EFFICIENCY OF VITAMIN E WITH SODIUM SELENITE IN REDUCING TOXICITY AND ACCUMULATION OF LEAD ACETATE IN COMMON CARP, *Cyprinus carpio* L.

S. A. Mustafa A. M. Al-Khalili

ABSTRACT

This study was, carried out to assess the efficiency of vitamin E and sodium selenite in reducing toxicity and accumulation of lead acetate in *Cyprinus carpio* L. For this purpose, 120 common carp with an average weight 65.50±0.25 g were randomly divided into 6 groups in duplicate (2 tank treatment^{-1}). Each of the six treatment groups was fed the formulated diets continuously for 10 weeks at a daily rate 3% of body weight throughout the experiment as the following: Treatment 1 (T1) serves as control group without additives; Treatment 2 (T2) were fed diet mixed with vitamin E (VE) 6 IU plus Sodium Selenite (Se) 240µg kg^{-1} d.w; Treatment 3 (T3) were fed diet mixed with Lead Acetate (Pb) (20 mg kg^{-1} d.w); Treatment 4 (T4) were fed diet mixed with Pb (30 mg kg^{-1} d.w); Treatment 5 (T5) were fed diet mixed with Pb (20 mg kg^{-1} d.w) and VE 6 IU plus Se 240µg kg^{-1} d.w; Treatment 6 (T6) were fed diet mixed with Pb (30 mg kg^{-1} d.w) with VE 6 IU plus Se 240µg kg^{-1} d.w. At the end of the feeding trial (i.e. 10 weeks) several endpoints at various levels of biological responses were assessed such as DNA damage using COMET assay, lipid peroxidation by TBARS assay, hematological indices (viz Hb content, PCV value, RBC and WBC count) and Pb accumulation in different organs. The results indicated that the highest level of DNA damage ($p<0.05$) was observed in T4 compared to T3. Also, there were significant differences among Pb treatment groups T3 and T4 and Pb with VE+Se T5 and T6. For lipid peroxidation the highest level of TBARS was observed in T3 and T4. In contrast, the concentrations of TBARS were significantly decreased ($p<0.05$) in Pb with VE plus Se groups T5 and T6 compared to T3 and T4. The count of WBC's showed significant increases in T3, T5, T6 compared to T1 and T2. While, RBC's count were significantly decreased ($p<0.05$) in Pb treatment groups compared to T1 and T2. The Pb accumulation in organs was in the following order: kidneys> liver> muscles. in conclusion, the results indicate that vitamin E and Se reduced DNA damage and protect against lead toxicity which could be useful in aquaculture application.

INTRODUCTION

Toxic pollutants, including the heavy metals (HMs) are natural components of the aquatic environment which could build up in the food chain and are responsible for negative effects on fish (9). These metals could be accumulated in aquatic organisms that occupy the highest levels in the food chain and become toxic when consumed by human beings (3, 6). Dallinger *et al.* (4) documented that the transfer of HMs via food chains could be considerable to reach high levels in fish organs and the dietary accumulation of heavy metal could be dominate the aqueous route. Therefore, it will become a source of concern not only as being threat to aquatic ecosystem, especially fish, but also due to the public health effects of such contaminants (22).

Part of M.Sc. thesis for the second author

*** College of Veter. Medicine, Baghdad Univ., Baghdad, Iraq.**

**** Veter. Direct., Ministry of Agric., Baghdad, Iraq.**

Lead (Pb) is one of the most dangerous pollutants in the environment which accumulates inside the body due to its low rate of elimination . Toxicity related to Pb may be resulted due to tissue oxidative damage. Previous data demonstrated that the antioxidants could play an essential role in decreasing some hazards of HMs (7, 8). Vitamins C, E, A, beta-carotene, are examples of antioxidants (32). Vitamin E (VE) is a fat soluble vitamin that has 8 forms. Each form has its own biological action, which is the measure of potency or functional use in the body (37). Also, VE has been shown to play an important role in immune function, DNA repair, protect membranous lipids from oxidative damage and other metabolic process (12). Furthermore, VE and selenium (Se) are antioxidants, which are considered as a defense mechanism for the body (11). Selenium is essential constituent of glutathione peroxidase that decreases potentially harmful oxygen radicals such as hydrogen peroxides and lipid hydroperoxides (16, 28). A biochemical role was recently established for Se as an element of an enzyme, GSH-Px, which function along with VE in the cells to protect against the peroxidation (38). Many researches were established to detect the role of antioxidants against toxicity of HMs exposure (7, 14, 17). However, there are few studies evaluating the impact of dietary HMs at various level of biological organization [DNA damage, lipid peroxidation (i.e. oxidative stress) hematological parameters and accumulation] (19, 20). Most of the recent studies have only been carried out for short-term aqueous exposure to HMs with a limited number of parameters. Therefore, the current study elucidated to assess the efficiency of VE and Se in reducing DNA damage (using the COMET assay), reducing lipid peroxidation (using TBARS assay) and in reducing the accumulation of Pb in specific tissues. Also, to investigate the effect of dietary Pb with and without Vitamin E+Se on hematological parameters in *C. carpio*.

