رقم البحث (56)

HEPATO-RENAL TOXIC EFFECT OF EXPOSURE TO CADMIUM AND TEMPERATURE IN *OREOCHROMIS NILOTICUS* FISH

BY

Sleem[a] F.R., Abou Elmagd[a] M.M. and Habotta[a] O.A.

[a]Department of Forensic Medicine and Toxicology

Faculty of Veterinary Medicine, Mansoura University

ABSTRACT

Heavy metals and temperature are common environmental stressors. Thus, they have considerable attention due to global climate change and anthropogenic pollution. Therefore, the present study focused on the influence of thermal change on liver and kidney damage induced by cadmium (Cd) in *Oreochromis niloticus* fish. The experiment was carried out on 120 apparently healthy Nile tilapia (60-70 gm) divided into 6 experimental groups, 20 for each exposed to Cd at different temperatures for 21 and 42 days. The first group used as control with water temperature of **22**˚C, the second group exposed to 3.44 mg/L $CdCl_2.H_2O$ (1/10 96hrs.LC_{50}) with water temperature of **22**˚C, the third group exposed to 1.77 mg/L $CdCl_2.H_2O$ (1/20 96hrs.LC_{50}) with water temperature of **26**˚C, the fourth group exposed to 3.44 mg/L $CdCl_2$ H_2O with water temperature of **26**˚C, the fifth group exposed to 1.77 mg/L $CdCl_2.H_2O$ with water temperature of **29**˚C and the sixth group exposed to 3.44 mg/L $CdCl_2.H_2O$ with water temperature of **29**˚C. The results of biochemical analysis revealed that the combined exposure to Cd and high temperatures lead to marked increase in the level of transaminases (AST and ALT), ALP, TP, urea and creatinine concentrations than exposure to Cd alone after 21 days. Meanwhile, after 42 days, the levels of TP and urea decreased with continued elevation of other parameters. Histopathological alterations in liver tissue revealed vacuolation, degenerative changes and necrosis. Kidney also showed necrosis, hemosiderosis and hemorrhage in renal tubules. These changes were more pronounced with temperature rising to 26˚C and 29˚C. These results confirm that the increase of temperature enhance Cd toxicity and needs to be taken into account for the accurate prediction and assessment of Cd-induced toxicity in fish.

INTRODUCTION

Pollution of aquatic environment by metals due to natural and anthropogenic sources is a worldwide environmental concern. Exposure to metals may lead to several toxic effects in aquatic organisms, including tissue damage, respiratory changes, alterations of biochemical and physiological mechanisms, and ultimate mortality (**Heath 1995**). Cadmium is one of the most toxic metals in the environment, does not serve a beneficial biologic function in higher organisms, and is extremely toxic even at very low concentrations (**Soares et al. 2008**). This metal can enter the environment from various anthropogenic sources, such as by-products from zinc refining, coal combustion, mine wastes, electroplating processes, iron and steel production, pigments, fertilizers, and pesticides (**USEPA, 2001**). Liver is the major site of metal storage and excretion in fish and as a result of its major role in metabolism and its sensitivity to metals in the environment, particular attention has been given to liver in toxicological investigations (**Parvez et al. 2006**). AST and ALT are the most important enzymes acting as transaminases involved in amino acid metabolism and they are known to be sensitive to metal exposures (**Gravato et al. 2006**). **Chowdhury et al. (2004)** have shown that the concentration of Cd rises more rapidly in the liver than in the kidneys following exposure, although the kidney is the primary organ of long-term Cd accumulation and storage in vertebrates including fish. This implies that some of the Cd accumulated in liver is transported later to the kidney for storage. **Thomann et al. (1997**) also demonstrated that the Cd concentration in the kidney continues to increase even when fish are no longer exposed to Cd, indicating the mobilization of Cd from other target tissues (e.g. liver) to kidney for long-term storage. The slow onset of global climate change (**IPCC, 2001**) has created concern about long-range effects of changing regional temperatures on aquatic organisms, most of which are poikilotherms (**Prosser, 1961**). Thermal changes can produce alterations in the toxicokinetics, bioavailability, biotransformation, homeostasis, absorption rate and elimination of different compounds (**Kőck et al., 1996**). Metal toxicity to fish is often reported to increase with increasing temperature which is often linked to increasing metal accumulation. There have been several reports of increasing cadmium toxicity with increasing temperature in aquatic organisms, juvenile mosquitofish and Gammarus pulex (**Sassi et al., 2010; Vellinger et al., 2012**) but the information about Tilapia are scarce. The present study, therefore, is an attempt to assess the possible influence of temperature on cadmium-induced hepato-renal toxicity in Nile tilapia.

