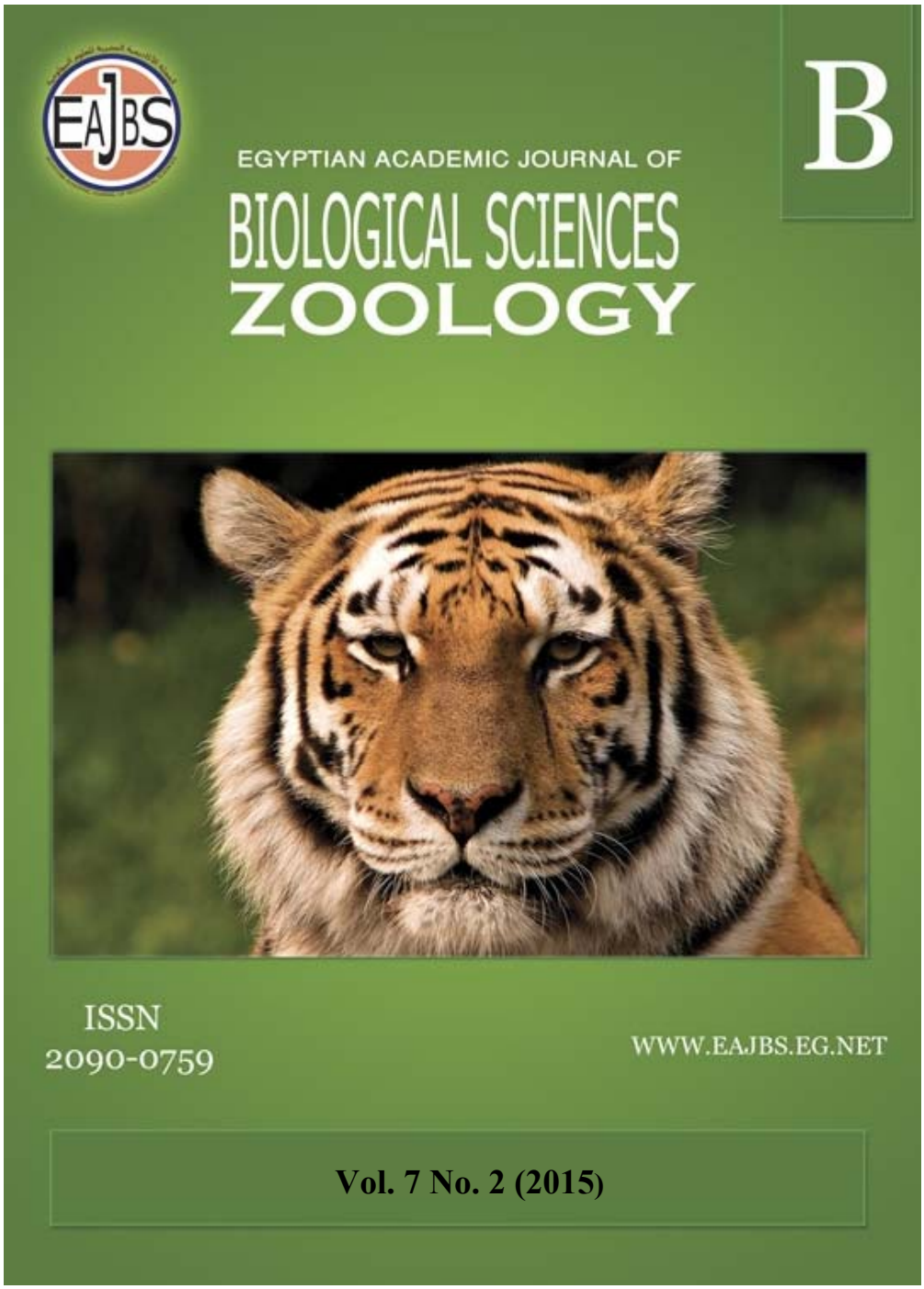


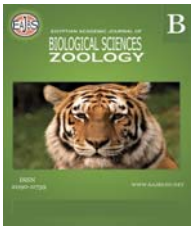
Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society of Biological Sciences, Department of Entomology ,Faculty of Sciences Ain Shams University .

The Journal publishes original research papers and reviews from any zoological discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematics, morphology, evolution, and general zoology

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 7(2): 1- 19 (2015)

Egypt. Acad. J. Biolog. Sci.
B. Zoology
ISSN: 2090 – 0759
www.eajbs.eg.net



The ability of vitamin E, selenium and water to improve and recover the hematological, biochemical and hormonal parameters of mercury-exposed catfish *Clarias gariepinus* (Burchell, 1822)

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ARTICLE INFO

Article History
Received:1/7/2015
Accepted:10/8/2015

Key words:
Clarias gariepinus
Mercury
Selenium
Vitamin E
Biochemical
Recovery

ABSTRACT

This study aimed to determine the toxic impacts of a sublethal dose of mercury chloride (0.10 mg/L for 30 days as exposure period) on hematological, biochemical parameters and thyroid hormones (T3 and T4) of the African catfish, *Clarias gariepinus*, and to evaluate the ability of vitamin E, selenium and water to improve and recover the previous parameters in mercury-treated fish (for 15 and 30 days as recovery periods). The exposure to mercury caused a significant ($p<0.05$) decrease in RBCs, Hb, Ht, MCH, MCHC, MCV, WBCs, lymphocytes, monocytes, neutrophils, potassium, albumin, Alb/glob ratio, triglycerides, glucose and thyroid hormones (T3 and T4) when compared with the control group. Also, exposure to mercury caused a significant increase ($p<0.05$) in levels of Na, urea, creatinine, ALP, AST, ALT, total proteins and globulins levels than those of the control fish. The ability of vitamin E and selenium to improve and recover the hematological and biochemical parameters and thyroid hormones (T3 and T4) studied were better than water alone. Also, the improvement was mostly better after 30 days than 15 days of recovering period. The recovery with water alone led to complete recovery only in MCHC. While, all the remaining of hematological parameters studied in addition to cholesterol and glucose levels are improved significantly (increased/decreased) to be relatively better than those of the mercury-treated fish. The levels of urea, creatinine, ALP, AST, ALT, TP, albumin, globulins and Alb/glob ratio, T3 and T4 are not improved well by the water. But, Na levels is not affected by the water. The levels of K and triglycerides are increased to high levels than that of the control fish. The recovery with vitamin E led to complete recovery in Hb, MCV, MCH, MCHC and Na to reach the control level ($p<0.05$); and significantly ($p<0.05$) improved RBCs, Hct, WBCs, neutrophils, lymphocytes and monocytes, urea, creatinine, ALP, AST, globulin, Alb/glob ratio, cholesterol, triglycerides and glucose to be better than those of the mercury-treated fish. K and albumin levels are fluctuated. While ALT, TP decreased significantly. But, T3 and T4 are not improved well by Vitamin E. The recovery with selenium led to complete recovery in MCH, neutrophils, lymphocytes, total proteins and globulins levels ($p<0.05$); and significantly helped improvement (increased/decreased) of RBCs, Hb, Hct, WBCs, monocytes, urea, creatinine, ALP, AST, ALT, Alb/glob ratio, cholesterol, triglycerides, glucose, T3 and T4 to be better than those of the mercury-treated fish. But, Na, and albumin are not improved well by selenium. Potassium levels showed fluctuations. ALT and TP decreased significantly to be less than that of the control values. Also, it was noted that, the improvement of T3 and T4 due to Selenium (Se) are better than vitamin E than the mercury-free water respectively.

