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Evaluation of Certain Blood and Biochemical Changes of Nile Catfish (*Clarias gariepinus*) Exposed to Mercuric Chloride with Particular Reference to Erythrocyte Morphological Anomalies

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Author's contribution

This whole work was carried out by author WAMM.

Original Research Article

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ABSTRACT

Sub-lethal toxicity of mercuric chloride (0.0002, 0.002, 0.02mg/L) in Nile catfish (*Clarias gariepinus*) after long-term (14 days) of exposure in the present study was investigated by measurement of some selective hematological and biochemical parameters with respect to erythrocyte morphological alterations. Experimental fish were set up with three groups for each concentration, plus the control group. Blood samples were collected from five individuals for each concentration after 14 days of exposure. The hematological parameters analyzed were: total red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin Concentration (MCHC), total platelets count, total white blood cell count (WBC) and differential leukocyte counts (neutrophil, eosinophil, lymphocyte, monocyte and basophils). Fish exposed to 0.02mg/L Hg showed a significant decrease in RBC count, Hb, MCHC and platelets number than control groups ($P<0.05$) with moderate elevation in PCV, MCV, WBC, neutrophil, eosinophil, lymphocyte , glucose and cortisol levels compared with the control groups ($P<0.05$). However, fish exposed to the other two low concentrations showed no toxic effects. Hg at the concentrations studied was toxic to Nile catfish (*Clarias gariepinus*) at the higher dose only (0.02mg/L) after long term exposure.

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1. INTRODUCTION

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 [1].

Mercury exists in nature primarily as elemental mercury or as a sulfide and is found in the earth's crust at approximately 0.5 parts per million. Atmospheric exposures occur from outgassing from rock or through volcanic activity. Human sources of atmospheric mercury include coal burning [2] and mining (mercury and gold in particular). Atmospheric elemental mercury settles in the water, where it is converted by microorganisms into organic (methyl or ethyl) mercury, which is ingested by smaller creatures which are eventually consumed by larger fish. Fish at the top of the food chain (e.g., tuna, swordfish, or shark) may concentrate considerable mercury in their tissues [3].

Environmental pollutants induced morphological and quantitative variations in blood parameters [4,5], because this reason, assessment of cellular and plasma component of blood is very important in detection of physiopathological changes in different stress conditions such as exposure to heavy metals [6]. Changes in hematological profile of the fish exposed to mercury have been observed in *Hoplias malabaricus* [7], *Tilapia mossambica* [8], *Oreochromis aureus* [9], *Acipenser baeri* [10], *Ctenopharyngodon idella* [11], *Pleuronectes platessa* [12], *Channa punctatus* [13], *Aphanius dispar* [14]. In the present study we study the hematological and biochemical abnormalities have been found in Nile catfish (*Clarias gariepinus*) following long-term exposure to sublethal concentrations of mercuric chloride.

2. MATERIALS AND METHODS

2.1 Preparation of Mercuric Chloride

Mercuric chloride (HgCl2 [99.5%] purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of 10mg/L mercury was made by dissolving the salt in double-distilled water. This solution was diluted directly into aquaria in sufficient amounts to provide the following mercury concentrations in water: 0.02, 0.002 and 0.0002mg/L as LC50/10, LC50/100 and LC50/1000 [15].

2.2 Animals and Experimental Design

Eighty specimens of Nile catfish (*Clarias gariepinus*) with average body weight (200-220 g) provided by the fisheries research laboratory. Fish were adapted to laboratory conditions for 7 days in a 400L tank with dechlorinated tap water. During adaptation, all fish were fed with trading pellet twice a day and were divided into 4 groups, each containing 20 fish. PH, dissolved oxygen, temperature and conductivity were monitored during the experiment.

Group I: this group was kept as a control in which fish were conserved in a fiberglass tank without accessing toxicant.

Group II: fish in this group were exposed to mercuric chloride at a concentration of 0.0002mg/L for 14 days according to [16].
Group III: fish in this group were exposed to mercuric chloride at a concentration of 0.002mg/L for 14 days.
Group IV: fish in this group were exposed to mercuric chloride at a concentration of 0.02mg/L for 14 days.

