Effect of vitamin E plus Selenium against lead exposure on histopathology of kidney and liver in common carp *Cyprinus carpio* L.

S. A. Mustafa[*] and A. M. Alkhalili[**]
[*]Dep. of Pathology- Collage of Veterinary Medicine/ University of Baghdad
[**] Dep. of Epidemiology- Veterinary Directorate/ Ministry of Agriculture

Abstract

The present study was undertaken to investigate the effects of lead (Pb) either alone or with Vitamin E (VE) plus Selenium (Se) on histopathological changes in *Cyprinus carpio*. For this purpose, 120 common carp with an average weight 65.50±0.25 g, and total length 17.4±2.5 cm were randomly divided into 6 treatment groups in duplicate. Each of the six treatment groups was fed the formulated diets for 10 weeks at a daily rate of 3% body weight throughout the experiment as the following: Treatment 1 (T1) serve as control group; Treatment 2 (T2) were fed diet mixed with VE + Se (300 mg kg-[1] d.w); Treatment 3 (T3) were fed diet mixed with 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) with VE+Se; Treatment 6 (T6) were fed diet mixed with Pb (30 mg kg^{-1} d.w) with VE+Se. After 10 weeks feeding trail 3 fish $tank^{-1}$ (6 fish $treatment^{-1}$) were dissected out liver and kidney were removed and fixed in 10% formaldehyde solution for histopathological studies. The liver of control group exhibited a quite normal architecture, while the fish exposed to Pb showed vacuolation and necrosis. These hepatic alterations were more evident in fish exposed to (30mg kg^{-1} dw). The kidney exhibited Bowman space dilatation, acute cellular degeneration and necrosis. The quantitative histopathology revealed abnormalities in kidney and liver in all dietary Pb treated groups alone and with VE+Se. The severity of lesion in both organs were significantly different ($P<0.05$) in Pb groups in comparison to Pb with VE+Se groups. These results indicated that histopathology could be used as a tool for assessing the risk of contaminants in the environment.

E-mail: sanaa.aziz@yahoo.com

Keywords: *Cyprinus carpio*, Lead, Selenium, Vitamin E, Histopathology

دراسة تأثير فيتامين هـ والسلينيوم ضد التعرض للرصاص على التغيرات النسجية في الكلية والكبد في اسماك الكارب الشائع *Cyprinus carpio* L

سناء عبد العزيز مصطفى[*] وعلي محمود الخليلي[**]
[*] فرع الأمراض وأمراض الدواجن– كلية الطب البيطري/ جامعة بغداد
[**] قسم الوبائيات– دائرة البيطرة/ وزارة الزراعة

الخلاصة

أجريت هذه الدراسة لغرض التقصي عن آثار التعرض للرصاص إما لوحده أو مع فيتامين هـ+عنصر السيلينيوم (مع العليقة)، على التغيرات النسجية المرضية في اسماك الكارب الشائع. لهذا الغرض، تم اخذ 120 سمكة من اسماك الكارب الشائع بمتوسط وزن 65.50 ± 0.25 غرام، وطول 17.4 ± 2.5 سم، قسمت هذه الأسماك بشكل عشوائي إلى 6 مجاميع علاجية وبصيغة مكررة (2 خزان للمعاملة الواحدة). كل مجموعة من المجاميع العلاجية تم تغذيتها لمدة 10 أسابيع بمعدل يومي 3% من وزن الجسم وكان الغذاء قد جُهز مسبقاً ليتماشى والغرض من التجربة وكما يلي: المجموعة الأولى (T1) مجموعة السيطرة تم تغذيتها بالعليقة الاعتيادية

