

Selective Haematological and Biochemical Parameters in Egyptian catfish (*Clarias lazera*) Exposed to Mercuric Chloride: With Respect to Erythrocyte Morphological Anomalies.

ABSTRACT

Aims: The study was undertaken to establish a sub-lethal concentrations (0.0002, 0.002, 0.02mg/L Hg) toxicity of mercuric chloride in Egyptian catfish (*Clarias lazera*) after long-term (14 days) exposure which achieved by investigation of some selective Haematological and biochemical parameters with respect to erythrocyte morphological alterations. **Methodology:** 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 Haematological parameters analyzed were: total red blood cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total platelets count, total white blood cell count (WBC) and differential leukocyte counts(neutrophil, eosinophil,lymphocyte , monocyte and basophils). **Results:**Fish exposed to 0.02 mg/L Hg for long time showed a significant decrease in RBC count , Hb , MCHC and platelets number than control groups ($P < 0.05$) in addition to moderate elevations in PCV, MCV, WBC, neutrophil, eosinophil,lymphocyte count , glucose and cortisol level compared to the control groups ($P < 0.05$), while fish exposed to other two low concentrations showed no toxic changes. **Conclusion:** Hg at the concentrations studied was toxic to Egyptian catfish (*Clarias lazera*) at the higher dose only (0.02 mg/L) after long term exposure.

Keywords: *Egyptian catfish (Clarias lazera) , Mercuric Chloride, Sublethal toxicity, Erythrocyte anomalies, Cortisol, Glucose*

1. INTRODUCTION

Mercuric chloride (HgCl_2) is one of the environmental pollutants that is 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 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 the the detection of physiopathological changes in different stress conditions such as exposure to heavy metals[6]. Changes in the Haematological 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 Haematological and biochemical abnormalities which can be induced in Egyptian catfish (*Clarias lazera*) when exposed to mercuric chloride.

2. MATERIALS AND METHOD

2.1. Preparation of mercuric chloride

Mercuric chloride (HgCl_2 [99.5%] purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of 10 mg/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 0.0002 mg/L as LC50/10, LC50/100 and LC50/1000 [15]

2.2. Animals and Experimental design

A total of 80 Egyptian catfish (*Clarias lazera*) with average body weight (200- 220 gm) 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 and were divided into 4 groups, presented in tank of 400L, each including 20 fish. For all experimental time were carried out three repeats. 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 (0.0002 mg/l) for 14 days according to [16]

Group III: fish in this group were exposed to mercuric chloride at a concentration of (0.002 mg/l) for 14 days.

Group IV: fish in this group were exposed to mercuric chloride at a concentration of (0.02 mg/l) for 14 days.

2.3. Sampling

Fish were without delay anesthetized with 200ppm clove power. Blood samples rapidly were taken from tail blood vessel by heparinized syringes. Blood indices were carried out on fresh blood. Another blood sample was collected without anticoagulant for serum separation and biochemical analysis.

2.4. Haematological Examination

2.4.1. Erythrogram: RBC count was carried out by the method of [17] using 1:30 dilution of diluting heparinized blood with Giemsa stain. Cells were counted using a haemocytometer Neubauer below the light microscope. Packed cell volume (PCV) were measured by putting new blood in glass capillary tubes and centrifuged in a microhematocrit centrifuge at 10000 rpm for 5 min (Hettich, Germany), then give the reading using With Hematocrite readerships. According to [18] by measuring the formation of cyanmethaemoglobin were found haemoglobin levels. Erythrocytes Indices (MCH or Mean Corpuscular Haemoglobin, MCHC or Mean Cell Haemoglobin Concentration and MCV or Mean Corpuscular Volume) were computed from RBC, PCV and Hb [18]

2.4.2.Erythrocyte anomalies determination:In all experimental groups blood smears were made ,stained with May Grunwald and Giemsa solutions and fixed with Canada balsam and cover glass and examined under microscope [19]

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

2.5.Biochemical Analysis:

Test kits of Bio Merieux (France).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.Result 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 causing serious health hazard in humans and animals [23].The toxicity data in this perusal showed that mercury chloride is toxic to Egyptian cat fish . In this report a series of Haematological, biochemical and Immunological parameters were examined in Sub-lethal concentrations (0.02, 0.002, 0.0002mg/L Hg) of mercuric chloride toxicity in Egyptian catfish (*Clarias lazera*) after long-term (14 days).Mercuric chloride at a dose of 0.02 mg/L in fish showed red skin erosions Fig.(1).