INTRODUCTION

Pollution of the aquatic environment by metals and other toxic substances is a great environmental problem and represents a cause of growing concern throughout the world.

Contamination of aquatic systems by heavy metals via natural and anthropogenic sources leads to appearance of undesired properties affecting animals and humans, and become a significant threat for public health (Özkan, *et al*., 2011); and constitutes an additional source of stress for aquatic organisms, and can lead to various physiological dysfunctions in fish (Shokr, 2015).

The toxicity of metals become more dangerous and be highly toxic when present in high concentrations due to their stability and persistent existence in the environment (Zarei *et al*., 2013). Harabawy and Mosleh (2014) have reported that, continuous exposure to high levels of the metals and metallic compounds pose potential health risk to various animals and even to human and can induce toxic impacts because they tend to bioaccumulate in different tissues. Many of pollutants are lipophilic or able to bound to proteins and accumulate in fish tissues to considered levels enough to disturb the internal environment of the animal body, so it is important to know which concentration above which the commercial fish species become unsuitable as food for human which may be affected (El-Ezaby, 1994; Hassaan *et al*., 2014). Also, toxicity of heavy metals is due to their ability to inactivate enzymes and/or functional proteins by directly binding to them (Tsuji *et al*., 2002). This may be achieved through formation of reactive oxygen species (ROS) resulting in oxidative damage (Stohs and Bagchi, 1995) which can lead to many pathological changes (Morakinyo *et al*., 2012).

Mercury is a well-known toxicant that comes to aquatic systems through natural and artificial sources (Zhang *et al*., 2013) and may be present in three basic forms (elemental, inorganic and organic mercury) with various toxic impacts (Oliveria *et al*. 2012). Mercury is generally found at very low concentrations and is very reactive in the environment (Mahmoud *et al*. 2012), and the soluble form of this metal is highly toxic to fish (Mahboob *et al*., 2014). Recently, great attention had been given to the potential risks of metals toxicity in human due to consumption of contaminated fishes (Authman *et al*. 2015).

The impacts of toxins on biota could be expressed by responses known as biomarkers. Biomarkers are defined as any alteration induced by xenobiotics in cellular or biochemical components or processes, structures or functions and be measurable in a biological system or sample (ATSDR 1994) in order to determine the extent of toxic impacts at a tissue or organ level before their appearance at a clinical/pathological level.

Fish tissues are sensitive indicators for aquatic pollution and have a high mercury bioaccumulation capacity for both organic and inorganic forms (Ribeiro *et al*., 2006). Hematological parameters (RBCs, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cells, lymphocytes, monocytes and neutrophils), biochemical parameters (ALT, AST, ALP, Albumin, globulin, total protein, cholesterol, triglycerides and glucose), several hormones (T3, T4, TSH, Testosterone and E2) are applied in diseases diagnosis as biomarkers to reflect the toxic impacts of pollutants on fishes. (Ibrahim, 2011; Harabawy and Ibrahim, 2014; Ibrahim and Harabawy 2014; Muttappa *et al*., 2015).

Exposures of fish to different Hg concentrations in the Lap have shown several alterations in the behavior, growth performance, hematology, hormones and

histopathological characteristics of the liver and kidney (Ibrahim, 2011). Also, many authors have reported that, mercury compounds can be retained in the animal tissues for long time, leading to irreversible damages, such as neurological impairment and lesions, behavioral and cognitive changes, ataxia, as well as convulsions, in addition to its harmful effect on gill arches, liver, kidney, hematological parameters, olfactory epithelium, and reproduction, alterations in enzymatic activities, problems during gonad development, reduction of eggs incubation success and survival during embryo-larval stages, decreased locomotors activity, reduction of escape capacity, brain lesions and death and genotoxic effects due to exposure of several fish species to Hg in the Lab (Raldúa *et al*., 2007; Rodrigues *et al*., 2010; Zaki *et al*., 2011; Authman *et al*., 2015).

The antioxidant defense against metal-free radical species and their toxicity is an important aspect of research in animal and human health. Selenium and vitamin E as antioxidants make the cell to act as a hunter of free radicals, thus preventing the autointoxication of immunological cells such as macrophages which represent the first processors of the information about the exotic bodies and providing maximum defense for the fish (Brake, 1997). Vitamin E (α-tocopherol) is one of the most significant antioxidant vitamins that acts as a fat-soluble antioxidant in biological membranes to protect lipids against peroxidative damages and maintaining the normal growth and metabolic functions of fish (Harabawy and Mosleh, 2014; Hassaan *et al*., 2014; Rengaraj and Hong, 2015). Mahmoud *et al*. (2012) have reported that, Vitamin E plays an essential role in elimination of mercury stress through antioxidant free radical mechanism. Selenium is also an essential element required in small amounts to maintain good health and playing an important role in antioxidant defenses and is a cofactor for the enzymatic antioxidant glutathione peroxidase (Khidr *et al*., 2008; Zubair *et al*. 2015). Also, Chen *et al*. (2006) have demonstrated that selenoproteins help eliminate reactive oxygen species caused by metals due to their antioxidant properties.

Considering the great aquaculture and commercial value of the African catfish, *C. gariepinus*, it is necessary to evaluate the role of Vitamin E and Selenium as antioxidants to attenuate the toxic impacts of mercury and thus improving the physiological status of the affected fish during recovering period. Therefore, the present study aims to study the toxic effects of mercury chloride on the hematological and biochemical characteristics and hormonal disrupting in mercury-exposed *C. gariepinus* for 30 days; and to evaluate the ability of the water, Vitamin E and Selenium, each alone, to decrease the toxicity of mercury through recovering periods (15 and 30 days) after mercury-exposure.

MATERIALS AND METHODS

Specimens Collection

Sixty four fish specimens of African catfish, *C. gariepinus* were collected from the Nile at Assiut (225.6±12.8 g in weight, 34.5±1.5 cm in length). Fish Specimens immediately were transported to the fish laboratory in the Department of Zoology, Faculty of Science, Assiut University. Fish specimens were acclimatized for two weeks in well-aerated glass tanks (160 L capacity). Fish were fed frequently a diet containing 32% crude protein at a rate of 3% of live body weight twice daily for 60 days. After application of the toxicant, siphoning a portion of water from each aquarium was done every 3 days for extract removal, and an equal volume of water containing the same concentrations of toxicants replaced it. Water temperature, pH

and dissolved oxygen (DO) concentrations were measured daily (24.3±0.5**°C**, **7.6**±0.45 pH and **6.5**±0.75 mg/L DO). Photoperiod was 12:12 Light: Dark.

Chemicals

Mercury chloride (HgCl2 [99.5% purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA), Sodium Selenite Na2SeO3 (98%) (Code No.28074 ADWIC) and DL-α-tocopherol (VE) acetate were obtained from Merck (Germany).