من دون إضافات علاجية؛ المجموعة الثانية (T2) تم تغذيتها بعليقة مضاف لها فيتامين هـ + عنصر السلينيوم وبجرعة (300 ملغ/كغم وزن جاف)؛ المجموعة الثالثة (T3) تم تغذيتها بعليقة مضاف لها الرصاص بجرعة (20 ملغ/ كغم وزن جاف)؛ المجموعة الرابعة (T4) تم تغذيتها بعليقة مضاف لها الرصاص بجرعة (30 ملغ/كغم وزن جاف)؛ المجموعة الخامسة (T5) تم تغذيتها بعليقة مضاف لها فيتامين هـ + عنصر السلينيوم (300 ملغ/كغم وزن جاف) مخلوطة مع الرصاص بجرعة (20 ملغ/كغم وزن جاف)؛ المجموعة السادسة (T6) تم تغذيتها بعليقة مضاف لها فيتامين هـ + عنصر السلينيوم (300 ملغ/كغم وزن جاف) مخلوطة مع الرصاص بجرعة (30 ملغ/كغم وزن جاف). بعد مرور 10 أسابيع من التجربة، تم اخذ 3 اسماك من كل حوض (6 اسماك لكل معاملة) لغرض إجراء الفحص النسجي المرضي لأنسجة الكلية والكبد، حيث تم استخراج الكبد والكلى لتلك الأسماك ووضعت في محلول 10% من الفورمالديهايد لغرض التثبيت. كان نسيج الكبد لمجموعة السيطرة طبيعيا تماما، في حين أظهر كبد الأسماك المعرضة للرصاص وجود فجوات وتنخر. وكانت هذه التغيرات المرضية أكثر وضوحا في الأسماك التي تعرضت للرصاص بجرعة (30 ملغ/كغ وزن جاف). أما نسيج الكلى فظهرت فيه فراغات ممتدة تدعىBoman space dilatation، مع ضمور خلوي حاد وتنخر. كشف التشريح المرضي الكمي تشوهات في الكلى والكبد في كل المجاميع التي تغذت على العليقة الحاوية للرصاص سواءً لوحده أو المخلوط مع فيتامين هـ+عنصر السلينيوم. كانت شدة الإصابة في كِلا العضوين (الكبد والكلى) ذات فرق معنوي كبير ($P<0.05$) بين المجاميع المعاملة بالرصاص فقط وبين المجاميع المعاملة بالرصاص المضاف له فيتامين هـ + عنصر السلينيوم. تشير هذه النتائج إلى أن التشريح النسجي المرضي يمكن أن يستخدم كدليل قوي في تقييم مخاطر الملوثات الموجودة في البيئة.

Introduction

Heavy metal contamination has been reported in aquatic organisms (1,2). This heavy metal could induce harmful toxicological effects, when present in high levels in aquatic ecosystem (3). Indeed, it is possibly toxic when the internal available level increases the ability of the physiological detoxification process. These pollutants build up in the food chain and are responsible for detrimental effects and death in the fish (4). Lead has the potential to harmfully affect the human and animal health. It exerts physiological, biochemical, and neurological dysfunctions in animal and humans (5). The optimum limits for lead in the food of animal origin ranged between 0.01-1.00 mg/kg. Dietary uptake of metals is a main cause of long-term contamination in wild fish (6), and there is a renewed interest in the nutritional and toxicological effects of metals in the food of fish (7). Data demonstrating that the antioxidant defense could be playing an essential role in decreasing some hazards of heavy metals (8, 9). Fish are widely used to assess the health of the aquatic environment and physiological changes act as biomarkers of environmental pollution. They are however, endangered by water borne pollutants transferred along the food chain (10). Because fishes are an important food resource and a major aquatic ecosystem component, it is important to assess the adverse effects of lead on fishes. The main pathway of vitamin E plus selenium in the defence by immune system is perhaps the protection of cell membranes and other cellular organelles of immune cells against lipid peroxidation (11). From the viewpoint of fish culture, the common carp is the most extensively cultivated species. Hence, this study was undertaken to examine the effect of dietary Pb alone or with VE plus Se on histological aspects of kidney and liver of common carp, *Cyprinus carpio.*