MATERIALS AND METHODS

DIET PREPARATION

A ten week feeding experiment were started using a commercial diet (GFC3- made in Iran), which composed of (32%) protein, (8%) fat, (6%) fibers, (10%) ash, (1%) phosphorous and (10%) moisture. Supplemented diets were used in this experiment with some alteration. Pb with VE plus Se supplemented diets were prepared using the same formulation of control diet except that mass of lead acetate [Pb(CH3COO)2] Pb were supplemented with 20 and 30 mg kg-1 dry weight and vitamin E (VE) 6 IU plus Sodium Selenite (Se) 240µg kg^{-1} d.w. A twelve kg of the pellets was grounded by grain grinder to obtain a powder, that powder divided into 6 aliquots, each one weighted 2 kg. The concentrations of Pb were determined on the basis of previous study, carried out by El-Shebly (7). The lead acetate was mixed properly with other feed ingredients and 300 ml DW. The resulting paste was extruded via a food mixer and dried at 40°C for 96 h. Then the diets were stored in a six separated dry plastic boxes at –20 °C.

EXPERIMENTAL DESIGN

A total of 150 healthy *C. carpio* fish with an average weight of 65.50±0.25 g, and length (17.4 ± 2.5 cm) were obtained from a commercial fish farm in Babil, Iraq. Fish were acclimated to the laboratory conditions for two weeks prior to the experiment. After the acclimation, 120 fish were randomly transferred to 12 tanks filled with chlorine free tap water (120 l^{-1}), each tank has contained 10 fishes, then the fish were divided to the tanks into 6 groups in duplicate (2 tank/treatment). Each of the six on groups was fed the formulated

diets continuously for 10 weeks at a daily rate of 3% body weight throughout the experiment as the following: Treatment 1 (T1) serves as control group without additives; Treatment 2 (T2) were fed diet mixed with vitamin E (VE) 6 IU plus Sodium Selenite (Se) 240µg kg^{-1} d.w; Treatment 3 (T3) were fed diet mixed with Lead Acetate (Pb) (20 mg kg^{-1} d.w); Treatment 4 (T4) were fed diet mixed with Pb (30 mg kg^{-1} d.w); Treatment 5 (T5) were fed diet mixed with Pb (20 mg kg^{-1} d.w) and VE 6 IU plus Se 240µg kg^{-1} d.w; Treatment 6 (T6) were fed diet mixed with Pb (30 mg kg^{-1} d.w) and VE 6 IU plus Se 240µg kg^{-1} d.w. The experimental fish were reared under a (12 h) light/dark cycle. Water of the tanks was changed every two days. The chemo-physical parameters of the water were measured during the experimental period as follows: [Temperature 23.53(ºC), DO (mg l^{-1}) 5.90, pH 7.30, Ammonia (mg l^{-1}) 0.002 and Hardness (mg l^{-1}) 210].

BIOLOGICAL SAMPLING

After ten weeks of feeding trail, 3 fish from each tank (n=6) were randomly selected for biological sampling. Blood samples were collected from the caudal vein. Blood samples were transferred immediately to two sets of test tubes containing ethylene diamine tetraacetic acid (EDTA) for determination of DNA damage using COMET assay and also for studying hematological parameters. After that, fish were dissected out, major organs were removed and weighed for Pb analysis and liver samples also were collected for measuring the lipid peroxidation using the 2-thiobarbituric acid reactive substances assay (TBARS assay).