2.3 Sampling

Fish were quickly anesthetized with 200ppm clove oil. Blood samples rapidly were taken from caudal vein by heparinized syringes for eryhtrogram and leukogram. Another blood sample was collected without anticoagulant for serum separation and serum glucose and cortisol analysis.

2.4 Hematological Examination

2.4.1 Eryhtrogram

Total red blood cell count (RBC) was carried out by the method of [17] using 1:30 dilution of diluting heparinized blood with Giemsa stain. Cells were counted using a Neubauer chamber below the light microscope. Packed cell volume (PCV) was measured, putting blood in glass capillary tubes and centrifuged in a microhematocrit centrifuge at 10000 RPM for 5 min (Hettich, Germany), then give the reading using hematocrite special reader. Hemoglobin concentration was measured by the cyanomethemoglobin method according to [18]. Erythrocytes indices; mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were computed from RBC, PCV and Hb [18].

2.4.2 Eryhtrocyte anomalies determination

In all experimental groups; blood smears were made, dried, fixed then stained with Giemsa solution and examined under microscope [19].

2.4.3 Leukogram

Total leukocyte count was carried by the method of [17] using haemocytometer Neubauer. Differential leukocyte count was carried out by the method of [20] by made peripheral blood smears stained with Giemsa. In addition, the calculation of the absolute number of neutrophils lymphocytes, eosinophil and monocyte was occurred.

2.5 Biochemical Analysis

Serum glucose was assessed according to [21]. A microtitre plate ELISA for measuring cortisol in fish according to [22].

2.6 Statistical Analysis

Results obtained were analyzed using student F-test to compare the means. Analysis was performed using computer database software from the statistical package for social sciences (version 16.0 SPSS). A P-value of <0.05 was considered statistically significant in all clinical comparisons at 95% confidence interval.

3. RESULTS AND DISCUSSION

Heavy metals are persistent contaminants in the environment that come to the forefront of dangerous substances such as cadmium, lead, mercury, copper and zinc which causing a serious health hazard in humans and animals [23]. The toxicity data in this work showed that mercuric chloride is toxic to Nile catfish. In this report, a series of hematological and biochemical parameters were examined for studying the toxicity induced by long-term exposure to sublethal concentrations of mercuric chloride in Nile catfish (*Clarias gariepinus*). Mercuric chloride at a dose of 0.02mg/L in group (4) showed red skin erosions (Fig.1).

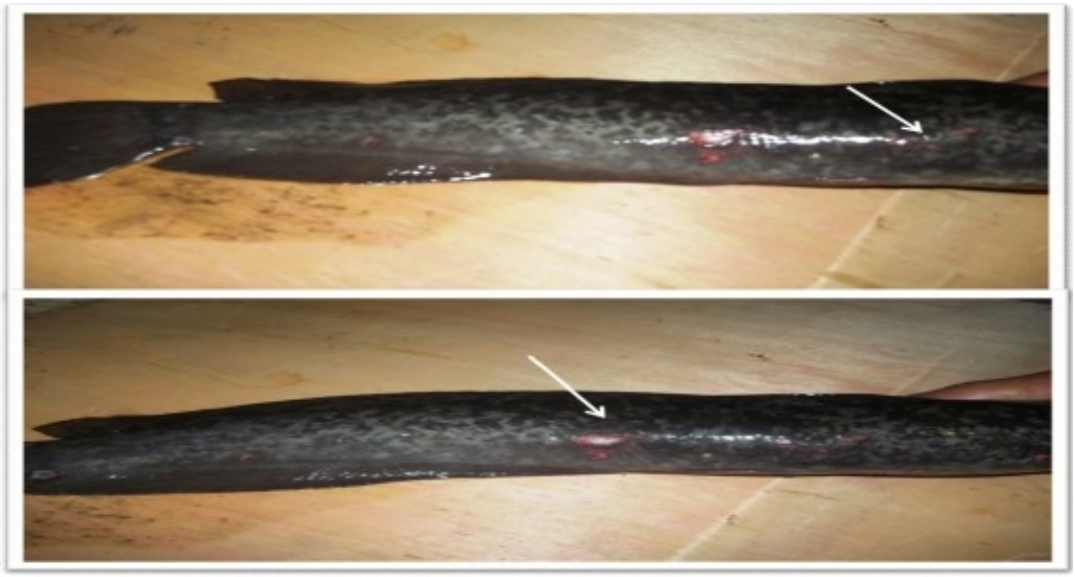

Fig. 1. Clinical signs of 0.02mg/L of mercuric chloride, showing red skin erosions