Materials and Methods

- **Experimental Design:** A hundred fifty healthy of *C. carpio* with an average weight 65.50±0.25 g, and length 17.4±2.5 cm was obtained from a commercial fish farm of Babil, Iraq. They 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; each tank with dimension (150 × 80 × 50 cm) has 10 fishes. Then the fish were divided to the tanks into 6 treatment groups in duplicate as described previously in diet preparation. Each of the six treatment groups were fed the formulated diets at a daily rate of (3%) body weight throughout the experiment (10 weeks) as the following: Treatment 1 (T1) serve as control group; Treatment 2 (T2) were fed diet mixed with VE + Se (300 mg kg^{-1} d.w); Treatment 3 (T3) were fed diet mixed with 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) with VE+Se; Treatment 6 (T6) were fed diet mixed with Pb (30 mg kg^{-1} d.w) with VE+Se. The experimental fishes were preserved under a 12 hour light/dark cycle. Every day after feeding (about one hour) the tanks were cleaned from the remnants. Water of the tanks was changed every two days. The chemo-physical parameters of the water were measured during the experimental period Water temperature was (23.53±0.80 ^{o}C) measured using a thermometer; pH (7.30±0.30) and dissolved oxygen (DO) (5.90±0.50) were measured using an electric meter. After 10 weeks feeding trail 3 fish $tank^{-1}$ (6 fish $treatment^{-1}$) were dissected out liver and kidney were removed and fixed in 10% formaldehyde solution for histopathological studies.
- **Histology:** The liver and kidney were removed carefully and were fixed in 10% formaldehyde solution for 24 h. According to (12), the samples were processed as usual in the standard method of dehydration through a series of alcohols to remove extra water (70% for 24 h, 90% industrial methylated spirit, then cleared in xylene to remove the extra alcohols and finally embedded in paraffin wax (58-60 °C for 1-2 h) before being sectioned at 5 μm using a rotary microtome. The specimens were stained with haematoxylin and eosin following usual method. Photographed were taken using a digital camera (Olympus camedia C-2020 Z) at total magnifications of × 400 (zoom on the camera was x2.5). Descriptions of histopathology were done for the images according to (13).
- **Statistical analysis:** Statistical analysis was achieved using Sigma Plot v11.0 software. Statistical differences between exposed groups and respective control group were analysed using ANOVA and multiple range test at a 5% significant level ($P<0.05$).

Results and Discussion

- **Histopathological studies:**

- **Kidney:** All tubules, glomeruli and other features of the kidney appeared normal, with well-developed and the histological study exhibited a typical structural organization of the nephros in both the control group (untreated fish) (Fig. 1 A) and in VE plus Se group (T2) (Fig. 1 B). Results of this study revealed that levels of Pb create a range of histopathological alterations in the kidneys of exposed fish (Fig. 1 C-F). These changes, including melanomacrophage infiltration in tubules, Bowman space dilation, degenerative changes in the form of cytoplasmic vacuolation, acute cellular degeneration of the tubular epithelium, hydrobic degeneration and necrosis in the tubular epithelium. However, the extent of the damage was not severe in the kidney in (T5 and T6) (Pb plus VE and Se) groups. Quantitative analysis showed that these changes were significantly increased ($P< 0.05$) in all treated groups (i.e., T3, T4, T5 and T6) compared to (T1 and T2). Also, there were significant differences ($P<0.05$) between (T3 and T4) (Pb alone without VE and Se) in comparison to (T5 and T6) (Table 1).