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

Regarding to the erythrogram as in Fig.(2) like RBC, Hb, PCV and other Haematological indices like MCV, MCH and MCHC, we found that the RBCs and Hb in catfish exposed to 0.02mg/L Hg for 14 days were reduced than that of the control ($p<0.05$) which reveal to an anemic situation may be due to inhibition of RBC manufacture by the mercury 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 anaemia to impaired erythropoiesis due

153 to the direct effect of metal on hematopoietic centers (kidney/spleen), accelerated
 154 erythroclasia due to altered membrane permeability and/or increased mechanical fragility
 155 and defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions.
 156 Our results are agreed with the result of [16,25,26 ,27,28]

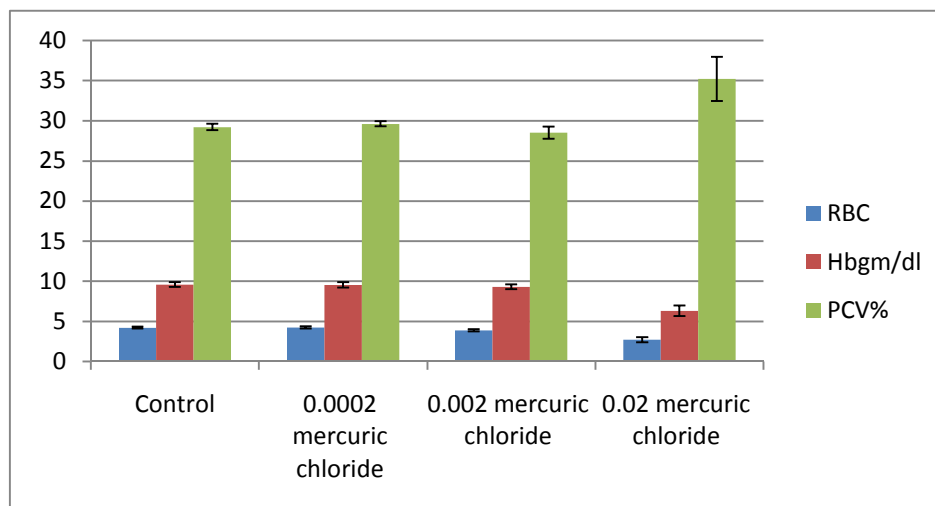


Fig.2. Changes in RBC (x10⁶cells/mm³) ,Hb (gm/dl) and PCV (%) in all experimental groups.

161 Morphological classification of anaemia was determined by calculation of blood
 162 indices as MCV, MCH, MCHC in Fig.(3) ,in the present work we found that 0.02 mg/l
 163 concentration of mercuric chloride induced an elevation in the value of PCV and MCV
 164 which may result from an expansion of unripe RBC [29] with reduction in MCHC
 165 indicating macrocytic hypochromic anaemia ,this may be due to the hemolysis and
 166 damaged osmoregulation which are resulted from injury of the structure of gill induced
 167 by toxicants which also lead to a diminution in RBC counts [30,31] . On the other hand,
 168 these parameters were still as control in catfish exposed to (0.002, 0.0002mg/L Hg) of
 169 mercuric chloride indicating no toxic effect of mercuric chloride at these both doses in
 170 Egyptian catfish (*Clarias lazera*), these results are parallel to the result proved by [16]