DETERMINATION OF DNA DAMAGE USING COMET ASSAY

DNA damage was determined using the COMET assay according to the method described by Tice *et al.* (36) and modified by Mustafa, (19). Briefly, erythrocytes were pelleted in a micro-centrifuge tubes and suspended in 180 µL of low melting point Agarose (0.5% in PBS). The cell suspension of 170 µL was placed on the frosted ends of glass slides covered with Agarose (1% in PBS), covered with a cover slip and placed on ice for 10 min. After that, the slides immersed in a lysing solution (4°C for 1 h). Then, the slides were left to unwind (15 min) at 4°C in freshly prepared alkaline electrophoresis buffer pH (12.3)]. Electrophoresis was performed at 25 V, 300 mA at 4°C in the dark for 20 min. Then, neutralization [Tris-HCl, pH (7.4), for (5 min.)]. Finally, to visualize COMET, (40 µl) of (0.2%) ethidium bromide stain was applied to each gel. Scoring was performed using fluorescence microscope (Leica DMR) using Komet (5.0 image analysis software) (Kinetic Imaging, Ltd., Liverpool, UK). Tail DNA (%) was chosen as a measure of single strand DNA breaks (18).

DETERMINATION OF LIPID PEROXIDATION USING TBARS ASSAY

Lipid peroxidation was determined using thiobarbituric acid-reactive substances (TBARS) assay according to the technique described by Casalino *et al.* (3). This assay measures various malonic dialdehyde (MDA) formed during acid hydrolysis of the lipid peroxide compounds.

HEMATOLOGICAL PARAMETERS

Blood was sampled from six fish per tank. Samples were taken from the caudal vein using a 25 gauge needle and 1-ml syringe. The hemoglobin determined by the cyanmethanemoglobin method. Hematocrit [(measured and read as % packed cell volume (PCV%)]. The Neubuers improved microscopic counter was used in counting the erythrocytes (RBC) and leucocytes (WBC)

counts after the blood was diluted with Dacies fluid. The hematological indices were calculated according to standard procedures as described by Rawling *et al.* (26).

LEAD ANALYSIS

Flame atomic absorption spectrophotometer (FAAS) was used to determine the concentration of Pb in tissues according to Szkoda and Żmudzki (35). Tissue samples (muscles, kidney and liver) were dried at 75 ºC for 24h, and then cooled to room temperature. Then Each piece of dried tissue (0.1g) was placed in 120 ml polyethylene screw-top tube and 1 ml of concentrated HNO_3 was added to each tube for digestion. Samples were digested at 70 °C for 2 h in water bath. After digestion was completed (the brown fumes stopped evolving), the tubes were allowed to cool, then diluted with 4 ml of distilled water. Lead concentration of tissues was expressed as µg/g dry weight according to the following equation: .

$$\text{Pb concentration (µg/g)} = \frac{\textit{sample volume } (ml) \textit{ x digest conc. } (mg/l)}{\textit{Sample weight } (g)}$$

STATISTICAL ANALYSIS

Statistical analysis was achieved using Sigma Plot v11.0 software. One way analysis of variance (ANOVA) was used to determine the significant differences among the variables. A probability level equal or less than $P < 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

DETERMINATION OF DNA DAMAGE USING COMET ASSAY

Dietary Pb exposure of *C. carpio* for 10 weeks was examined to exert a damage effect on the erythrocytes DNA (measured by the length of a DNA tail %) (Fig. 1). DNA damage was relatively low in control (T1) and in VE plus Se groups. All Pb treated groups (T3, T4, T5 and T6) were exhibited significant increases ($p<0.05$) in average tail length values over control and VE plus Se groups. As the concentration of Pb increased the level of DNA damage increased significantly ($p<0.05$). The mean score of DNA damage ranged between 35 to 44% in Pb treated fish. The highest level of DNA damage was observed in T4, which was significantly increased ($p<0.05$) compared to T3 (Fig. 2). Also, there was a significant difference among Pb groups T3 and T4, and Pb plus VE and Se T5 and T6. These results indicated that COMET assay could act as bio-indicator for measuring the genotoxicity in aquatic organism.

The high level of DNA damage in Pb treated groups can be explained on the basis of the principle pathway of DNA damage caused by HMs ions, which participated to genotoxicity. This pathway is via the generation of HMs large number of free radicals that assault DNA double chains and caused their damaged. If the damaged DNA strands cannot be repaired auspicious, it will affect the capacity of DNA and result in genotoxicity. The decreased damage in DNA of Pb plus VE and Se groups compared to Pb groups at both concentrations indicated that VE could act as antioxidant, which play an essential role in protecting the cells against the impacts of the generated free radicals, which are possibly damaging by products of energy metabolism, or from environmental exposure like radiation (10). Similar findings recorded previously by Shi *et al.* (31).