Regarding to the erythrogram as in (Fig. 2) like RBC, Hb, PCV and other hematological indices like MCV, MCH and MCHC, we found a significant decrease in the total red blood cells count and Hb concentration following 2 weeks of exposure to 0.02mg/L of Hg comparing with the control ($p<0.05$) which reflect an anemic situation that may be due to inhibition of RBC manufacture by the mercuric chloride or may be due to the decreased rate of production of red blood cells or an increased loss of these cells [24]. Have attributed anemia to impaired erythopoiesis due to the direct effect of metal on hematopoietic centers (kidney/spleen), accelerated erythroclasia due to altered membrane permeability and/or increased mechanical fragility and defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions. Our results are agreed with the result of [16,25-28].

Morphological classification of anemia was diagnosed by calculation of blood indices representative in MCV, MCH and MCHC as in (Fig. 3). In the present work we found a significant increase in the values of PCV and MCV following the exposure to mercuric chloride at a concentration of 0.02mg/L which may result from an expansion of unripe RBC [29]. However, a significant decrease of MCHC was noticed with indication to macrocytic hypochromic anemia which induced by hemolysis and damaged osmoregulation which are resulted from gill injury caused by toxicants which also lead to a diminution in RBC counts [30,31]. On the other hand, no significant changes were noticed in the above mentioned parameters after 2 weeks from exposure to 0.002 and 0.0002mg/L concentations of mercuric

chloride reflecting no toxicity induced by these both doses in Nile catfish (*Clarias gariepinus*), these results are parallel to the result proved by [16].

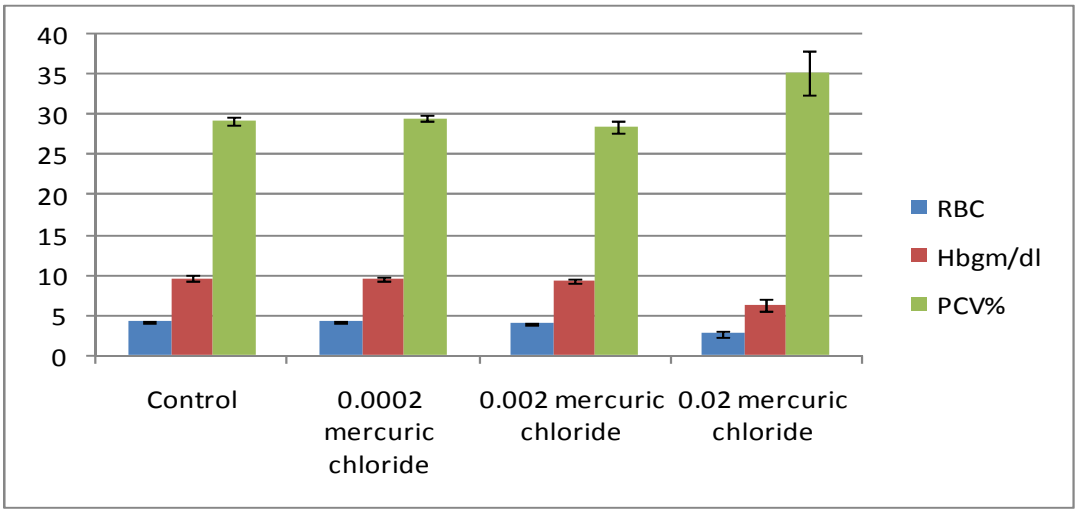

Fig. 2. Changes in RBCs count (x10 cells/mm3), Hb concentration (gm/dl) and PCV (%) in all experimental groups