MATERIAL AND METHODS

Tested compound:

Cadmium chloride monohydrate was purchased from LOBA chemie, Co. (Mombai, India).

Fish acclimation:

Apparently healthy Nile tilapia (*Oreochromis niloticus)* of body weight (60-70 gm) was obtained from private aquaculture farm, Manzalah, Dakahlia, Egypt. They were transported in large plastic bags containing oxygen to the laboratory of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Mansoura University. They were adapted for two weeks before starting the experiment. During the acclimation period, fish were reared in glass aquaria (70x40x40cm) provided with heaters and aerators and fed twice daily based on 3% of body weight with standard laboratory pelleted diet. They were under controlled conditions (12 hrs. light and 12 hrs. dark). Also, the water was changed twice weekly with clean dechlorinated tap water.

Exprimental design

One hundred and twenty acclimated fish were divided into 6 experimental groups 20 for each group divided into two aquria. The first group used as control with water temperature of **22**˚C, the second group exposed to 3.44 mg/L (1/10 96hrs.LC_{50}) $CdCl_2.H_2O$ with water temperature of **22**˚C, the third group exposed to 1.77 mg/L (1/20 96hrs.LC_{50}) $CdCl_2.H_2O$ with water temperature of **26**˚C, the fourth group exposed to 3.44 mg/L $CdCl_2$ with water temperature of **26**˚C, the fifth group exposed to 1.77 mg/L $CdCl_2.H_2O$ with water temperature of **29**˚C and the sixth group exposed to 3.44 mg/L $CdCl_2.H_2O$ with water temperature of **29**˚C. (The value of 96hrs.LC50 was calculated as 34.65mg/L under our experimental conditions, unpublished data).

Samples collection

At day 21 and 42, samples were collected from tested fish after their immobilization on absorbent paper and kept motionless. The body surface was then cleaned and blotted dry. For plasma separation, fresh blood samples were collected from the heart with disposable 3cc syringe and 21 gauge needles containing heparin, the samples were kept in Epindorff tubes in

standing position in refrigerator for 24hrs., and then centrifuged at 3000 rpm for 15 minutes and the clear plasma were separated carefully and stored in Epindorff tubes at -20˚C until biochemical analysis. For histopathological study, specimen from liver and kidney were collected and preserved in 10% neutral buffered formalin (groups exposed to 29˚c was sampled after 21days only due to high mortalities).

Biochemical analysis

Plasma samples were analyzed for total protein was determined according to **Kingsley et al (1939).** Urea was determined by colorimetric method according to **Vassault et al (1986)**. Creatinine determined by photometric colorimetric test for kinetic measurement according to **Henry (1974).** Aminotransferases (ALT and AST) were determined according to **Tietz (1976).** Alkaline phosphatase (ALP) determined by kinetic method according to **Young et al (1972).**

Histopathological Examination

Sections of 5 micron thickness were prepared from liver and kidney stained by hematoxyline and eosin (H&E), then examined microscopically according to **Bancroft et al (1990).**

Statistical analysis:

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 15, USA). Means and standard error for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same column show a significance ($P<0.05$).

RESULTS

Plasma biochemical Parameters of fish exposed to cadmium at different temperatures for 21 days as shown in table (1):

Total protein results revealed significant decrease between all exposed groups and the control. There are significant decreases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+29˚C exposed group while there are no significant difference in compare to

Cd1/10LC_{50}+26˚C. Also, there are significant decreases in Cd1/20LC_{50}+26˚c exposed group in compare to Cd1/20LC_{50}+29˚C exposed group Fig. (1).

Urea results revealed significant increase between all exposed groups and the control. There are significant increases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C and Cd1/10LC_{50}+29˚C exposed groups although there is no significant difference between Cd1/10LC_{50}+26˚C and Cd1/10LC_{50}+29˚C exposed groups. Moreover, there is significant decrease in Cd1/20LC_{50}+26˚C exposed group in compare to Cd1/20LC_{50}+29˚C exposed group Fig. (2).