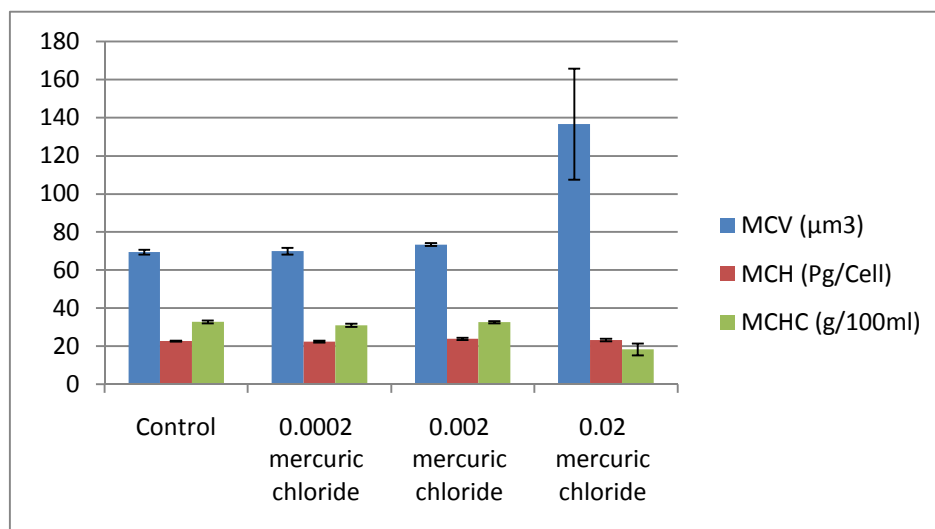
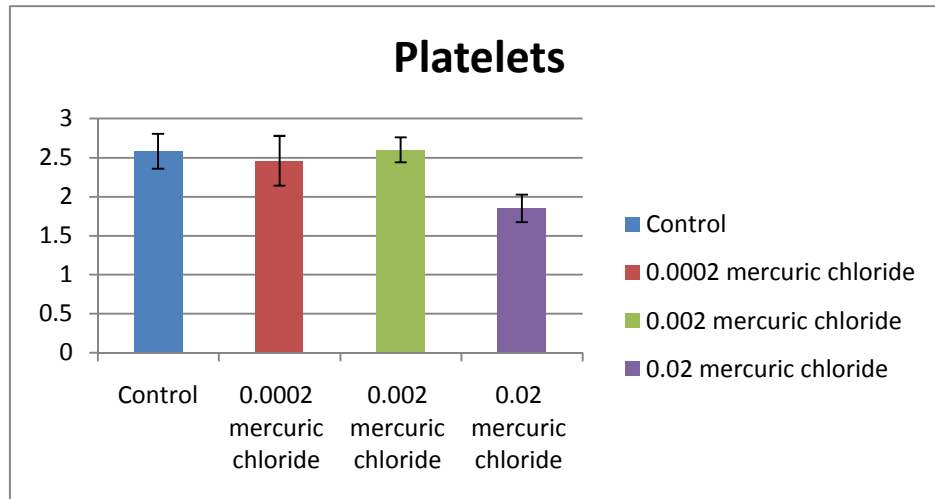


Fig.3. Changes in MCV , MCH and MCHC in all experimental groups.