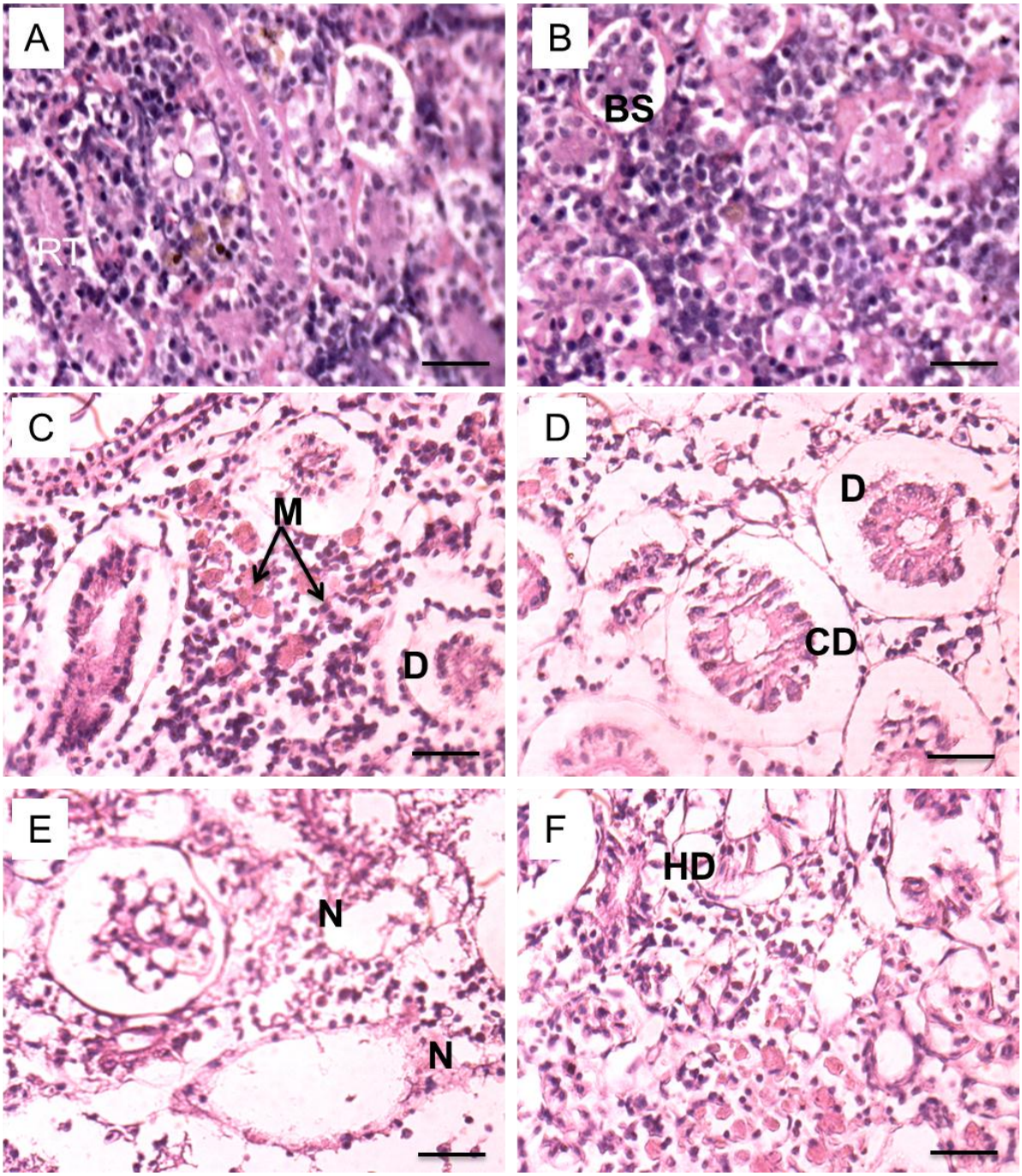

Fig. (1) Photomicrographs of the kidney of *C. carpio* affected by dietary lead either alone or with VE plus Se at 5 µm thickness. [A] Control kidney showing normal renal tubules (RT) and the Bowman's space (BS) well defined [B] T2 showing normal histology of the kidney; [C&F] dietary Pb treatment groups showing melanomacrophages aggregate (M), Bowman's space dilation (BS), cellular degeneration (CD), necrosis (N) and hydrobic degeneration (HD) in renal tubules. H&E, Scale bars: 50 µm.

Table (1) Histopathological changes presented as a percentage in the kidney of *C. carpio* L. exposed to dietary Pb alone or with VE plus Se for 10 weeks

Lesion (%) / Treatments	Bowman space dilation (BS)%	Melanomacrophage infiltration (M)%	Acute cellular degeneration (CD)%	Necrosis (N)%
T1	5.0 ±0.02[a]	14.0±0.02[a]	2.0±0.00[a]	0.0±0.00[a]
T2	3.0 ±0.07[a]	10.0±0.04[a]	0.0 ±0.00[a]	0.0±0.00[a]
T3	34.0 ±0.00[b]	38.0±0.09[b]	22.0 ±0.08[b]	18.0 ±0.90[b]
T4	38.0 ±0.05[b]	42.0 ±0.01[b]	31.0 ±0.30[b]	22.0 ±0.10[b]
T5	27.0 ±0.02[c]	27.0 ±0.05[c]	17.0 ±0.02[c]	11.0 ±0.08[c]
T6	25.0 ±0.05[c]	34.0 ±0.03[b]	13.0 ±0.00[d]	14.0±0.05[c]

Data are mean ± S.E. Groups with different alphabetic superscripts indicate significantly different at $P < 0.05$.