Experiment

LC_{50} of mercury chloride after 96 hour was 0.412 ppm, according to Ibrahim (2011). One sublethal concentration of mercury chloride (0.10 ppm), one concentration of Selenium (100 ppm) and one concentration of Vitamin E (100 mg/kg) were taken. Water was changed every day in the control and the treated groups, the concentrations of mercuric chloride remained as the same during the experimental period. Fish specimens were exposed to 0.10 ppm of HgCl2 for 30 days. Then, the recovery began for 15 and 30 days using water, selenium and vitamin E separately.

Experimental Design

After two weeks of acclimatization, fishes were weighed, measured and randomly classified into 5 groups. The first two containing 8 fish/tank, one control and one group for treatment with 0.10 ppm mercury, and the other three groups containing 16 fish/tank for recovery with water, vitamin E and selenium as shown in Table (1). Stock solution (1,000 ppm) of Mercury as HgCl2 was prepared and stored in clean glass bottles and diluted to concentrations of 0.10 mg/L as 1/4 of LC_{50} (Ibrahim, 2011). Mercury doses were prepared and added constantly to the aquarium for 30 days.

Table 1: The fish groups exposed to mercury (Hg=0.10 mg/L) and recovering by water, selenium (Se=0.1 mg/L) and vitamin E (100 mg/kg body weight).

Group no	Treatment for 30 day	Recovery for 15 days after treatment	Recovery for 30 days after treatment
1	Control (only water, n=8)	**-----**	**----**
For Treatment			
2	Low mercury dose **(**Hg, n=8)	**-----**	**-----**
For recovery from mercury dose (Hg=0.10 mg/L)			
3	High mercury dose (Hg, n=16)	Water only (n=16)	Water only (n=8)
4	High mercury dose (Hg, n=16)	Selenium only (n=16)	Selenium only (n=8)
5	High mercury dose (Hg, n=16)	Vitamin E only (n=16)	Vitamin E only (n=8)

Then, water, selenium and vitamin E were used for the next 30 days for recovery. The test water was replaced daily with the required amount of stock solution to prevent deterioration of water quality and replenish mercury levels. Fish in the first group was the control, and group 2 exposed to mercury for 30 day as treatment-period. Fish in groups 3-5 were exposed to mercury for 30 day and then were left for recovery with mercury-free water, Se and VE, respectively. Blood samples of the control, treated and recovered fish (8 fish/group) were collected.

Methods

1-Hematological parameters

A-Red Blood cells count

After 30 days, blood samples of the control (group 1) and treated fish (group 2) (8 fish/group) were collected from the caudal vein of the fish in a small plastic tubes containing heparin solution (0.2 ml/ml blood) as anticoagulant. After 15 and 30 days of recovering period of exposure to mercury (groups 3-5), blood samples (8 fish/group) were collected from the caudal vein of the fish in a small plastic tubes

containing heparin solution (0.2 ml/ml blood) as anticoagulant. The RBC's, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and hemoglobin (Hb) concentration were determined by using automated technical analyzer (Mindray Bc-2800).

B-White Blood Cells count

Total white blood cells (WBCs) were counted using an improved Neubaur haemocytometer (Mgbenka *et al*., 2003 and Shah and Altindağ, 2005). Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer. 4 large (1 sq mm) corner squares of the haemocytometer were counted under the microscope (Olympus) at 640 X. The total number of WBC was calculated in mm^3 $x10^3$ (Wintrobe, 1978) and differential count was displayed by staining blood films with gemsa stain.

2-Biochemical parameters

After 30, 45 and 60 days period of exposure to mercury and its recovery, blood samples of the control and treated fish (8 fish/group) were collected from the caudal vein of the fish in small plastic tubes and left to coagulate for 15 minutes and centrifuged at 3000 rpm to separate serum. The fresh serum was subjected to biochemical analysis. Biuret method described by Wotton (1964) was used for the determination of serum total protein, albumin, aspartic amino transferase (AST, U/L), alanine amino transferase (ALT, U/L) and alkaline phosphates (ALP, U/L) using assay kits supplied by Diamond Diagnostic, Egypt. Serum glucose (mg/dl), total cholesterol (mg/dl) and triglycerides (mg/dl) were estimated in serum using assay kits supplied by Diamond Diagnostic, Egypt. Also serum urea and creatinine were estimated using kits supplied from Biomerieux (France). The samples were measured by Microlab 200 spectrophotometer. Potassium and Sodium concentrations were determined by atomic absorption spectrophotometer (Varley *et al*., 1980).

3-Hormone assays

Blood samples (1 ml) were collected from the control and treated fish and centrifuged (20 min at 1,500 x g), followed by collection of serum, freezing and storing at -70 ºC before hormone analysis. Concentration of T3 and T4 were determined using competitive chemiluminescent enzyme immunoassay (Immulite 1000, Siemens, Los Angeles, CA). All samples were run in duplicate and assayed at the same time, in a single run with a single lot number of reagents and consumables employed by a single operator, with intra-assay coefficients of variation for all variables less than 5%.

Statistical Analysis

The basic statistics, means and standard errors of the measured parameters were estimated. The patterns of variation due to mercury then recovery with selenium and vitamin E doses and water only were tested by using one-way and two-way ANOVA which determined the effects of mercury doses and its recovery with selenium, Vitamin E and water as the factors simultaneously tested. The differences between means were done by using The Tukey-HSD test. The post-hoc test was used to compare between means at $P \leq 0.05$. The software SPSS, version 10 (SPSS, 1998) was used as described by Dytham (1999).

Ethical statement

All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the Committee of the Faculty of Science, Assiut University, Egypt.

RESULTS

Haematological parameters

Treatment with mercury (0.10 mg/L) for 30 days

The mean hematological indices, RBCs, Hb, Hct, MCV, MCH and MCHC, of control and treated African catfish *Clarias gariepinus* exposed to sublethal concentration of mercury, 0.10 mg/L for 30 days were shown in Table 2. From the analyses, the differences between selected parameters of control and treated fish were statistically important. Exposure of *C. gariepinus* to mercury caused a highly significant ($P<0.001$) decrease in RBCs count, Hb, Ht, MCH and MCHC values when compared with the control group. While, MCV values showed significant ($p<0.05$) increase when compared with the control group.

Table 2: The basic data (Mean ± S.E.; N=8) of blood constituent parameters of *Clarias gariepinus* exposed to sublethal concentration of mercury (Hg=0.10 mg/l) for 30 days, then recovering with water, vitamin E (VE=100 mg/kg body weight) and selenium (Se=0.1 mg/l), each alone, for 15 and 30 days.