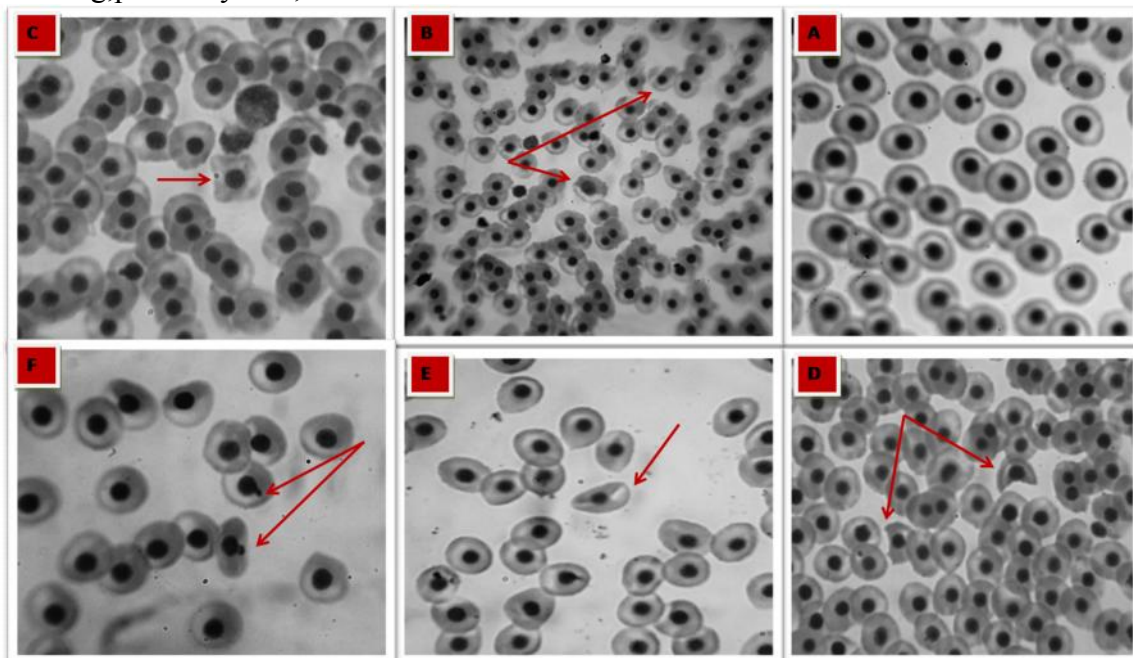
174 Regarding to the result of thrombocyte as in Fig.(4) we found a decrease in there
 175 number in catfish exposed to 0.02mg/L Hg for 14 days, this result agreed with [32,33]
 176 .On the other hand, these parameters were still as control in catfish exposed to (0.002,
 177 0.0002mg/L Hg) of mercuric chloride indicating no toxic effect of mercuric chloride at

178 these both doses in Egyptian catfish(*Clarias lazera*),these results are parallel to the result
179 proved by [16]



180 **Fig.4.**Changes in Platelets number($\times 10^5$ cells/mm³) in all experimental groups.
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182

183 Erythrocyte morphological alterations and anomalies in Fig.(5)showing fragmented,
184 crenated, enlarged RBC with giant nucleus, star shape RBC, semi-lunar shaped RBC
185 with vacuolated cytoplasm, elliptical RBC with hypochromasia and presence of micronucleus
186 indicating toxicity in fish group treated with 0.02 mg/L mercuric chloride. Our result
187 parallel to the result of Gill and Pant who reported erythrocyte anomalies in fish
188 subjected to intoxication with heavy metal, these anomalies represented by erythrocyte
189 swelling,poikilocytosis, vacuolation and deterioration of the cell membrane.



190 **Fig.5.** Blood smear for morphological anomalies.[A] showing normal RBC morphology
191 of the control group , while [B] showing fragmented, crenated and enlarged RBC with
192 giant nucleus , [C] showing star shape RBC,[D] showing semi-lunar shape RBC with
193 vacuolated cytoplasm ,[E] showing elliptical RBC with hypochromasia and [F] showing
194 presence of micronucleus indicating toxicity in fish group treated with 0.02 mg/L
195 mercuric chloride.
196
197

Regarding to the leukogram results as in Figs.(6,7,8) , we found increase in the number of WBC,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 lymphocyte ,this may be due to the effect of toxicants 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 .