Rresults of this study indicated that exposure to dietary levels of Pb create a variety of histopathological alterations in the kidney of exposed fish, including: Bowman space dilation, melanomacropgage infiltration, acute cellular degeneration and necrosis. Similar lesions on Tilapia fish were recorded by (14). Kidney is the basic organ to be influenced by contaminants in the water (15). Due to the vital role of the kidney in the elimination of injurious substances, the numerous histological changes observed in the kidney could be a sequence from Pb toxicity which indicated the contribution of kidney in excretion of Pb from the body. The hydropic swelling observed in this study was very in line to those detected in Nile tilapia (*Oreochromis niloticus*) which exposed to contaminated sediments(16). The occurrence of tubule degeneration, together with the necrosis in the kidney in the present study shows that there was a damage in the kidney after exposure to Pb. (17, 18), observed that the polluted kidneys of carp fish appear acute degeneration, interstitial nephritis and renal necrosis and mononuclear cell infiltration. These changes are partially seen in this study.

- **Liver:** Liver of control fish (T1) and (T2) groups revealed a normal structure (architecture) and there were no pathological alterations. The hepatocytes exhibited a homogenous cytoplasm and a large subcentral or central spherical nucleus (Fig. 2 A and B). The histopathological changes of the liver after exposure to Pb (Fig. 2 C, D, E and F) revealed important changes including: hypertrophy of hepatocyets, nuclear piknosis, necrosis, mononuclear cells (MNCs), infiltration (monocytes/ macrophages and lymphocytes) (Fig. 2 E). As well as, necrosis and cytoplasmic vacuolation. The extent of damage was not severe in (T5 and T6). Quantitative analysis showed that these changes were significantly increased (P<0.05) in all treated groups (T3, T4, T5 and T6) compared to (T1 and T2) groups. Also, there were significant differences (P<0.05) between (T3 and T4) groups relative to (T5 and T6) groups (Table 2).