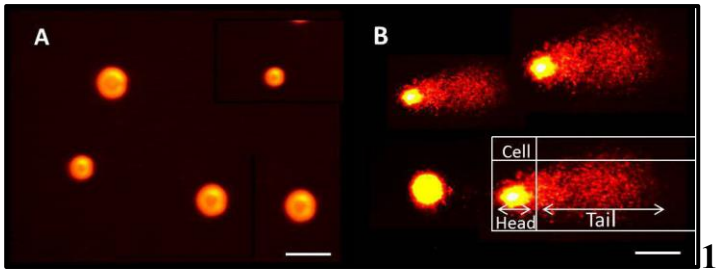

Fig. 1: Representative COMET images of erythrocytes of *C. carpio* following dietary exposure to Pb and VE plus Se. (A) Control cells; (B) Damaged cells. Cells are stained with Ethidium Bromide and examined under fluorescence microscopy. Total magnification= 400x; Scale bars= 50 µm.

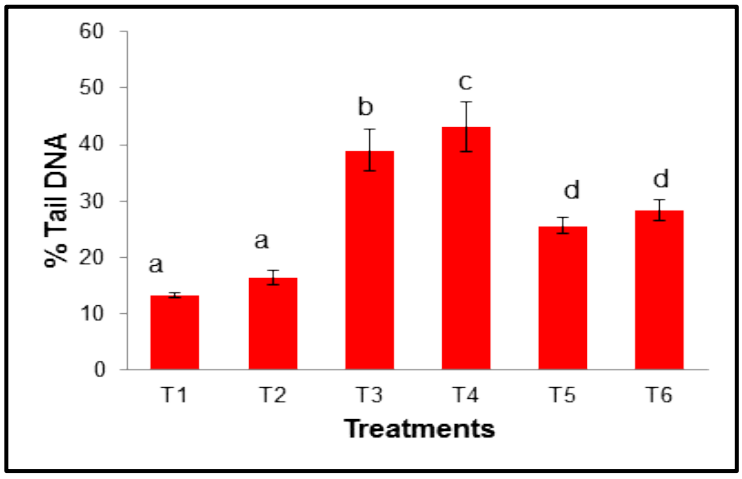

Fig. 2: Induction of DNA damage (represented as a percentage of tail DNA) in *C. carpio* erythrocytes after 10 weeks exposure to dietary Pb and VE plus Se. Values are mean ± SE; Different alphabetic letters indicated significantly different at *P*<0.05; n=6.

DETERMINATION OF LIPID PEROXIDATION USING TBARS ASSAY

The Pb treated groups revealed significantly higher values for TBARS levels, approximately 5 fold increased as compared to control and VE plus Se groups. In contrast, the concentration of TBARS decreased in Pb with VE plus Se groups (T5 and T6). However, there were no significant differences between Pb treated groups (i.e. T3 and T4), also, there was no significant difference between Pb with VE plus Se groups (T5 and T6; p>0.05) (Fig.3).

This apparent elevation in lipid peroxidation in liver Pb treated groups, could be due to the accumulation of HMs in the organs (as current data showed a significant increases of dietary Pb concentration in several organs) and thus could lead to high levels of oxygen free radicals in fish liver and as a result of lipid peroxidation (3). Previous studies were demonstrated the pathway of lipid peroxidation and genotoxicity; HMs exposure causes an increase in the generation of oxygen free radicals, or Reactive oxygen species (ROS) via Fenton reaction (2, 34). This can result in widespread damage to cells because of lipid peroxidation and genotoxicity. The results of the present study were in line with the results obtained by Olaifa *et al*. (23), who found that Cd, Zn, As, Pb and Cu were accumulated highly in the gill, kidneys, liver and heart of (*Clarias gariepinus*) from the (Ogun River), which led to the generation of lipid

peroxidation and modification in the antioxidant defense system in the organs of the fish. The protective effect of vitamin E was suggested to be due to its ability to support detoxification and scavenge tissue damaging free radicals (29). Seung *et al.* (30) documented that "Vitamin E seems to provide defense capability against oxidative damage triggered by Pb toxicity".