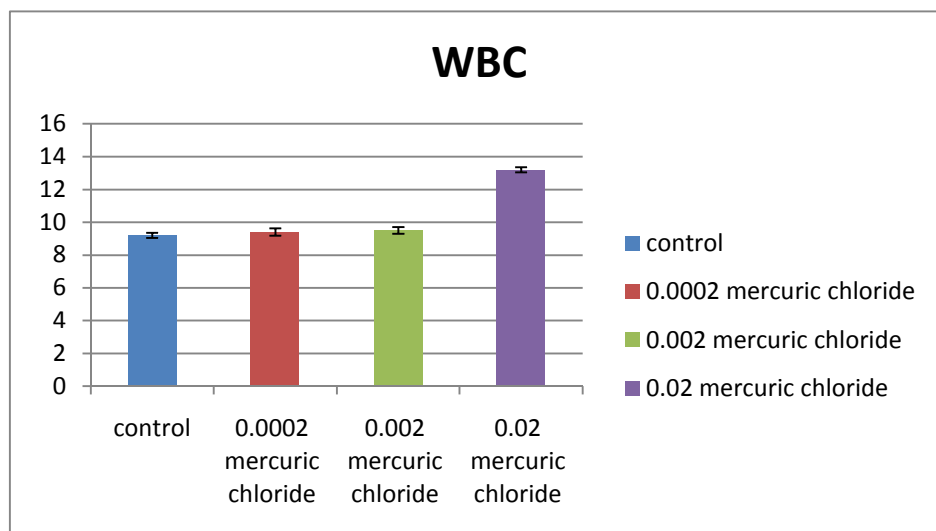


Fig.6:Changes in WBC (x10³cells/mm³) in all experimental groups.