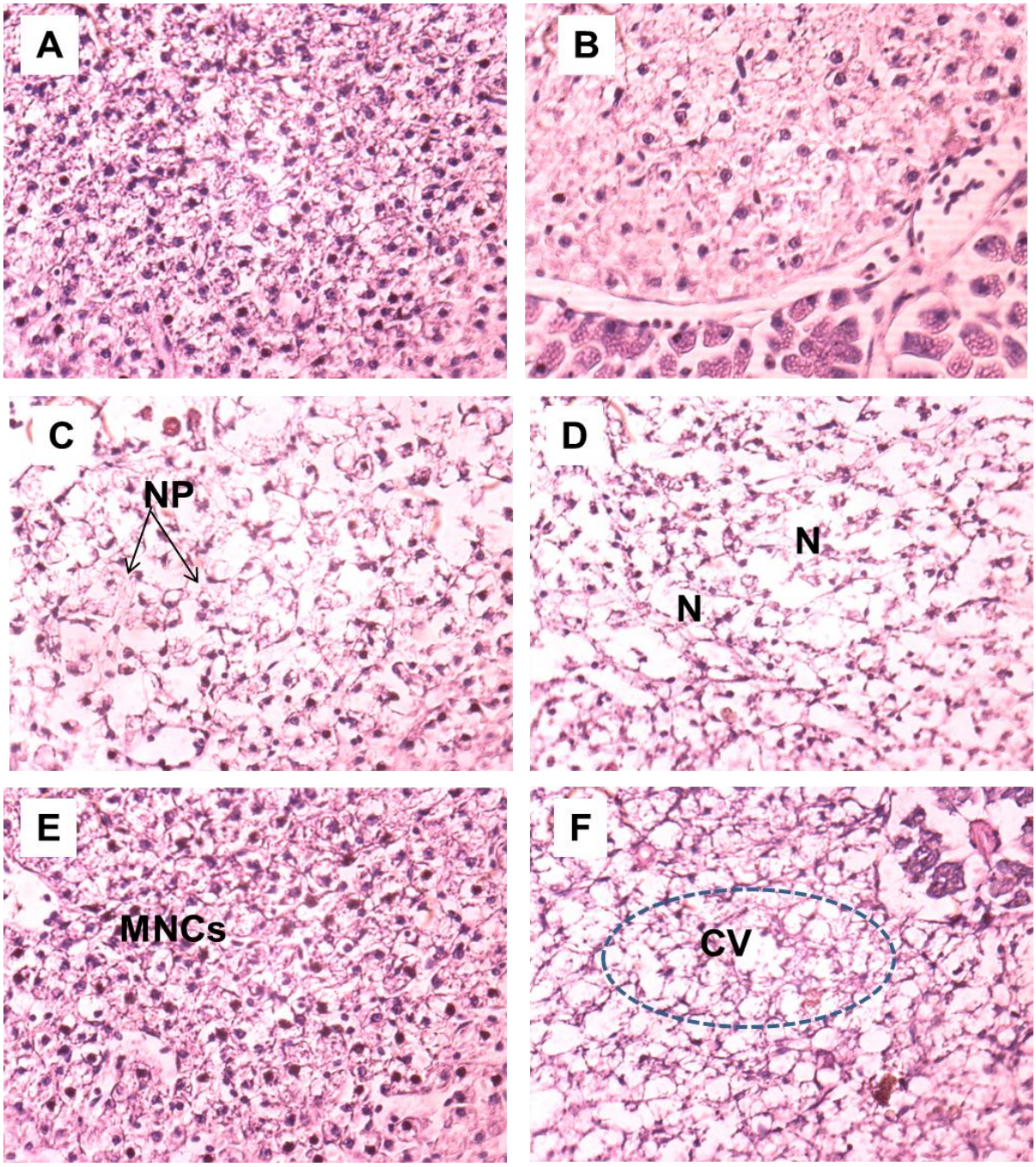

Fig. (2) Photomicrographs of the liver of C. carpio affected by dietary lead either alone or with VE plus Se at 5 µm thickness. [A] Control liver showing normal hepatocytes [B] T2 show normal histology of the liver; [C&F] dietary Pb treatment groups showing nuclear piknosis (NP), necrosis (N) mononuclear cells infiltration (MNCs) and cytoplasmic vacuolation (CV). H&E, Scale bars: 50 µm.

Table (2) Histopathological changes presented as a percentage in the liver of *C. carpio* L. feeding dietary Pb alone or with VE plus Se for 10 weeks

Lesion (%)	Nuclear piknosis (NP)%	Necrosis (N)%	Mononuclear cells infiltration (MNCs)%	Cytoplasmic vacuolation (CV)%
T1	5.0±0.01^{a}	0.0±0.00^{a}	3.0±0.05^{a}	0.0±0.00^{a}
T2	7.0±0.08^{a}	0.0 ±0.00^{a}	2.0 ±0.01^{a}	0.0 ±0.00^{a}
T3	28.0 ± 0.90^{b}	42.0 ± 5.00^{b}	16.0 ± 2.00^{b}	67.0 ± 0.05^{b}
T4	25.0 ± 1.50^{b}	34.0 ± 7.00^{b}	14.0 ± 1.50^{b}	70.0 ± 3.50^{b}
T5	23.0 ± 0.60^{b}	28.0 ± 0.40^{c}	13.0 ± 0.60^{b}	38.0 ± 0.90^{c}
T6	34.0 ± 0.80^{c}	25.0 ± 0.90^{c}	17.0 ± 0.80^{c}	32.0 ± 2.60^{c}

Data are mean ± S.E. Groups with different alphabetic superscripts indicate significantly different at $P < 0.05$.