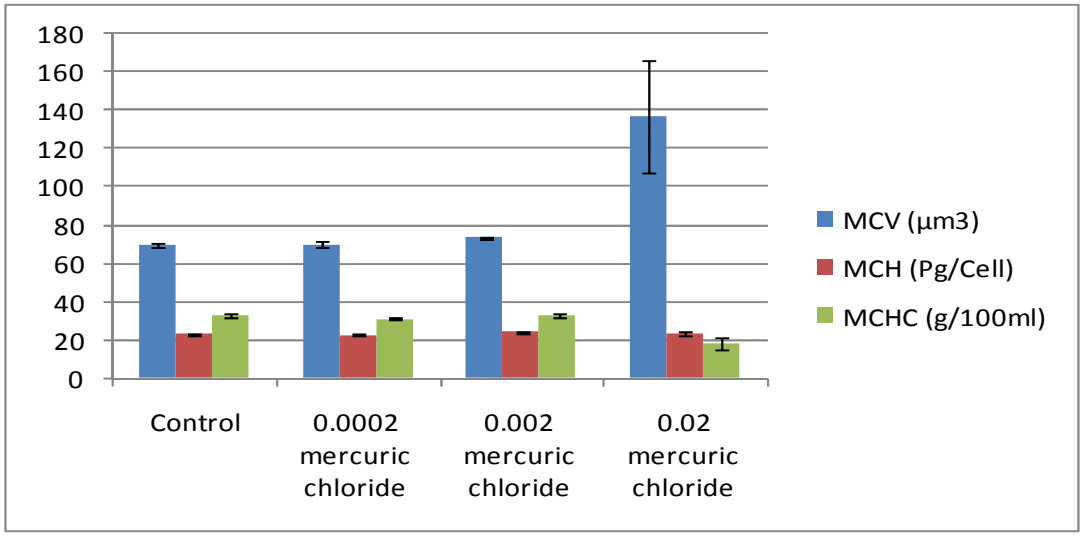

Fig. 3. Changes in MCV, MCH and MCHC values in all experimental groups

As shown in (Fig. 4) regarding to the result of total platelets count, we found a moderate decrease in their number in group 4, this result agreed with [32,33]. On the contrary, groups 2 and 3 showed no significant deviation in platelets number than the normal level and this agreed with the result of [16].

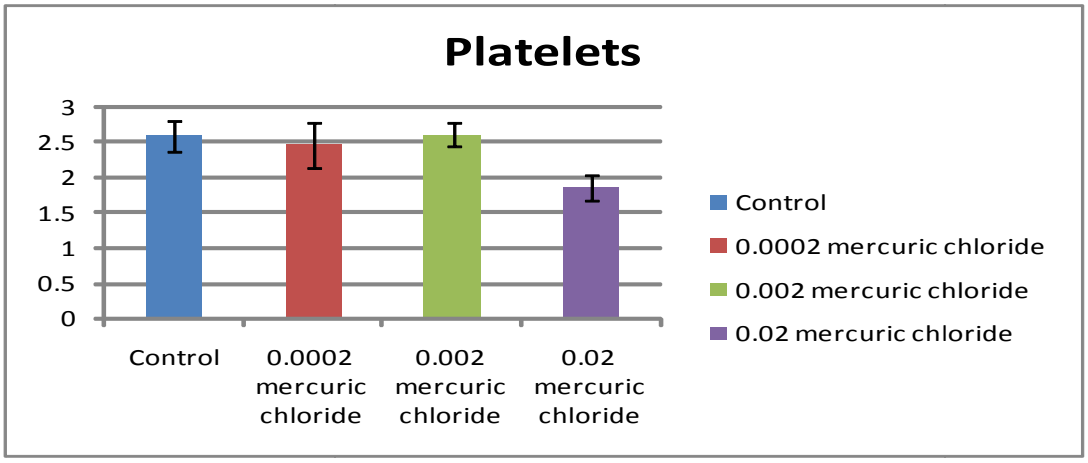

Fig. 4. Changes in platelets number (x10 cells/mm3) in all experimental groups

Erythrocyte morphological alterations and anomalies in (Fig. 5) showing fragmented, crenated, enlarged RBC with giant nucleus, star and semi-lunar shaped RBC with vacuolated cytoplasm, also elliptical RBC with hypochromasia in addition to micronucleus were seen in group 2 treated with mercuric chloride at a concentration of 0.02mg/L. Our findings were parallel to the result of Gill and Pant who found erythrocyte anomalies in fish subjected to intoxication with heavy metal, these anomalies represented by erythrocyte swelling, poikilocytosis, vacuolation and deterioration of the cell membrane.