Treatment and recovery	periods	RBCs ($X10^6$/ml)	Hb (Mg/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBCs ($X10^3$/ml)	NEUTRO (%)	LYMPHO (%)	MONO (%)
control		1.91± 0.01A	11.75± 0.05A	24.31± 0.11A	142.66± 0.67A	57.93± 0.27A	40.86± 0.19A	28.41± 0.15A	44.85± 0.24A	52.33± 0.28A	2.49± 0.01A
Treatment Hg	30 days	0.88± 0.00F	6.47± 0.03G	15.56± 0.07E	173.09± 0.81F	52.62± 0.25D	29.85± 0.14D	95.93± 0.51E	19.83± 0.10F	74.50± 0.39D	5.33± 0.03G
Recovery By water	15 days	1.11± 0.01E	8.87± 0.04F	18.24± 0.09D	163.04± 0.76E	63.66± 0.30C	36.12± 0.17C	78.34± 0.41D	23.72± 0.13E	71.36± 0.38D	4.58± 0.02F
	30 days	1.19± 0.01E	13.16± 0.06D	19.34± 0.09C	150.53± 0.70D	59.73± 0.28B	40.05± 0.19A	63.51± 0.34C	34.34± 0.18C	61.44± 0.32BC	3.89± 0.02E
Recovery With Selenium	15 days	1.22± 0.01D	11.18± 0.05B	19.23± 0.09C	137.32± 0.64B	58.11± 0.27 A B	44.49± 0.21B	78.63± 0.42D	36.23± 0.19BC	59.60± 0.32B	3.84± 0.02E
	30 days	1.39± 0.01BC	9.11± 0.04E	20.04± 0.09BC	122.28± 0.57C	57.17± 0.25A	43.89± 0.21B	60.37± 0.32C	41.11± 0.22A	55.46± 0.29A	3.09± 0.02B
Recovery With Vitamin E	15 days	1.33± 0.01C	10.78± 0.05C	19.53± 0.09C	161.27± 0.75E	57.61± 0.27A	34.18± 0.16C	75.46± 0.40D	32.69± 0.17D	63.44± 0.34C	3.54± 0.02D
	30 days	1.44± 0.01B	11.94± 0.06A	20.95± 0.10B	142.00± 0.66A	57.21± 0.27A	40.88± 0.19A	55.01± 0.29B	38.42± 0.20B	57.91± 0.31B	3.34± 0.02C

Different letters indicate significant difference at $p<0.05$

Red blood cells (RBC), Hemoglobin (Hb), Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), White blood cells (WBCs), Neutrophils (Neutro), Lymphocytes (Lympho) and Monocytes (Mono).

The results in Table 2 show that the total number of WBCs, and percentages of lymphocytes and monocytes increased significantly ($p<0.05$) in blood of the exposed fishes compared with the control ones. While, a significant decrease ($p<0.05$) in neutrophils percentage was recorded.

Recovering after exposure to mercury (for 15 and 30 days):

The effects of mercury-free water, vitamin E (VE=100 mg/kg body weight) and selenium (Se=0.1 mg/l), each alone, on hematological indices during recovering periods (15 and 30 days) after exposure to mercury (0.10 mg/L) are shown in Table 2.

Water recovery

In comparison with the mercury-treated fish and control group, Table 2 shows that, the recovery with mercury-free water led to an improvement (after 15 days) and complete recovery (after 30 days) in MCHC to the normal range of the control group ($p<0.05$). While, all the remaining of hematological parameters studied are significantly ($p>0.05$) changed (increased/decreased) to be relatively better than those

of the mercury-treated fish, but still far from those of the control group. It was noted that, the improvement was better after 30 days than 15 days of recovering period.

Recovery with supplementation of Vitamin E

Table 2 shows that, supplementation of Vitamin E led to complete ($p>0.05$) recovery in the levels of Hb, MCV, MCH and MCHC to reach the normal range of the control level; and significant ($p>0.05$) improvements in all the remaining of hematological parameters studied to be relatively better than those of the mercury-treated fish, and the improvements are better after 30 days than 15 days recovering period.

Recovery with Selenium (Se=0.1 mg/L)

The recovery with selenium led to complete recovery ($p<0.05$) in MCH (after 15 and 30 days), neutrophils and lymphocytes (after 30 days). While, RBCs, Hb, Hct, WBCs and monocytes are significantly ($p>0.05$) improved (increased/decreased) to be relatively better than those of the mercury- treated fish. On the other hand, MCV decreased significantly ($p>0.05$) to be lesser than that of the control value, while MCHC increased significantly ($p>0.05$) to be higher than that of the control value (after 15 and 30 days).

In general, the results revealed that, after exposure to mercury (0.10 mg/L), the ability to improvement and recovery potential of vitamin E and selenium (respectively) were better than that of the mercury-free water.

Biochemical parameters

Treatment with mercury (0.10 mg/L) for 30 days

The mean values of the biochemical parameters including Na, K, urea and creatinine as kidney functions parameters; ALP, ALT, AST as liver functions parameters; and total protein, albumin, globulin and Alb/glob ratio of the control and treated catfish *C. gariepinus* exposed to a sublethal dose of mercury, 0.10 mg/L for 30 days are given in Table 3. The results revealed that, the treatment with mercury caused a highly significant ($P<0.001$) decrease in K and significant increases ($p<0.05$) in Na, urea and creatinine level.

Table 3: The basic data (Mean ± S.E.; N=8) of blood constituent parameters (kidney and Liver) of *Clarias gariepinus* exposed to sublethal concentration of mercury (Hg=0.10 mg/l) for 30 days, then recovering with water, vitamin E (VE=100 mg/kg body weight) and selenium (Se=0.1 mg/l), each alone respectively, for 15 and 30 days.

Treatment		Na (Meg)	K(Meg)	Urea (mg/dL)	CREAT (mg/dL)	ALP (U/L)	AST (U/L)	ALT (U/L)	TP (mg/dL)	ALB (mg/dL)	GLOB (mg/dL)	ALBGLOB (%)
Control		181.02± 0.86A	3.43± 0.02A	14.16± 0.07A	0.26± 0.00A	9.28± 0.04A	40.42± A	19.96± A	4.37± A	1.77± A	2.60± A	0.69± A
Hg	30 days	567.31± 2.68E	2.83± 0.01B	106.21± 0.49G	1.93± 0.01F	26.68± 0.13G	463.14± 2.15G	60.97± 0.28G	5.72± 0.14F	1.02± 0.00C	4.69± 0.14E	0.22± 0.01F
Recovery By water	15 days	517.47± 2.44CD	5.93± 0.03F	40.36± 0.19E	1.32± 0.01C	22.25± 0.01F	322.32± 1.51F	39.43± 0.19F	4.75± 0.21B	0.90± 0.00D	3.78± 0.21D	0.26± 0.01E
	30 days	589.85± 2.77E	6.65± 0.03F	38.42± 0.18D	1.42± 0.01D	19.47± 0.09D	188.27± 0.88D	30.56± 0.14D	3.28± 0.11D	0.93± 0.00D	2.94± 0.10B	0.36± 0.01D
Recovery With Selenium	15 days	545.93± 2.56D	1.88± 0.01D	46.33± 0.22F	1.59± 0.01E	20.58± 0.10E	207.00± 0.97E	26.61± 0.12D	4.59± 0.20AB	0.90± 0.00D	3.69± 0.20D	0.25± 0.01E
	30 days	395.42± 1.86B	5.10± 0.02E	29.16± 0.14C	1.18± 0.01BC	18.59± 0.09D	141.94± 0.67C	12.98± 0.06B	3.41± 0.13D	0.97± 0.00D	2.54± 0.13A	0.35± 0.02D
Recovery With Vitamin E	15 days	194.07± 0.91A	4.78± 0.02C	19.11± 0.09B	1.00± 0.00B	15.17± 0.07B	60.13± 0.28B	11.48± 0.05C	3.94± 0.07C	1.23± 0.00B	2.01± 0.07C	0.46± 0.01B
	30 days	503.98± 2.38C	2.65± 0.01B	26.82B± 0.13C	1.44± 0.01D	16.27± 0.08C	67.03± 0.31B	9.98± 0.05E	3.01± 0.09E	1.00± 0.00C	2.15± 0.09C	0.40± 0.02C

Different letters indicate significant difference at $P<0.05$

Sodium (Na), Potassium (K), Urea, Creatinine (Creat), Alkaline phosphates (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST). Total Protein (TP), Albumin (Alb), Globulin (Glob) and Albumin/globulin ratio (Alb/glob).