Two factors were detecting the detrimental effect of HM pollution on histology of fish liver, the period of the exposure and the concentration of the specific metal and that fish liver histology might be used as a model for demonstraing the interactions between environmental factors and hepatic structures and functions (19). The monitor of histological abnormalities in fish liver is a greatly sensitive and accurate method to evaluate the effects of xenobiotic compounds in the field and investigational researches (20). Since the liver expected to be the primary target organ with the detoxification and biotransformation process this, given an exceptional bioindicator of aquatic contamination (21). The most common changes of liver after Pb exposure were reported to include nuclear pyknosis, cytoplasmic vacuolation and moderate mononuclear cells aggregation and necrosis. The presence of vacuoles in hepatocyte in the cytoplasm which could be lipids and glycogen, and can be related to the normal metabolic function of the liver (22). (23), explained that increased vacuolization as a cellular defence mechanism against injury of contaminants to hepatic cells and this process are responsible for accumulating injurious substances and stopping them from interfering with the biological actions of these cells. Some researchers suggested that vacuoles formation in the hepatic cells acts as a marker of degenerative changes due to metabolic damage, which possibly related to exposure to contamination (24). In addition, the cytoplasmic vacuolization could be indicated to an imbalance between the rate of production of lipids and glycogen in the parenchymal cells and the release of these substances in the circulation (25). The aggregation of inflammatory cells could be indicated to the reaction of melanodialdehyde in oxidative stress developed by Pb exposure as evidenced by increased of TBARS. The present observations are in line with those found by numerous researchers who studied the impacts of different contaminants in fish (26, 27). Also, the necrosis and the cellular degeneration could be attrabuited to metal accumulation consequence in hepatic tissue. Fish generally accumulate higher levels of metals in liver than other organs (28). (29) reported that the contaminants have the ability of inducing disarrangement of cell membranes resulting in their degradation. In addition, (30), described that the necrosis in the liver cell might be due to inhibition of production of DNA required for the development and growth of the liver. Our results are also in agreement with (31), how reported the similar changes in *C. carpio* exposed to dietary Cu for 10 weeks. The current study approves the worth of histopathology in evaluating the effects of HM in the aquatic ecosystem. In addition, the use of different parameters, histology being one of them, may contribute in environmental monitoring. In conclusion, histopathological changes in liver and kidney tissues following exposure to dietary Pb alone or with VE plus Se indicating that the histopathology are a powerful tool for evaluating the environmental contamination. Additionally, VE plus Secould be used as a feed additive in diet to produce that effect and act as an antioxidant.

References

1. Adham, K. G.; Hamed S. S.; Ibrahim H. M. & Saleh, R. A. (2002). Impaired functions in nile tilapia, *Oreochromis niloticus* (L.,1757), from polluted waters. Acta Hydrochem. Hydrobiol., 29:278-288.
2. Olojo, E. A. A.; Olurin, K. B.; Mbaka, G. & Oluwemimo, A. D. (2005). Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to lead. Afr. J. Biotech., 4:117-122.
3. Pelgrom, S.; Lamers, L.; Lock, R.; Balm, P. & Wendelaar Bonga, S. (1995). Integrated physiological response of tilapia, *Orechromis mossambicus*, to sublethal copper exposure. Aquat. Toxicol., 32:303-320.

4. Farkas, A.; Salanki J. & Specziar, A. (2002). Relation between growth and the heavy metal concentration in organs of bream, *Abramis brama* L. populating lake Balaton. Arch. of Environ. Contam. Toxicol., 43:236-243.
5. Nordberg, G. F.; Fowler, B. A.; Nordberg, M. & Friberg, L. (2007). Handbook on the Toxicology of Metals. (3rd eds.), Academic press, Amesterdam, ISBN-10:0123694132, 1024.
6. Dallinger, R.; Prosi, F.; Segner, H. & Back, H. (1987). Contaminate food and uptake of heavy metals by fish: A review and a proposal for further research. Oecol., 3: 91-98.
7. Handy, R. D. (1996). Dietary exposure to toxic metals in fish. In: Taylor. EW, editor, Toxicology of aquatic pollution, New York: Cambridge University Press, PP. 29-60.
8. El-Sokkary, G. H.; Abdel-Rahman, G. H. & Kamel, E. S. (2005). Melatonin protects against lead-induced hepatic and renal toxicity in male rats. Toxicology., 213: 25-33.
9. El-Shebly, A. A. (2009). The role of antioxidant (Vitamin E) in the control of lead (Pb) pollution and enhancement of growth within Nile Tilapia (*Oreochromis niloticus*). Int. J. Appl. Res. Vet. Med., 7: 97-101.
10. Kock, G.; Triendl, M. & Hofer, R. (1996). Seasonal patterns of metal accumulation in Arctic char (*Salvelinus alpinus*) from an oligotrophic Alpine lake related to temperature. Can. J. Fish. Aquat. Sci., 53:780-786.
11. Bendich, A. (1990). Antioxidant vitamins and their functions in immune responses. Adv. Exp. Med. Biol., 262: 35-55.
12. Myers, M. S.; Johnson, L. L.; Hom, T.; Collier, T. K.; Stein, J. E. & Varanasi, U. (1998). Toxicopathic hepatic lesions in subadult English sole (*Pleuronectes vetuls*) from Puget Sound, Washington, USA: Relationships with other biomarkers of contaminant exposure. Mar. Environ. Res., 45: 47-67.
13. Bernet, D.; Schmidt, H.; Meier, W.; Burkhardt-Holm, P. & Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. of Fish Dis., 22: 25-34.
14. Hutton, M. (1983). Evaluation of the relationships between cadmium exposure and selected indicators of renal function. In Heavy Metals in the Environment, Heidelberg, CEP Consultants Ltd, Edinburgh. PP. 274-277.
15. Thophon, S.; Kruatrachue, M. E.; Upathan, S.; Pokethitiyook, P.; Sahaphong, S. & Jarikhuan, S. (2003). Histopathological alterations of white seabass, *Lates calcarifer* in acute and subchronic cadmium exposure. Environmental Pollution, 121: 307-320.
16. Peebuaa, P.; Kruatrachuea, M.; Pokethitiyooka, P. & Kosiyachindaa, P. (2006). Histological effects of contaminated sediments in Mae Klong River Tributaries, Thailand, on Nile tilapia, *Oreochromis niloticus*. Science Asia, 32: 143-150.
17. Hamouda, E. E. (1993). Pathological studies on fish of some chemically polluted areas at Alexandria province. MVSc Thesis, Department of Pathologyand Parasitology, Faculty of Veterinary Medicine, Alexandria University, Egypt.
18. Abdullah, A. E. E.; Mehana, A. & Meki. (2008). Evaluation of lead and cadmium levels in freshwater fish farms at Qassim region, KSA. J. Agric. and Vet. Sci., 1(2): 59-69.
19. Mobarak, Y. M. S. & Sharaf, M. M. (2011).Lead acetate-induced histopathological changes in the gills and digestive system of silver Sailfin molly (*Poecilia latipinna*). Int. J. Zool. Res., 7: 1-18.