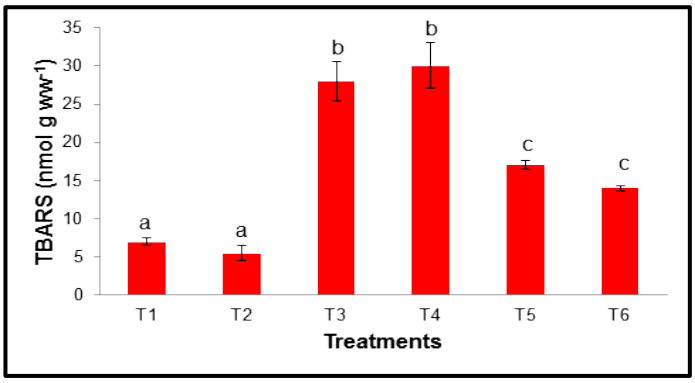

Fig. 3: Effects of dietary exposure to Pb alone and with VE plus Se on levels of TBARS in the liver of *C. carpio.* Values are mean ± SE; Different alphabetic letters indicated significantly different at (p<0.05); n=6.

HAEMATOLOGICAL PARAMETERS

The results of hematological parameters are illustrated in Table 1. WBCs count in T3, T5 and T6 showed significant increases (p<0.05) compared to T1, T2 and T4. The increase was more pronounced in group (T6) which was significantly different (p<0.001) compared to other groups (T1, T2, T3, T4 and T5). Also, T3 was significantly increased compared to T4. Regarding RBC cell counts, a significant increase (p<0.05) in this parameter has been shown among T1 (control group) compared to all other treatment groups respectively (T2, T3, T4, T5 and T6).

The current study showed that, there was a general increase (p<0.05) in PCV in all Pb treatment groups (T3, T4, T5 and T6) compare to control groups (T1) and (T2). Also, there were a statistically differences (p<0.05) among Pb groups (T3, T4) and Pb and vitamin plus Se at both concentrations (T5, T6). Hb concentration was increased in Pb and vitamin plus Se groups (T5, T6) only, but was not affected in the only Pb treated groups (T3, T4). There was a significant increase (p<0.05) in T5 and T6 groups compared with other treatment groups.

Haematological variables within the fish are appropriate parameters for assessing the prospective risk of contaminants (27). The current findings had shown an increase in total WBC count in Pb exposed groups and other groups. The increase in WBCs count could associated with stimulating the immune response and with an increase in antibody production (15).

regarding RBC counts there is generally a decrease in RBC counts in Pb treated groups, whether they were supplemented by vitamin plus Se or not, could be a result of severe anaemic state or haemolysing power of HMs, particularly on the red cell membrane and also to the destruction of mature erythrocytes with inhibition of erythrocytes production (13). These results are in agreement with Drastichova *et al.* (5), who reported a decrease in RBC count in *C. carpio* following exposure to Cd. While PCV% in this study was shown to be increased in all treated groups when compared to the control groups, by linked to the observed reduction in the total RBC count, this increase in PCV% might

be due to the total increase caused by microcytic hypochromic anemia. A study is disagreement with our findings. Adeyemo (1), reported decreased RBC count and haematocrit values in *C. gariepinus* exposed to Pb nitrate. In regard to Hb concentrations, our results showed no significant changes in Pb treated group with the control groups, but there was a significant increase in Hb concentrations in all VE plus Se treated groups with and without Pb. Although other studies showed a decline in Hb concentration in HMs treated groups (1 and 39). Pamila *et al*. (24) suggested that this may be due to the inhibitory effect of toxic substance on the enzyme system responsible for Hb synthesis.

Table 1: Haematological parameters of *C. carpio* following 10 weeks dietary trial containing Pb either alone or Pb with vitamin plus Sodium Selenite.