Also, the liver-functions parameters studied, ALP, AST and ALT are increased significantly ($p<0.05$) than those of the control fish. The total protein and globulin levels increased significantly ($p<0.05$), while, albumin and frequently Alb/glob ratio decreased significantly ($p<0.05$).

Recovering after exposure to mercury:

The effects of mercury-free water, vitamin E (VE=100 mg/kg body weight) and selenium (Se=0.1 mg/l), each alone, on the biochemical parameters during recovering periods (15 and 30 days) after exposure to mercury are shown in Table 3.

Recovering with mercury-free water:

Table 3 showed that, Na is not affected by mercury-free water throughout the recovering period. K levels is strongly increased ($p<0.05$) to be higher than the levels of control fish throughout the recovering period. The mercury-free water slightly ($p<0.05$) helped improvement of urea, creatinine, ALP, AST, ALT, TP, albumin, globulin and Alb/glob ratio to be relatively better than those of the mercury-treated fish. But, total protein TP (after 30 days recovery) decreased significantly ($p<0.05$) to be less than that of the control fish.

Recovery with supplementation of Vitamin E

Supplementation of vitamin E through recovering period (after exposure to mercury, 0.10 mg/L) led to a complete recovery in Na level only after 15 days; and improved urea, creatinine, ALP, AST, globulin and Alb/glob ratio significantly ($p<0.05$) along the recovering periods to be relatively better than that of the mercury-treated fish. Potassium and albumin levels are fluctuated throughout the recovery time. While ALT, TP decreased significantly to lower level than that of the control ($p<0.05$).

Recovery with Selenium (Se=0.1 mg/L)

The recovery with selenium after exposure to mercury (0.10 mg/L) led to complete recovery ($p<0.05$) in total protein (after 15 days) and globulins levels (after 30 days) to be as those of the control fish. While urea, creatinine, ALP, AST, ALT and the albumin globulin ratio are significantly ($p<0.05$) improved to be relatively better than those of the mercury-treated fish, but still far from those of the control group. But, Na, and albumin are not improved well by selenium; and potassium levels showed fluctuations through the recovery period with selenium, it decreased significantly ($p<0.05$) after 15 days and then increased significantly ($p<0.05$), at 30 days of recovery, to be higher than that of control value. Also, it was noted that, ALT and TP decreased significantly ($p<0.05$) after 30 days of recovery to be less than that of the control values.

Lipogram, glucose and thyroid hormones

Table 4 shows the normal values of cholesterol, triglycerides, glucose and thyroid hormones (T3 and T4) of *C. gariepinus* which exposed to mercury (0.10 mg/L) for 30 days and then recovered for 15 and 30 days with mercury-free water, Vitamin E (VE) and Selenium (Se).

The results revealed that, Mercury treatments led to significant ($p<0.05$) decreases in all pervious parameters except cholesterol when compared with the control group.

Recovering after exposure to mercury:

The recovery with mercury-free water revealed that, cholesterol and glucose levels are improved significantly ($p<0.05$) along the recovering periods to be better than that of the mercury-treated fish. But, T3 and T4 are not improved well by the mercury-free water throughout the recovering period. While, triglycerides increased

significantly ($p<0.05$) along the recovering periods to be higher than that of the control level.

Table 4: The basic data (N=8) of blood constituent parameters (lipid profile, glucose and thyroid hormones T3 and T4) of *Clarias gariepinus* exposed to sublethal concentration of mercury (Hg=0.10 mg/l) for 30 days, then recovering with water, vitamin E (VE=100 mg/kg body weight) and selenium (Se=0.1 mg/l), each alone, for 15 and 30 days.

Treatment		CHO (mg/DL)	TRI (mg/DL)	GLU (mg/DL)	T3 (ng/ml)	T4 (ng/ml)
Control		192.83±0.91A	134.88±0.64A	58.81±0.31A	1.58±0.01A	4.15±0.02A
Hg	30 days	330.71±1.57F	58.93±0.28F	12.96±0.07E	0.22±0.00E	1.46±0.01E
Recovery By water	15 days	282.78±1.33E	177.26±0.86D	34.88±0.18D	0.42±0.00D	1.87±0.01D
	30 days	246.29±1.16CD	377.74±1.77G	42.36±0.22B	0.43±0.00D	1.90±0.01D
Recovery With Selenium	15 days	295.72±1.40E	87.38±0.42E	39.37±0.21C	0.84±0.00B	3.48±0.02B
	30 days	259.23±1.24D	117.39±0.56B	45.35±0.24B	0.94±0.00B	3.35±0.02B
Recovery With Vitamin E	15 days	268.74±1.28D	84.43±0.40E	33.89±0.18D	0.62±0.00C	2.69±0.01C
	30 days	214.29±1.02B	101.42±0.48C	44.85±0.24B	0.52±0.00C	2.19±0.01C

The data are presented as Means±S.E.
Different letters indicate significant difference at $P<0.05$
Cholesterol (CHO), Triglyceride (TRI), Glucose (GLU), Triiodothyronine (T3) and Thyroxin (T4).

Also, the recovery with vitamin E showed that, cholesterol, triglyceride and glucose are improved significantly ($p<0.05$) along the recovering periods to be better than that of the mercury-treated fish. But, T3 and T4 are not improved well by vitamin E throughout the recovering period. The recovery with selenium revealed that, all pervious parameters are improved significantly ($p<0.05$) along the recovering periods to be better than that of the mercury-treated fish. It was noted that, selenium and vitamin E respectively were better than the mercury-free water in improvement of the levels of cholesterol, triglyceride, glucose, triiodothyronine (T3) and thyroxin (T4).,

DISCUSSION

Under the stress of pollutants, the responses of the fish could be through a series of biochemical, physiological, hormonal or behavioral alterations in an attempt to meet the requirements needed to cope the stress. Harabawy and Ibrahim (2014) have reported that, blood is a pathophysiological reflector of the whole body; and the hematological profile represents a good indicator to detect the physiological variations after exposure to pollutants and reflect the overall health status of fish. The results of the present work showed a significant decrease in RBCs, Hb and Ht of *C. gariepinus* after exposure to a sublethal concentration of mercury, 0.10 mg/L for 30 days. These results are agreed with those of (Ibrahim, 2011) on *C. gariepinus*, and Maheswaran, *et al*. (2008) on *Clarias batrachus*. The reduction in the RBCs count after exposure to pollutants may be attributed to the inhibition of erythropoiesis, haemosynthesis or due to an increased rate of erythrocyte destruction in the hematopoietic organs (Vani *et al*., 2011). This may indicate that, the fish of the present work, have suffered from anemic conditions and may reflect the negative impacts of mercury on O_2 carrying capacity of blood which may lead to inability of the fish to maintain the gas transfer. (Kori-Siakpere *et al*., 2011) stated that the reduction of blood parameters after exposure to pollutants leads to anemia and reduce the physical activities of the fish due to insufficient amounts of the oxygen provided to the tissues. Many authors referred to the role of heavy metals in reducing haemoglobin and haematocrit values

(Muttappa *et al*. 2015). Such decrease in the haemoglobin and haematocrit values may be attributed to several reasons including the internal bleeding and haemolysis from the damaged tissues in the vital organs such as kidney, gills and liver as a result of pollutant bioaccumulation; elimination of RBCs as a result of extravasations of blood; disturbance of the osmotic pressure inside and outside the blood cells because of gain of water in the extracellular fluid with a later increase in size (Ibrahim 2011, Muttappa *et al*. 2015).