20. Figueiredo-Fernandes, A.; Ferreira-Cardoso, J. V.; Garcia-Santos S.; Monteiro, S. M.; Carrola, J.; Matos, P. & Fontaínhas-Fernandes, A. (2007). Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus* exposed to waterborne copper. Pesq. Vet. Bras., 27(3): 103-109.
21. Braunbeck, T. & Volkl, A. (1993). Toxicantinduced cytological alterations in the fish liver as biomarkers of environmental pollution. A case study on hepatocellular effects of dinitroo-cresol in golden ide *Leuciscus idus melanotus*. In: Fish Ecotoxicology and Ecophysiology. Preceedings of an International Symposium, Heidelberg, September 1991. (Eds.) T. Braunbeck, W. Hanke, & H. Segner. VCH Verlagsgesellschaft mbH. Weinheim.
22. Camargo, M. M. P. & Martinez, C. B. R. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotrop. Ichthyol., 5(3): 327-336.
23. Mollendroff, (1973). Cytology and cell physiology. 3rd Ed. Academic press. New York.
24. Pacheco, M. & Santos, M. A. (2002). Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel, *Anguilla anguilla* L. Ecotoxicol. and Environ. Saf., 53: 331-347.
25. Gingerich, W. H. (1982). Hepatic toxicology of fishes. In: Aquatic toxicology. (LJ Weber, Ed), Raven Press, New York., PP. 55-105.
26. Arellano, J. M.; Storch, V. & Sarasquete, C. (1999). Histological changes and copper accumulation in liver and gills of the Senegales Sole, *Solea senegalensis*. Ecotoxicol and Environ Safe., 44: 62-72.
27. Shaw, B. J. & Handy, R. D. (2006). Dietary copper exposure and recovery in Nile tilapia, *Oreochromis niloticus*. Aquatic Toxicol., 76: 111-121.
28. Al-Yousuf, M. H.; El-Shahawi, M. S. & Al-Ghais, S. M. (2000). Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. Science of The Total Environment, 256: 87-94.
29. Byczkowski, J. (1976). The mode of action of P, P-DDT on mammalian mitochondria. Toxicology, 6: 309-314.
30. Sanad, S. M.; El-Nahass, E. M.; Abdel-Gawad, A. M. & Aldeeb, M. (1997). Histochemical studies on the liver of mice following chronic administration of sodium barbitone. J. Ger. Soc. Zool., 22(C): 127-165.
31. Mustafa, S. A.; Davies, S. J. & Jha, A. N. (2012). Determination of hypoxia and dietary copper mediated sub-lethal toxicity in carp, *Cyprinus carpio*, at different levels of biological organisation. Chemosphere, 87: 413-420.