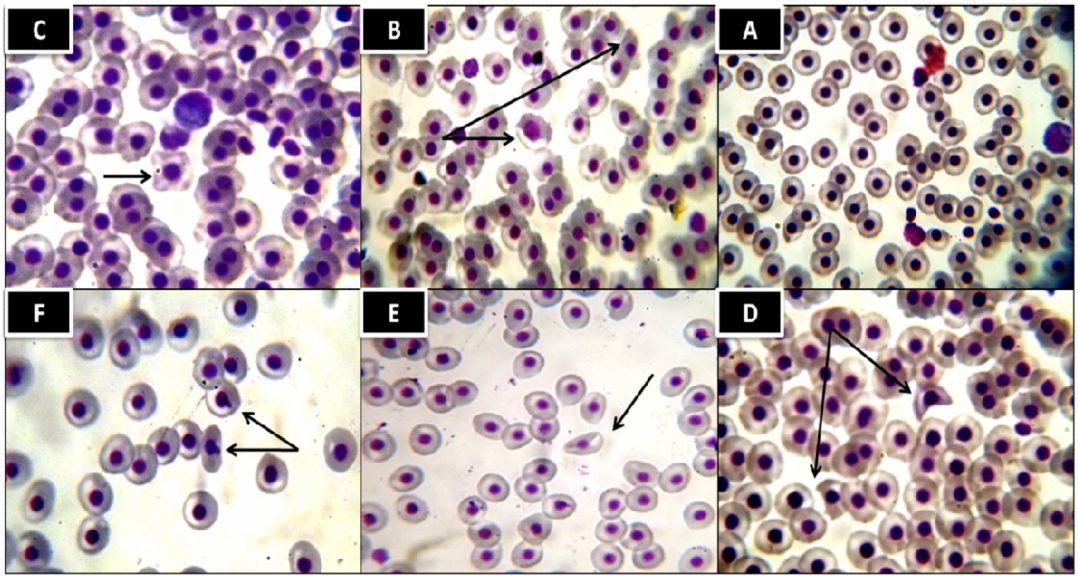

Fig. 5. Blood smear for morphological anomalies. [A] showing normal RBC morphology of the control group, while [B] showing fragmented, crenated and enlarged RBC with giant nucleus, [C] showing star shape RBC,[D] showing semi-lunar shape RBC with vacuolated cytoplasm, [E] showing elliptical RBC with hypochromasia and [F] showing presence of micronucleus indicating toxicity in fish group treated with 0.02mg/L mercuric chloride

Regarding to the leukogram results as in (Figs. 6, 7 and 8) we found an increase in the total number of leukocytes, lymphocyte, monocyte, neutrophil and eosinophil which may be attributed to the stimulation of immune system in response to tissue damage caused by Mercuric chloride [27,31,34]. In addition to the elevation in the number of lymphocytes may be due to the toxicant effects on lymphomyeloid tissue as a protection mechanism may also redound to expansion in WBC count in fish [35] or may be due to the stimulation of the immune system caused an increase in lymphocytes by an injury or tissue damage caused by heavy metals in fish [36]. Our results are agreed with [32] who found an increase in lymphocyte, monocyte, neutrophil and eosinophil cells in *Heteropneustes fossilis*, *Channa punctatus* and *Mastacmebalus puncalus* on long-term exposure to least effective concentrations of mercuric chloride. Also our results parallel to the result of [26,33,37] who observed the effect of mercuric chloride on the differential leucocyte counts and found lymphocytosis, neutrophilia, monocytosis, eosinophilia and in *Anabas testudineus*, *Tinca tinca* and *Hoplias malabricus* respectively.