Hg-induced variations towards increase of MCV and decrease of MCH and MCHC values were recorded in the present work. Similar findings were recorded by Omitoyin (2006) and Shalaby (2007) due to exposure of *C. gariepinus* and *Oreochromis niloticus* to cadmium. Many authors have recorded significant variations (increase or decrease) in MCV, MCH and MCHC under the influence of heavy metals and pesticide stress in different fish species (Köprücü, *et al*., 2006; Adeyemo, 2007; Harabawy and Ibrahim, 2014; Shokr, 2015). (Saravanan *et al*. 2011) revealed that, significant decrease in MCH may be due to high percentage of immature RBC; and the decrease in MCHC in treated fish could be due to swelling of RBC (Milligan and Wood 1982).

White blood cells play a major role in the defense mechanism of the fish. In the present work, WBCs count increased significantly in comparison with the control group. The increase in WBCs may be attributed to a stimulation of the immune system in response to tissue damage caused by mercury chloride. Similar results were observed in fish exposed to mercury by (Ibrahim, 2011) in *C. gariepinus*; (Shah and Altindağ, 2005) in *Tinca tinca*; Maheswaran *et al*. (2008) in *Clarias batrachu*; and (Ribeiro *et al*. 2006) in *Hoplias malabaricus* exposed to subchronic and dietary doses of methyl mercury. The results of the present study showed significant increases in percentages of lymphocytes and monocytes and decrease in neutrophils. (Ibrahim, 2011) reported that, the stimulation of the immune system causes an elevation in lymphocytes by injury or tissue damage; and the increase of lymphocytes number may be attributed to the response of lymphoid tissues to the destruction of circulating lymphocytes (Shah and Altindağ, 2005). (Kumar *et al*., 2004), have found that exposure to mercuric chloride induced changes in the differential leucocytes count and caused lymphocytosis, neutrophillia, monocytosis, eosinophilia and thrombocytopenia in *Anabas testudineus*.

The presence of pollutants in aquatic environment exerts its impacts at cellular or molecular levels which leads to significant alterations in biochemical compositions of the organisms (Vaseem and Banerjee, 2012). Biochemical parameters such as urea, creatinine, Na and K are biomarkers for kidney function, and represent the best indicators of stress impacts caused by mercury (Mahmoud *et al*. 2012). In the present work, the treatment with mercury caused a significant decrease in potassium (K) and significant increases in mean values of Na, Urea and Creatinine. These negative impacts on the kidney functions parameters (increases or decreases) due to exposure to various pollutants are recorded in different fish species and could reflect the kidney dysfunction (Hadi *et al*., 2009; Zaki *et al*., 2010; Mahmoud *et al*., 2012; Harabawy and Ibrahim, 2014). Hadi *et al*. (2009) showed that, elevation of creatinine level may be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrates metabolism.

In the present work, the liver-functions parameters, ALP, ALT and AST are increased significantly due to mercury exposure. ALT, AST and ALP are metabolic enzymes participate in amino acid metabolism (Bhattacharya *et al*., 2008). Mahmoud *et al*. (2012) have reported that, the increase of ALT and AST levels has been proven

to reflect liver damage, while increase in the ALP level may be indicator for renal and liver damage; and the alterations in enzymes activities in the serum directly indicates major pathologic changes in permeability of cell membrane or hepatic cell rupture. Also, the increase in the activity of ALP and AST in blood might be attributed to the necrosis of liver and kidney as reported by Ochmanski and Barabasz (2000) and Zaki *et al*. (2013).

One of the most problematic impacts in the body is the reaction of the pollutants with the cell nucleoproteins and nucleic acids and consequently they affect protein synthesis and the overall integrity of the cell as reported by (Sharf-Eldeen and Abdel-Hamid, 2002). Harabawy and Ibrahim (2014) have reported that, blood proteins are important indicators to monitoring the negative impacts of pollutants on fish as well as physiological homeostasis; and considered a good tool to evaluate the physiological, biochemical and ecological status of fish; they play a key role in transportation of different metabolites and exogenous chemicals, protect the organism against infections, parasites and xenobiotics. Determination of total protein contents or albumin/globulin ratio are very important to assess the liver functions and should be estimated because the increase, decrease or normal albumin/globulin ratio helps in evaluation of health status of the liver (Mahmoud *et al*., 2012). In the present work, significant increases in total protein and globulin were recorded in *C. gariepinus* exposed to sublethal concentration of mercury chloride. Many authors have obtained similar results, and revealed that heavy metals and pesticides have impacts on total protein and globulin levels (Hasheesh *et al*., 2000; Abbas and Mahamoud, 2002; El-Fayoumi and Abd-Allah, 2003; Mahmoud *et al*., 2012). The increased levels of blood proteins and globulins after exposure to mercury may be attributed to high rates of protein synthesis making the fish able to use the produced proteins as a source of energy to meet the increased energy demand to detoxify the pollutants. These increases in total protein and globulin reflect malabsorption, liver dysfunction and sever kidney damage due to mercury doses (Abu *et al*., 2009; Zaki *et al*., 2010; Mahmoud *et al*., 2012). The results obtained in the present study showed decline in the albumin level and frequently albumin-globulin ratio in mercury-exposed *C. gariepinus* suggesting that mercury may have caused renal disease and liver insufficiency. Similar findings were recorded by many authors in different fish species exposed to various toxic agents (Alam *et al*., 2014, Mahmoud *et al*., 2012). The decline in albumin level can reflect that the pollutants could have caused malnutrition, renal disease, liver inefficiency and may induce harmful impacts on immune system (Alam *et al*., 2014). Therefore, the depletion of albumin levels may reflect the disturbance in the colloidal osmotic pressure of the blood and transportation mechanisms of fatty acids and hormones; reduction in blood viscosity; inflammation and hepatic and renal damage; and these disturbances may be attributed to liver necrosis due to pollutants that leads to leakage from liver into the blood (Mahmoud *et al*., 2012; Sharf-Eldeen and Abdel-Hamid, 2002). Alam *et al*. (2014) have reported that, many animals can mobilize proteins as an energy source during the oxidation process of amino acids; and the decrease in albumin levels probably due to tissue repair, increased energy cost of homeostasis, impaired food intake and detoxification mechanisms during stress.

Glucose is an important fast source of energy for organisms and is used as an indicator for stress resulting from pollution and the physical factors (Manush *et al*., 2005; Authman *et al*., 2013). In the present study, the pattern of glucose metabolism of *C. gariepinus* showed a decreasing trend in its level under the stress of mercury. Similar conclusions were recorded by Zaki *et al*. (2009) and Mahmoud *et al*. (2012)

who working on different fish species and using different pollutants of heavy metals including mercury and pesticides. The blood glucose level could be affected by the rate of carbohydrates metabolism under hypoxia and stress conditions (Mahmoud *et al*., 2012); and the hypoglycemia may be attributed to the rapid utilization of glucose during stress stimuli.