Treatment Groups	WBC cell $10^3 \mu l^{-1}$	RBC cell $10^6 \mu l^{-1}$	PCV%	Hb g dl^{-1}
T1	22.73 ± 1.31^c	1.13 ± 0.06^a	27.70 ± 1.18^c	8.90 ± 0.4^b
T2	24.12 ± 0.47^c	1.09 ± 0.03^a	27.00 ± 1.58^c	8.60 ± 0.52^b
T3	28.75 ± 1.96^b	1.07 ± 0.08^b	31.70 ± 0.85^b	10.20 ± 0.29^b
T4	24.87 ± 1.66^c	0.70 ± 0.06^b	30.70 ± 0.94^b	9.20 ± 0.30^b
T5	28.00 ± 1.74^b	1.08 ± 0.05^b	34.00 ± 1.63^a	10.90 ± 0.54^a
T6	38.00 ± 0.54^a	0.95 ± 0.07^b	35.20 ± 0.47^a	11.40 ± 0.15^a

Data are mean ± SE. Groups with different alphabetic superscript vertically indicate significantly different at P<0.05.

LEAD ACCUMULATION IN INTERNAL ORGANS

The accumulation of Pb in the kidney, liver and muscle of *C. carpio* are presented in Table (2). The order of Pb accumulation was in the kidney > liver > muscles. Pb accumulation in *C. carpio* livers were significantly ($p<0.01$) increased in T3 and T4 groups respectively as compared to T1 and T2 groups. Also, Pb level increased 2 fold in T4 than T3 and there was a significant difference ($p<0.05$) between these two groups (T3 and T4). Furthermore, the Pb level in T5 and T6 were significantly decreased ($p<0.05$) compared to T3. However, no significant difference were found between T6 and T4.

Interestingly, this study showed that accumulation of Pb in muscles was in order level lower than in the liver and kidney. Pb treated groups i.e., T3, T4, T5 and T6 showed highly significant ($p<0.001$) increased compared to T1 and T2.The concentration of Pb in the kidney were approximately in order of level higher than in the liver and muscle. All Pb treated groups were significantly higher than T1 and T2. The Pb accumulation level was elevated with increasing the Pb concentration in the diet. There was a significant increase in Pb concentrations of liver and kidney between T3 and T4. It was reported that the highest concentration of bioaccumulation was observed in kidneys and liver then muscles, which was explained as a consequence of detoxification of toxic compounds by the liver, since Pb is a toxic metal, while the level found in the kidney may be as a consequence of the excretory function of the kidney, in which some toxic compounds are mobilized from the body tissues and are sent to the kidney to excrete.

Kojadinovic *et al*. (17) also supported this finding, when documented that the general principle of accumulation of metal (i.e., higher in the liver, followed by the gills, kidneys then muscles). In general, gills and liver are targets of greater level of metals accumulation when compared with other organs (6 and 8) also recorded high levels of Pb in kidney and liver compared to other organs.

Surprisingly, the results of the current study showed that the Pb concentration in muscles was in lesser amount compared to other tissues (i.e., kidney or liver). Also, Javed (14), reported the Pb accumulation in such order: kidney > liver > gill > brain > muscle. However, the accumulation was significantly higher in treatment groups relative to control and to VE plus Se groups. Many authors have studied the roles of Se in the prevention and treatment of toxic effects of Pb and providing potent evidence of the defensive role of Se in preventing the harmful effects of HMs (21 and 25). The data of the present work indicated that the intake of Vitamin E in Pb-exposed fish can prevent the accumulation of Pb in tissues.

Data obtained through this integrated study using concurrent applications of several parameters (i.e. multiple biomarkers) will offer a clear evaluation of the toxicological impact of environmental heavy metal (HM). This might have an important clinical implications for human health.

Table 2: Tissue-specific accumulation of Lead (µg Pb g-1) in *C. Carpio* following 10 weeks dietary trial containing different levels of Pb (mg kg dry wt.-1) alone or with VE plus Se

Treatment groups	Pb concentratlon ug^{-1}		
	Liver	Muscle	Kidney
T1	UDL*	UDL*	UDL*
T2	UDL*	UDL*	UDL*
T3	22.01 ± 0.22^{b}	10.68 ± 0.22^{b}	14.30 ± 1015^{b}
T4	38.98 ± 1.33^{e}	13.13 ± 1.33	50.84 ± 5.26^{e}
T5	15.21 ± 2.74^{d}	10.50 ± 2.74^{b}	15.06 ± 0.608^{b}
T6	36.44 ± 1.34^{e}	12.66 ± 1.34^{b}	40.16 ± 6.63^{e}

Data are mean + S.E. Group with different alphabetic superscripts vertically indicate significant difference at P<0.05; (n=6).

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فعالية فيتامين E مع سلينات الصوديوم في تقليل سمية وتراكم خلات الرصاص في اسماك الكارب الشائع *Cypriuns carpio* L.