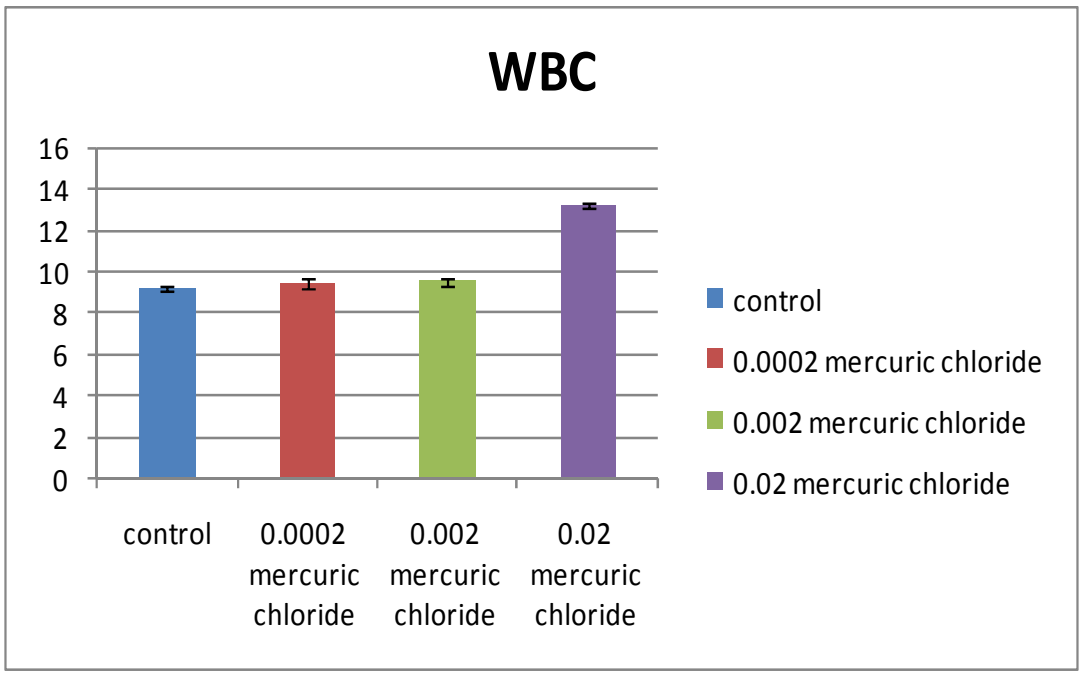

Fig. 6. Differences in WBCs count (x10 cells/mm3) in all experimental groups

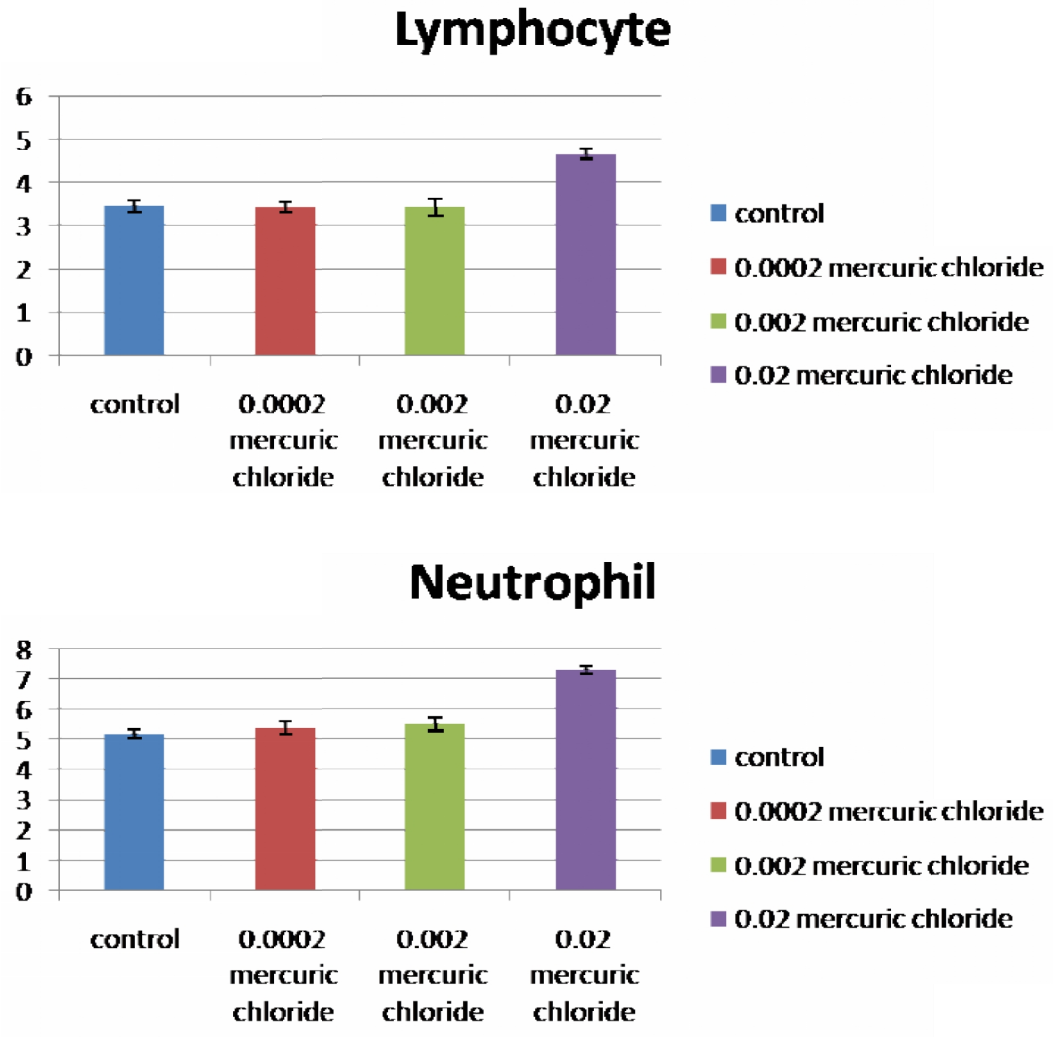

Fig. 7. Variables in lymphocyte and neutrophil numbers (x10 cells/mm3) in all experimental groups

Some biochemical parameters may be useful tools to evaluate the health and/or stress condition of the fish [38-40]. Stress has been reported to elevate plasma cortisol [41-43] and glucose levels [44,45], many researchers considered that fishes undergoing stressful situations exhibit increases of plasma cortisol and glucose levels [46-48]. Regarding to the biochemical result as seen in Fig. 9, we found a significant increase in blood glucose and cortisol levels in catfish exposed to 0.02mg/L Hg for 14 days. These parameters showed no difference in catfish exposed to 0.002 and 0.0002mg/L of mercuric chloride when compared with the control one, these results are parallel to the result reported by [16]. Hyperglycemia in the current study might be caused by gluconeogenesis to supply energy for the increased metabolic claims instituted by mercury chloride stress. A significant increase in cortisol level may be due to some stress which activate the hypothalamus pituitary- internal (HPI) axis, resulting in a cortisol liberates that causes secondary stress response [16].