Results of the present work revealed that, cholesterol increased significantly in blood of *C. gariepinus* after exposure to mercury. Similar results were recorded in the serum of *Channa punctatus* (Kaur and Kaur, 2006) and *Cirrhina mrigala* (Kumar *et al*., 2005), *Labeo rohita* (Vaseem and Banerjee, 2012) due to exposure to other pollutants. The increase of cholesterol level may be attributed to dysfunction of liver causing release of additional amounts of cholesterol into the blood (Vaseem and Banerjee, 2012). The elevation of cholesterol levels due to pollutant stress leads to weakness in the body and swimming ability of the fish (Osman and Harabawy, 2010).

Data of the present work revealed that, mercury treatments led to a significant decrease in triglycerides in *C. gariepinus* in comparison with the control group. Similar results are recorded in *C. gariepinus*, under mercury toxicity (Ibrahim, 2011); also, increased and decreased levels in triglycerides levels are recorded by Mekkawy *et al*. (1996) under the stress of the herbicide atrazine in *Oreochromis niloticus and Chrysichthyes auratus* respectively. These alterations in triglycerides levels could reflect the disturbance in energy storage mechanisms under the stress of different pollutants including heavy metals and herbicides; reflect the chronic renal failure and damage of pancreatic cells; also, the decreased levels of triglycerides may reflect the impaired absorption of nutrients in the intestine of some fishes and sever liver damages. Kori-Siakpere *et al*. (2011) have stated that, under stress the fish mobilizes triglycerides to meet the increased demand for energy to cope with toxic conditions and to meet energy needed to sustain the physical activity, bio-transformation and excretion of xenobiotic.

Some xenobiotics have structural similarity to the thyroid hormones (T4 and T3), they behave as endocrine disruptors and can interfere with the thyroid axis (Hedayati and Arsham, 2012). The action of these disruptors is based on its ability to bind to transporter proteins such as transthyretin that transport TH in the blood (Morgado *et al*., 2007). In the present study, *C. gariepinus*, under mercury toxicity, exhibited a significant reduction in thyroid hormones (T3 and T4) when compared with the control group. Similar results are recorded in *C. gariepinus*, under mercury toxicity (Ibrahim 2011); in *Sarotherodon mossambicus* after exposure to dimecron pesticide (Thangavel *et al*., 2005); and in *Clarias gariepinus* exposed to carbofuran pesticide (Ibrahim and Harabawy, 2014). Ibrahim (2011) has mentioned that, mercury is able to block thyroid hormone production, causing hypothyroidism, through occupying the iodine binding sites; as well, mercury causes autoimmune thyroiditis and able to impair the conversion of T4 to the active form T3; also it may disrupt the hypothalamic-pituitary-thyroid (HPT) axis.

The present study revealed that, after exposure to mercury (0.10 mg/L), the recovery with water led to complete recovery only in MCHC. While, all the remaining of hematological parameters studied, cholesterol and glucose levels are improved to be relatively better than those of the mercury-treated fish. At the same time, the levels of urea, creatinine, ALP, AST, ALT, TP, albumin, globulins and Alb/glob ratio, T3 and T4 are not improved well by water throughout the recovering period. The water has no effects on Na levels; but led to increases in the levels of K and triglyceride to high levels than that of the control fish. While, the recovery with supplementation of vitamin E led to complete recovery in the levels of Hb, MCV, MCH, MCHC and Na

to reach the normal range of the control level; and significantly improved the levels of RBCs, Hct, WBCs, neutrophils, lymphocytes and monocytes, urea, creatinine, ALP, AST, globulin, the albumin globulin ratio, cholesterol, triglyceride and glucose to be relatively better than those of the mercury-treated fish. Potassium and albumin levels are fluctuated. ALT, TP decreased significantly to lower level than that of the control. But, T3 and T4 are not improved well by vitamin E throughout the recovering period. Vitamin E is found in high quantities in vegetable oils; and after absorption of vitamin E, it is stored mainly in the liver, and due to its fat-soluble nature, it is incorporated in lipid storage organelles and plasma membranes, therefore it is widely distributed throughout the body as reported by Rengaraj and Hong (2015). Harabawy and Mosleh (2014) have stated that, vitamin E is considered as a strong agent to improve the internal antioxidant system in the body to prevent the attacks of ROS, and making ROS unable to react with the vital macromolecules such as lipid, protein, carbohydrate and nucleic acid in cells preventing the cytotoxicity and genotoxicity of pollutants. Also, the later authors confirmed the powerful protective potential of the vitamin E as antioxidant helped in decreasing of genotoxicity and cytotoxicity of Cd, Cu, Pb and Zn in the erythrocytes of Nile tilapia, *Oreochromis niloticus* to become less than those recorded in metals-treated fish. Vitamin E is required to regulate the heme-biosynthesis; it plays an important role in the transportation of amino acids and possibly lipids in the intestine (Rengaraj and Hong 2015). Ibrahim (2011) has reported that, vitamin E is closely related to the immunological system performance and has antioxidant properties, favoring integrity and fluidity of membranes; controls the oxidizing reactions of fatty acids, keeps cellular respiration and helping in avoiding the cell death. As well, vitamin E as a lipid soluble antioxidant, is playing a crucial role in protection of biological membranes, lipoproteins and lipid stores against oxidation (Mekkawy *et al*., 2013; Hassaan *et al*., 2014); it plays an important role in scavenging lipid peroxyl radicals which are the chain-carrying species and propagate lipid peroxidation ((El-Demerdash *et al*., 2004); and necessary to improvement and maintenance of fish immunity, normal resistance of erythrocytes to haemolysis (Halver, 2002; Hassaan *et al*., 2014).

The results of the present work revealed that, the recovery with selenium led to complete recovery in MCH, neutrophils, lymphocytes, total proteins and globulins levels. and significantly helped improvement (increased/decreased) of the levels of RBCs, Hb, Hct, WBCs, monocytes, urea, creatinine, ALP, AST, ALT, the albumin globulin ratio, cholesterol, triglyceride, glucose, T3 and T4 to be relatively better than those of the mercury-treated fish. But, Na, and albumin are not improved well by selenium; and the potassium levels showed fluctuations through the recovery period with selenium. Also, ALT and TP decreased significantly to be less than that of the control values. Selenium is an essential element in nutrition for human and animals including fish (Pacitti *et al*., 2015). The effects of selenium are mediated mainly through incorporation into several Se-containing proteins (selenoproteins), which exert important biological functions within the organism (Papp *et al*., 2007; Pacitti *et al*., 2015). Mariotti *et al*. (2012) have reported that, selenoproteins have been involved in cancer prevention, modulation of the aging process, male reproduction, and immune response. Selenium may be taken to help prevent the toxic impacts of cadmium, mercury, or arsenic or to correct their toxicity (Karahan *et al*., 2005). The detoxification potential of selenium on mercury toxicity seems to be due to a formation of a biologically inactive complex (Ibrahim, 2011) which will become unable to pass through biological barriers and is stored in the liver and the spleen (Hansen *et al*., 1981). The obtained results showed that, T3 and T4 are better

improved by selenium than vitamin E than the water respectively. This may be attributed to the fact that, selenium is an essential component of the iodothyronine 5'-deiodinase enzymes which are responsible for the deiodination of L-thyroxine (T4), converting it to its more active form, 3, 5, 3'-triiodo-Lthyronine (T3); also, Se is an important component (a cofactor) of glutathione peroxidase, the main enzyme responsible for protecting thyroid cells against oxidative damage as reported by (Ibrahim, 2011). Regarding human, Benstoem *et al*. (2015) have reported that, balanced selenium levels are required for many biological functions in the human body including thyroid hormone metabolism, the antioxidant defense systems of the body, the adaptive and acquired immune system and prevention of certain cancers. Also, selenium supplementation can affect and control the migration, adherence and phagocytosis of leucocytes (Ahrens *et al*., 2008).