سناء عبد العزيز مصطفى* علي محمود الخليلي**

الملخص

هدفت هذه الدراسة الى تقويم تأثير فيتامين E وسلينات الصوديوم في تقليل سمية والتأثير التراكمي لخلات الرصاص في اسماك الكارب الشائع. اخذت 120 سمكة من اسماك الكارب الشائع بمتوسط وزن 65.50 ± 0.25 غم/سمكة. قسمت الاسماك عشوائيا إلى ست مجاميع اختبارية بواقع مكررين/معاملة و5 سمكة/مكرر (10 سمكة للمعاملة الواحدة). وغذيت لمدة 10 أسابيع بنسبة 3% من وزن الجسم: المجموعة الاولى (T1) مجموعة السيطرة غذيت بالعليقة الاعتيادية من دون اضافات علاجية، المجموعة الثانية (T2) تمت تغذيتها بعليقة مضاف لها فيتامين E (6 وحدة دولية) + عنصر السلينيوم 240 مايكروغرام/كغم وزن علف جاف ، المجموعة الثالثة (T3) غذيت بعليقة مضاف اليها الرصاص بجرعة 20 ملغ/كغم وزن علف جاف ، المجموعة الرابعة (T4) غذيت بعليقة مضاف لها الرصاص بجرعة 30 ملغ/كغم وزن علف جاف ، المجموعة الخامسة (T5) غذيت بعليقة مضاف اليها فيتامين E (6 وحدة دولية)+عنصر السلينيوم 240 مايكروغرام/كغم وزن علف جاف مخلوطة مع الرصاص بجرعة 20 ملغ/كغم وزن جاف ؛ المجموعة السادسة (T6) غذيت بعليقة مضاف اليها فيتامين E (6 وحدة دولية)+ عنصر السلينيوم 240 مايكروغرام/كغم وزن علف جاف مخلوطة مع الرصاص بجرعة 30 ملغ/كغم وزن جاف. تم تقويم الاستجابات البيولوجية في نهاية التجربة (بعد 10 أسابيع) تضمنت: فحص تلف الحمض النووي لخلايا الدم الحمراء باستخدام (COMET assay) و فحص بيروكسيد الدهون lipid peroxidation لنسيج الكبد باستخدام (TBARS) والفحوص الدمية وايضا التحري عن تراكم الرصاص في الانسجة المختلفة (الكبد والكلية والعضلات). اظهرت النتائج أعلى مستوى من التلف في الحامض النووي في مجموعة T3 ، وهي زيادة معنوية ($p \leq 0.05$) مقارنة مع المجموعة T4. كما ان هناك فرق معنوي بين المجاميع المعاملة بالرصاص فقط (T3 وT4) وبين المجاميع المعاملة بالرصاص المضاف اليه فيتامين هـ + عنصري السلينيوم (T5 وT6). اشارت النتائج إلى قدرة فيتامين E + عنصر السلينيوم مضاداً للأكسدة. كما لوحظ ان مستوى تركيز TBARS فيما يخص بيروكسيد الدهون عالياً في المجموعتين T3 و T4، في المقابل كان تركيز TBARS منخفضاً بشكل معنوي ($p \leq 0.05$) في المجموعتين T5 و T6، مقارنةً مع المجموعتين T3 وT4. أظهر عدّ كريات الدم البيضاء زيادة معنوية في المجاميع T3، T5 وT6 على التوالي نسبةً إلى المجموعتين T1 وT2. في حين أظهرت المجاميع المعاملة مع الرصاص نقصاً في عدد الكريات الحمراء بشكل معنوي ($p \leq 0.05$) مقارنةً مع المجموعتين T1 وT2. ولوحظ ان تراكم الرصاص في الاعضاء كان على الترتيب التالي: الكلى > الكبد > العضلات. ان البيانات التي تم الحصول عليها خلال هذه الدراسة ومن خلال استخدام الفحوص المرافقة على بعض المؤشرات الحيوية ، قدمت تقويما كاملاً للآثار السمية لعناصر المعادن الثقيلة، وهذا قد يترتب عليه آثاراً طبيةً مهمة في صحة الإنسان.

*كلية الطب البيطري،جامعة بغداد،بغداد،العراق.

**دائرة البيطرة ،وزارة الزراعة، بغداد،العراق.