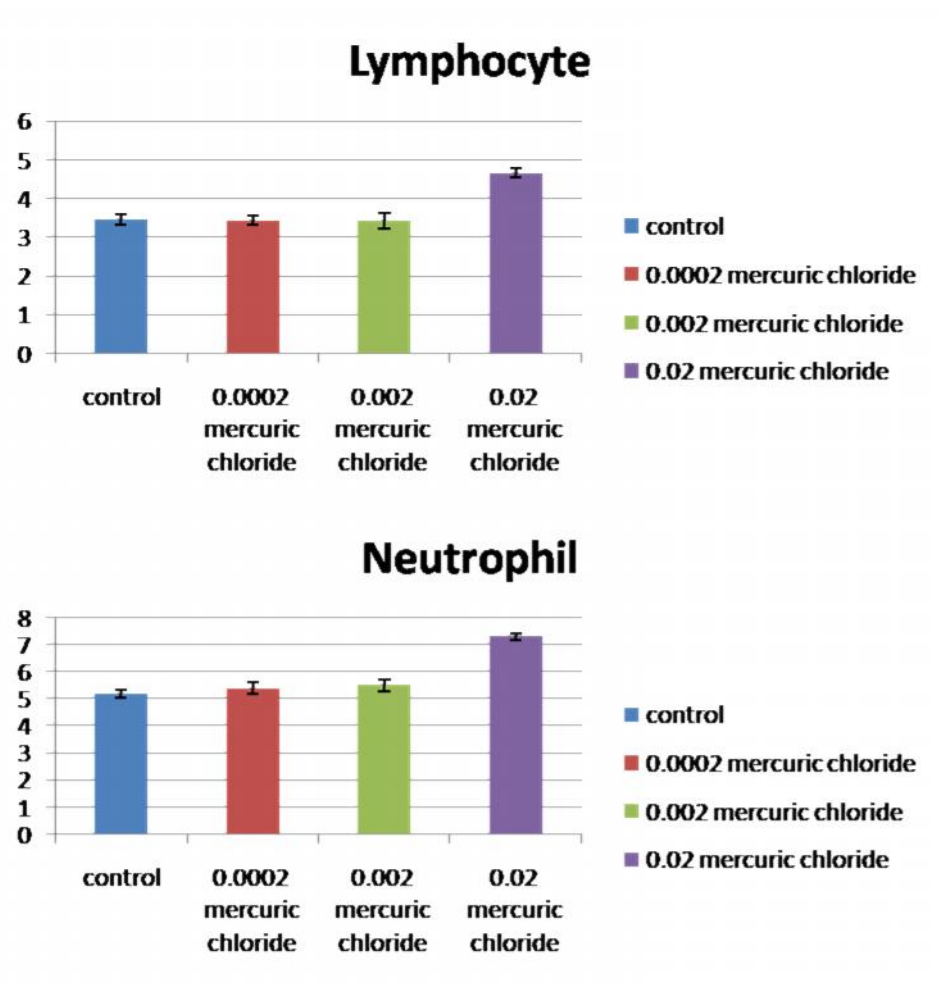


Fig.7: Changes in Lymphocyte and Neutrophil (x10³cells/mm³) in different experimental groups.

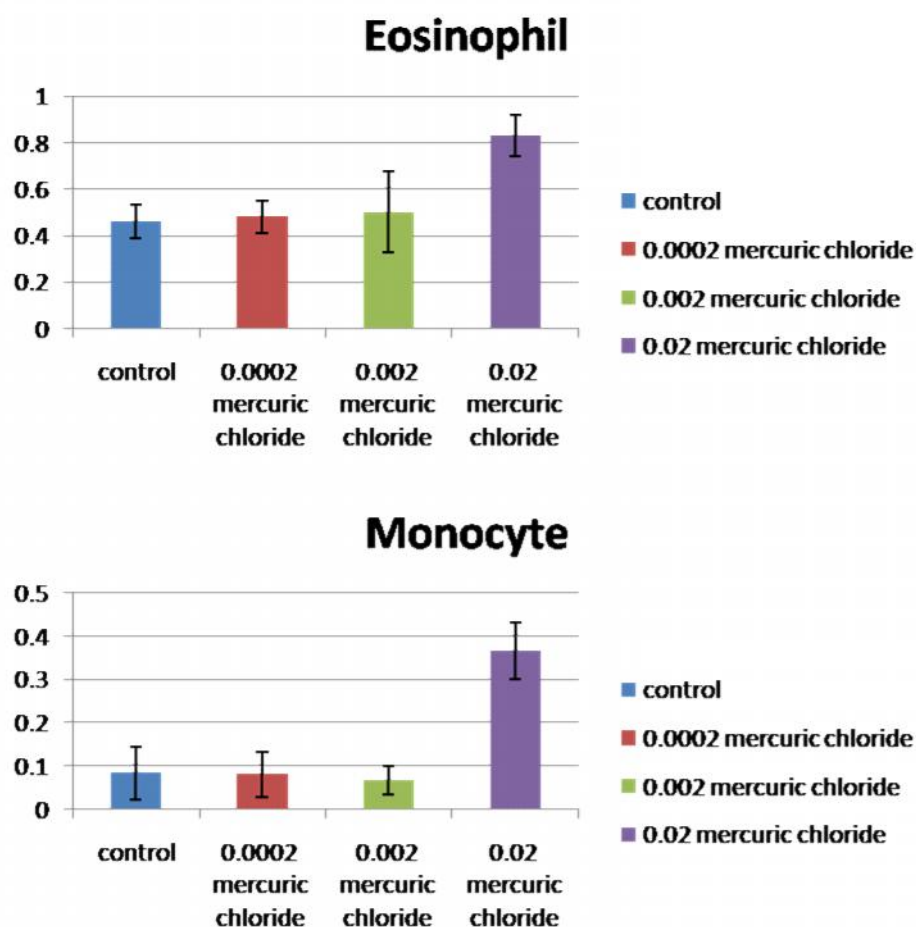


Fig.8:Changes in Eosinophil and Monocyte ($\times 10^3$ cells/mm³) in all experimental groups.