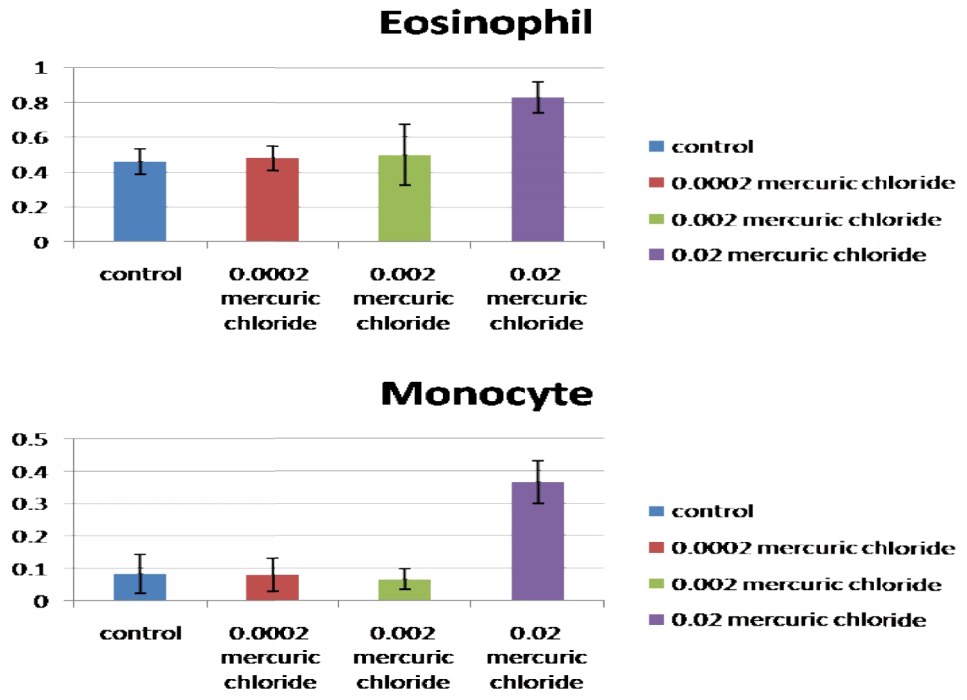

Fig. 8. Changes in eosinophil and monocyte (x10 cells/mm3) in all experimental groups

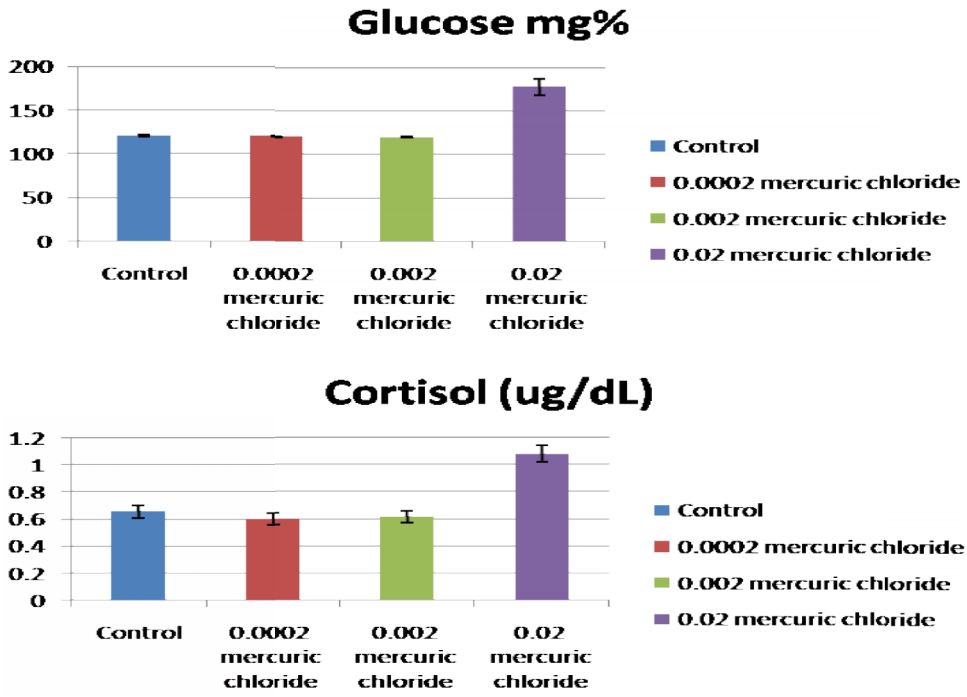

Fig. 9. Changes in serum glucose and cortisol levels in all experimental groups

4. CONCLUSION

The LC50/100 and LC50/1000 of mercuric chloride in the present study were insufficient to cause hematological and biochemical changes in Nile catfish. Whereas the LC50/10 induced moderate changes in cellular and plasma constituents of blood. Therefore, further studies of Hg toxicity would be needed to be carried out at higher concentrations or using long exposure times.

CONSENT

Author declares that 'written informed consent was obtained from the fisheries research laboratory for publication of this case report and accompanying images.

ETHICAL APPROVAL

Author hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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