphocyte in high concentrations is appropriate biomarkers of mercury chloride in silver carp *(H. molitrix)*.

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