CONCLUSION

The exposure of the African catfish, *C. gariepinus* to mercury (0.10 mg/L for 30 days) led to a significant decrease ($p<0.05$) in RBCs, Hb, Ht, MCH, MCHC, MCV, WBCs, lymphocytes, monocytes, neutrophils, K, albumin, Alb/glob ratio, triglyceride, glucose and thyroid hormones (T3 and T4) when compared with the control group. Also, exposure to mercury caused a significant increase ($p<0.05$) in levels of Na, urea, creatinine, ALP, AST, ALT, total proteins and globulins levels than those of the control fish. The present study revealed that, after exposure to mercury (0.10 mg/L), the ability of vitamin E and selenium to improve and recover the hematological and biochemical parameters and thyroid hormones (T3 and T4) studied were better than water alone. Also, the improvements were mostly better after 30 days than 15 days of recovering period.

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ARABIC SUMMARY

قدرة فيتامين هـ ،السيلينيوم والمياه على تحسين واستعادة القياسات الدموية، البيوكيميائية والهرمونية لسمك القرموط كلاريس جاريبينس المعرضة للزئبق.

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تهدف هذه الدراسة لتقدير التأثيرات السامه لجرعة تحت مميتة من كلوريد الزئبق (٠.١٠ مجم/ل لمدة ٣٠ يوما) على القياسات الدموية والبيوكيميائية وهرمونات الغدة الدرقية فى سمكة القرموط وكذلك معرفة مدى قدرة فيتامين هـ والسيلينيوم والماء (كل على حده) على تحسين واعادة مستوى هذه القياسات تحت الدراسة خلال فترة الاسترجاع (لمدة ١٥ و ٣٠ يوما بعد التعرض للزئبق). وقد اظهرت النتائج أن تعرض سمكة القرموط للزئبق لمدة ٣٠ يوما أدى الى نقص معنوى ($p<0.05$) فى كلا من عدد كرات الدم الحمراء،الهيموجلوبين، الهيماتوكريت، متوسط تركيز الهيموجلوبين، متوسط تركيز الهيموجلوبين لكل كرة دم حمراء، متوسط حجم كرات الدم الحمراء، وخلايا الدم البيضاء، الخلايا اللمفية، الخلايا الأحادية، الخلايا المتعادلة، البوتاسيوم، الألبيومين، نسبة الألبيومين/الجلوبيولين، الدهون الثلاثية، الجلوكوز، وهرمونات الغدة الدرقية (T4 , T3) وذلك بالمقارنة بالمجموعة الضابطة. كما أظهرت النتائج أن التعرض للزئبق أدى إلى زيادة معنوية ($p<0.05$) فى مستوى كلا من الصوديوم، اليوريا، الكرياتينين، ALP, AST, ALT ، البروتين الكلى، الجلوبيولين ليصل الى مستويات أعلى من مستوى المجموعة الضابطة. أما بالنسبة إلى قدرة فيتامين هـ والسيلينيوم والماء (كل على حده) على تحسين واعادة مستوى هذه القياسات تحت الدراسة خلال فترة الاسترجاع لمدة ١٥ و ٣٠ يوما بعد التعرض للزئبق فإن النتائج تشير الى أن فيتامين هـ والسيلينيوم ساعدا على تحسين مستوى هذه القياسات بشكل أفضل من استخدام الماء وحده. كما أوضحت النتائج أن معدلات التحسن فى هذه القياسات كان أفضل بعد ٣٠ يوما عما كان عليه بعد ١٥ يوما من فترة الاسترجاع. حيث وجد أن فترة تعافى الأسماك فى الماء أعاد متوسط تركيز الهيموجلوبين لكل كرة دم حمراء (MCHC) فقط الى مستوى المجموعة الضابطة وتحسنت باقى قياسات الدم نسبيا بحيث أصبحت أفضل من مستوى الأسماك المعالجة بالزئبق. ولم تتحسن بشكل جيد مستويات كلا من اليوريا، والكرياتينين، ALP، AST، ALT، TP، الألبيومين، نسبة الألبيومين/الجلوبيولين، T3 و T4 بالماء. أما مستويات الصوديوم فلم تتأثر بالماء. وزادت مستويات البوتاسيوم والدهون الثلاثية إلى مستويات أعلى من المجموعة الضابطة. فى حين أن اضافة فيتامين هـ فى فترة الأسترجاع أدى الى اعادة كاملة فى مستويات كلا من الهيموجلوبين ، MCV، MCH، MCHC والصوديوم ليصل إلى مستوى المجموعة الضابطة، كما أن فيتامين هـ أدى الى تحسن معنوى ($p<0.05$) فى كرات الدم الحمراء، الهيماتوكريت، الكريات البيضاء، الخلايا المتعادلة، الخلايا اللمفية، الخلايا الأحادية، واليوريا، والكرياتينين، ALP، AST، نسبة الألبيومين/الجلوبيولين ، والكولستيرول، الدهون الثلاثية والسكر لتصبح فى مستويات أفضل من تلك المسجلة فى الأسماك المعالجة بالزئبق. وتذبذبت مستويات البوتاسيوم والألبيومين. في حين انخفضت مستويات الـ ALT و البروتين الكلى بشكل كبير. ولم تتحسن مستويات الـ T3 و T4 بشكل جيد فى وجود فيتامين هـ. وقد أدى استخدام السيلينيوم فى فترة النقاهه الى الاسترجاع الكامل لمتوسط تركيز الهيموجلوبين، الخلايا المتعادلة، الخلايا اللمفاوية ،البروتين الكلي ومستويات الجلوبيولين . وقد ساعد بشكل ملحوظ فى تحسين (زيادة/نقص) كلا من كرات الدم الحمراء ، الهيموجلوبين ، الهيماتوكريت ، الكريات البيضاء ، الخلايا الأحادية ، اليوريا، الكرياتينين ، ALP، AST، ALT ، نسبة الالبيومين/ الجلوبيولين، الكولستيرول، الدهون الثلاثية، الجلوكوز، T3 و T4 وأصبحت أفضل من تلك المعالجة بالزئبق . ولكن الصوديوم و الألبومين لم يتحسنا بشكل مقبول عند استخدام السيلينيوم. فى حين أظهرت مستويات البوتاسيوم تقلبات. وحدث انخفاضاً ملحوظاً فى ALT و البروتين الكلى ليصبح أقل من قيم المجموعه الضابطة. أيضا لوحظ أن الاسترجاع لقيم T3 و T4 كان الافضل فى وجود السيلينيوم يليه فيتامين هـ ثم المياه.