Some plasma chemicals may be useful tools to evaluate the health and/or stress condition of the fish [38,39,40]. Because stress has been reported to elevate plasma cortisol [41,42,43] and glucose levels [44,45], many researchers considered that fishes undergoing stressful situations exhibit plasmatic increases of cortisol and glucose [46,47,48]. Regarding to the biochemical result in Fig.(9), we found significant increase in blood glucose and cortisol levels in catfish exposed to 0.02 mg/L Hg for 14 days. While, these parameters were still as control in catfish exposed to (0.002, 0.0002 mg/L Hg) of mercuric chloride indicating no toxic effect of mercuric chloride at these both doses in Egyptian catfish (*Clarias lazera*), these results are parallel to the result proved 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 stressors which activate the hypothalamus-pituitary-interrenal (HPI) axis, resulting in a cortisol liberates that causes secondary stress answers [16].

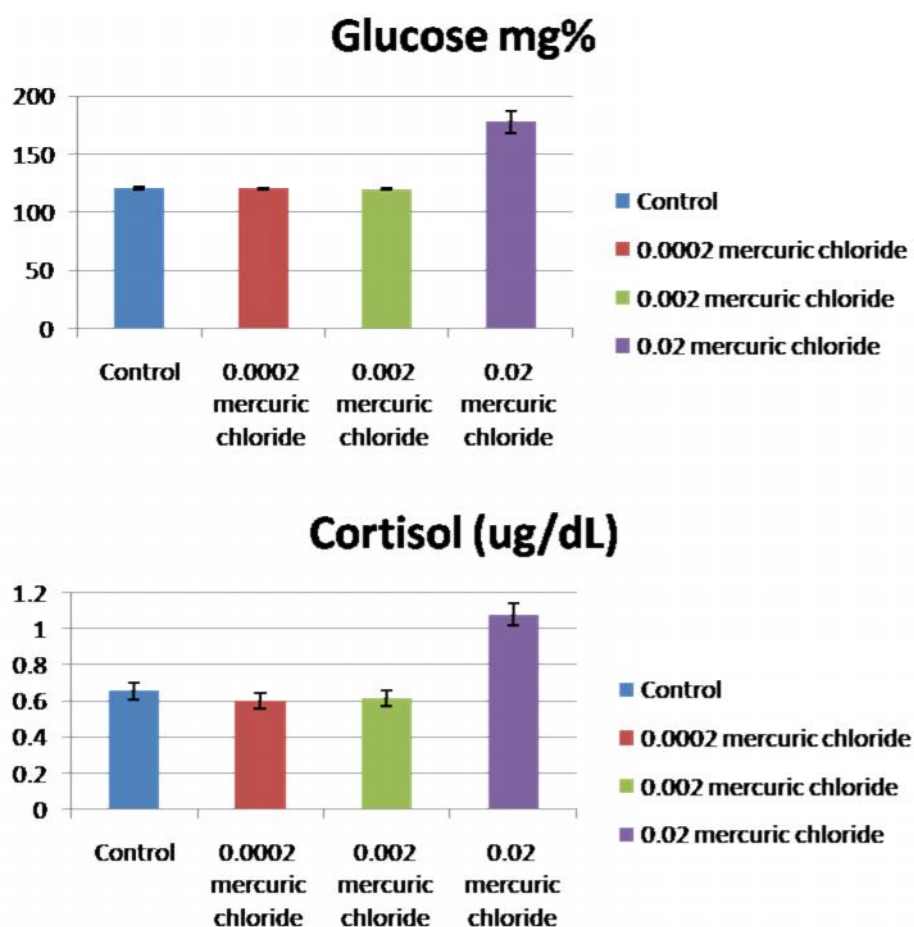


Fig.9:Changes in glucose and cortisol in all experimental groups.

4.Conclusion

The sublethal Hg (HgCl₂) concentrations(0.0002,0.002 mg/L) used in the present study were insufficient to cause hematological changes in Catfish . While, the concentration of (0.02 mg/ L) induce moderate hematological and biochemical toxicity .Therefore, further studies of Hg toxicity would be need to be carried out at higher concentrations or using long exposure times.

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