Creatinine results revealed significant increases in all exposed groups in compare to the control except Cd1/20LC_{50}+26˚C exposed group. There are significant decreases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C and Cd1/10LC_{50}+29˚C exposed groups although there is no significant difference between Cd1/10LC_{50}+26˚C and Cd1/10LC_{50}+29˚C exposed groups. Moreover, there is significant decrease in Cd1/20LC_{50}+26˚C exposed group in compare to Cd1/20LC_{50}+29˚C exposed group Fig. (3).

ALT levels revealed significant increases between all exposed groups and the control. There are significant decreases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C and Cd1/10LC_{50}+29˚C exposed groups although there is no significant difference between Cd1/10LC_{50}+26˚C and Cd1/10LC_{50}+29˚C exposed groups. Moreover, there is significant decrease in Cd1/20LC_{50}+26˚C exposed group in compare to Cd1/20LC_{50}+29˚C exposed group Fig. (4).

AST levels revealed significant increase between all exposed groups and the control. Also, there are significant differences between all exposed groups Fig. (5).

ALP results revealed significant increases in all exposed groups except Cd1/10LC_{50}+22˚C and Cd1/20LC_{50}+26˚C exposed groups in compare to the control. There are significant decreases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C and Cd1/10LC_{50}+29˚C exposed groups in addition to significant increase in Cd1/10LC_{50}+26˚C in compare to Cd1/10LC_{50}+29˚C exposed groups. Moreover, there is significant decrease in Cd1/20LC_{50}+26˚C exposed group in compare to Cd1/20LC_{50}+29˚C exposed group Fig. (6).

Table (1): Plasma biochemical Parameters of fish exposed to cadmium at different temperatures for 21 days (Means ± S.E).

Group	TP g/dl	Urea mg/dl	Creatinine mg/l	ALT U/L	AST U/L	ALP U/L
Control	5.7125^{a} ±0.174	16.487^{e} ±0.407	0.7888^{d} ±0.028	8.5625^{e} ±0.429	15.325^{f} ±0.4977	29.525^{d} ±0.595
Cd 1/10LC_{50}+22˚c	3.6375^{c} ±0.106	27.125^{b} ±1.347	0.9938^{c} ±0.053	17.100^{c} ±0.594	27.800^{e} ±0.3234	32.650^{d} ±0.814
Cd 1/20LC_{50}+26˚c	3.9125^{c} ±0.091	18.675^{d} ±0.548	0.9025^{cd} ±0.022	12.687^{d} ±0.413	31.287^{d} ±0.831	31.800^{d} ±0.716
Cd 1/10LC_{50}+26˚c	3.6875^{c} ±0.1306	22.687^{c} ±0.564	3.2275^{a} ±0.066	61.762^{b} ±1.136	37.337^{c} ±0.715	115.79^{a} ±2.589
Cd 1/20LC_{50}+29˚c	4.5938^{b} ±0.103	42.012^{a} ±0.782	2.5538^{b} ±0.083	72.187^{a} ±0.730	49.325^{b} ±0.856	55.312^{c} ±1.031
Cd 1/10LC_{50}+29˚c	4.3250^{b} ±0.054	23.975^{c} ±0.644	3.1750^{a} ±0.052	59.075^{b} ±2.490	67.200^{a} ±0.696	61.000^{b} ±1.100

The means in the same column having the same superscript are not significantly different (P<0.05).

Plasma biochemical Parameters of fish exposed to cadmium at different temperatures for 42 days as shown in table (2):

TP results revealed significant decreases in all exposed groups in compare to the control. There are significant increases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C and Cd1/20LC_{50}+26˚C exposed groups. Moreover, there is no significant difference between Cd1/10LC_{50}+26˚C and Cd1/20LC_{50}+26˚C exposed groups Fig. (1).

Urea results revealed significant increase in all exposed groups in compare to control. There are significant increases in Cd1/10LC_{50}+22˚c exposed group in compare to Cd1/10LC_{50}+26˚c exposed group. Also, there is significant increase in Cd1/20LC_{50}+26˚c in compare to Cd1/10LC_{50}+26˚c exposed group Fig. (2).

Creatinine levels revealed significant increase in all exposed groups and the control. There are significant decreases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C. Moreover, there is significant decrease in Cd1/20LC_{50}+26˚C in compare to Cd1/10LC_{50}+26˚C exposed group Fig. (3).

ALT levels revealed significant increase in all exposed groups in compare to the control. There are significant decreases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C. Moreover, there is significant decrease in Cd1/20LC_{50}+26˚C in compare to Cd1/10LC_{50}+26˚C exposed group Fig. (4).

AST levels revealed significant increase in all exposed groups in compare to the control. There are significant decreases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C. Moreover, there is significant increase in Cd1/20LC_{50}+26˚C in compare to Cd1/10LC_{50}+26˚C exposed group Fig. (5).

ALP levels revealed significant increase in all exposed groups in compare to the control. There are significant decreases in Cd1/10LC_{50}+22˚C exposed group in compare to Cd1/10LC_{50}+26˚C. Moreover, there is significant decrease in Cd1/20LC_{50}+26˚C in compare to Cd1/10LC_{50}+26˚C exposed group Fig. (6).

Table (2): Plasma biochemical Parameters of fish exposed to cadmium at different temperatures for 42 days. (Means ± S.E).

Group	**TP g/dl**	**Urea mg/dl**	**Creatinine** mg/dl	**ALT U/L**	**AST U/L**	**ALP U/L**
Control	**5.7125^{a} ±0.158**	**15.975^{d} ±0.232**	**0.7888^{d} ±0.034**	**8.7750^{d} ±0.265**	**15.462^{d} ±0.509**	**29.362^{d} ±0.569**
Cd 1/10LC_{50}+22˚c	**3.8625^{b} ±0.101**	**25.550^{b} ±0.722**	**1.5838^{b} ±0.060**	**20.562^{c} ±0.446**	**20.862^{c} ±0.740**	**48.262^{b} ±1.963**
Cd 1/20LC_{50}+26˚c	**3.3125^{c} ±0.091**	**32.237^{a} ±0.788**	**1.2762^{c} ±0.085**	**30.562^{b} ±0.539**	**66.500^{a} ±0.468**	**41.862^{c} ±0.850**
Cd 1/10LC_{50}+26˚c	**3.2562^{c} ±0.053**	**23.462^{c} ±0.669**	**5.0000^{a} ±0.092**	**44.787^{a} ±0.801**	**36.387^{b} ±1.080**	**60.712^{a} ±0.880**

The means in the same column having the same superscript are not significantly different ($P<0.05$).

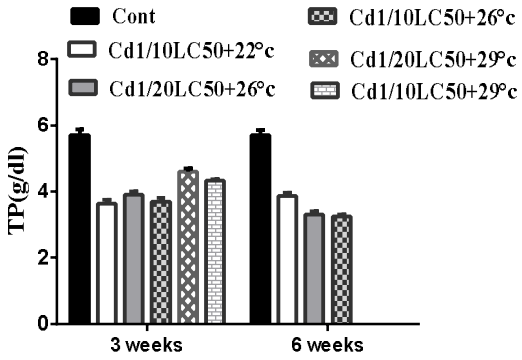

Fig. (1): Total Protein values of fish exposed to cadmium at different temperatures.

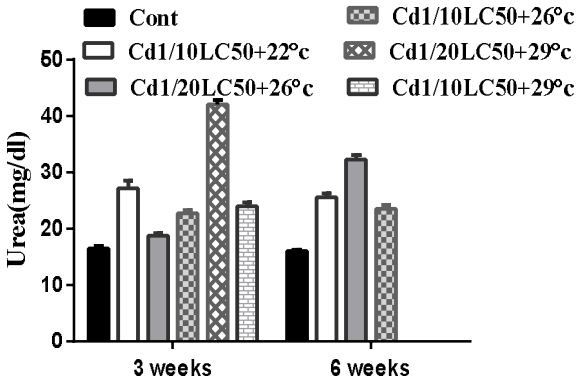

Fig. (2): Urea values of fish exposed to cadmium at different temperatures.

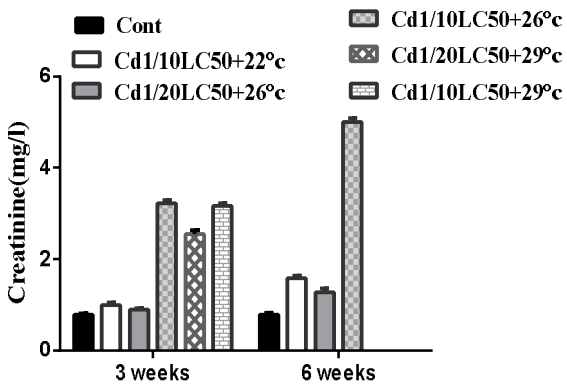

Fig. (3): Creatinine values of fish exposed to cadmium at different temperatures

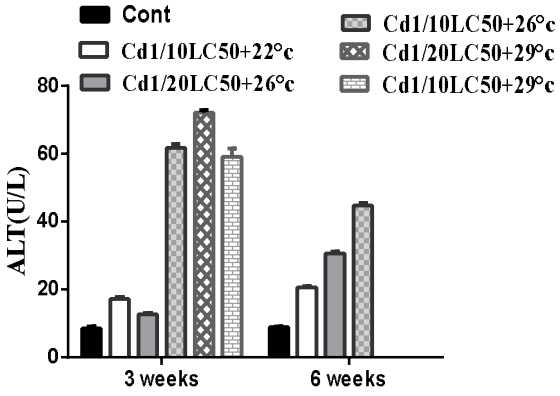

Fig. (4): ALT values of fish exposed to cadmium at different temperatures

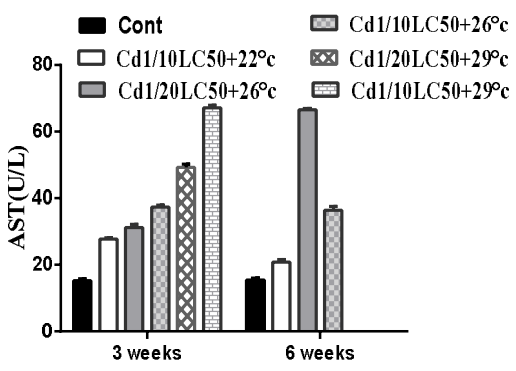

Fig. (5): AST values of fish exposed to cadmium at different temperatures.

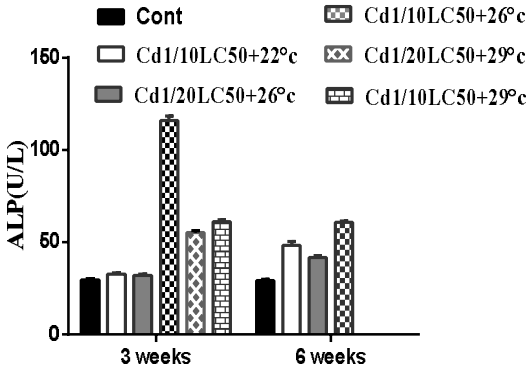

Fig. (6): ALP values of fish exposed to cadmium at different temperatures.

Histopathological findings of fish exposed to cadmium at different temperatures for 21 days:

In Cd1/10LC_{50} at 22˚C exposed group, severe congestion with vacuolation and necrosis of hepatocytes Fig. (7). Also, kidney showed Brown to black pigment deposition and congestion, besides vacuolation and necrosis in renal tubules were seen Fig. (8).

In Cd1/20LC_{50} at 26˚C exposed group, Vacuolation of hepatocytes with normal hepatopancease was seen. Furthermore, kidney showed Hemorrhages with brownish hemosiderin pigment deposition were observed.

In Cd1/10L_{C50} at 26˚C exposed group, numerous glycogen vacuolation in hepatocytes was observed. Also, kidney Hemorrhage and coagulative necrosis in renal tubules were seen Fig. (8).

In Cd1/20LC_{50} at 29˚C exposed group, liver showed severe congestion and necrosis of hepatocytes were seen Fig. (7). Kidney showed hemorrhage and necrosis in renal tubular epithelium were observed.

In Cd1/10LC_{50} at 29˚C exposed group, liver showed Congestion with normal hepatopancrease, beside mild vacuolation of hepatocytes was detected Fig.(7). Necrosis in renal tubules and dissolution in renal glomeruli were seen Fig.(8).

Histopathological findings of fish exposed to cadmium at different temperatures for 42 days:

In Cd1/10LC_{50} at 22˚C exposed group, liver tissue showing lytic necrosis in hepatocytes. Moreover, kidney showed hemorrhage and hemosiderosis with degenerative changes in renal tubules.

In Cd1/20LC_{50} at 26˚C exposed group, liver showed congestion in central vein. In addition, kidney showed necrosis and dissociation of the renal tubular epithelium. Fig.(8).

In Cd1/10LC_{50} at 26˚C exposed group, liver showed severe congestion with periportal necrosis. Fig. (7). Also, kidney showed lymphocytic exudates in interstitial tissue.

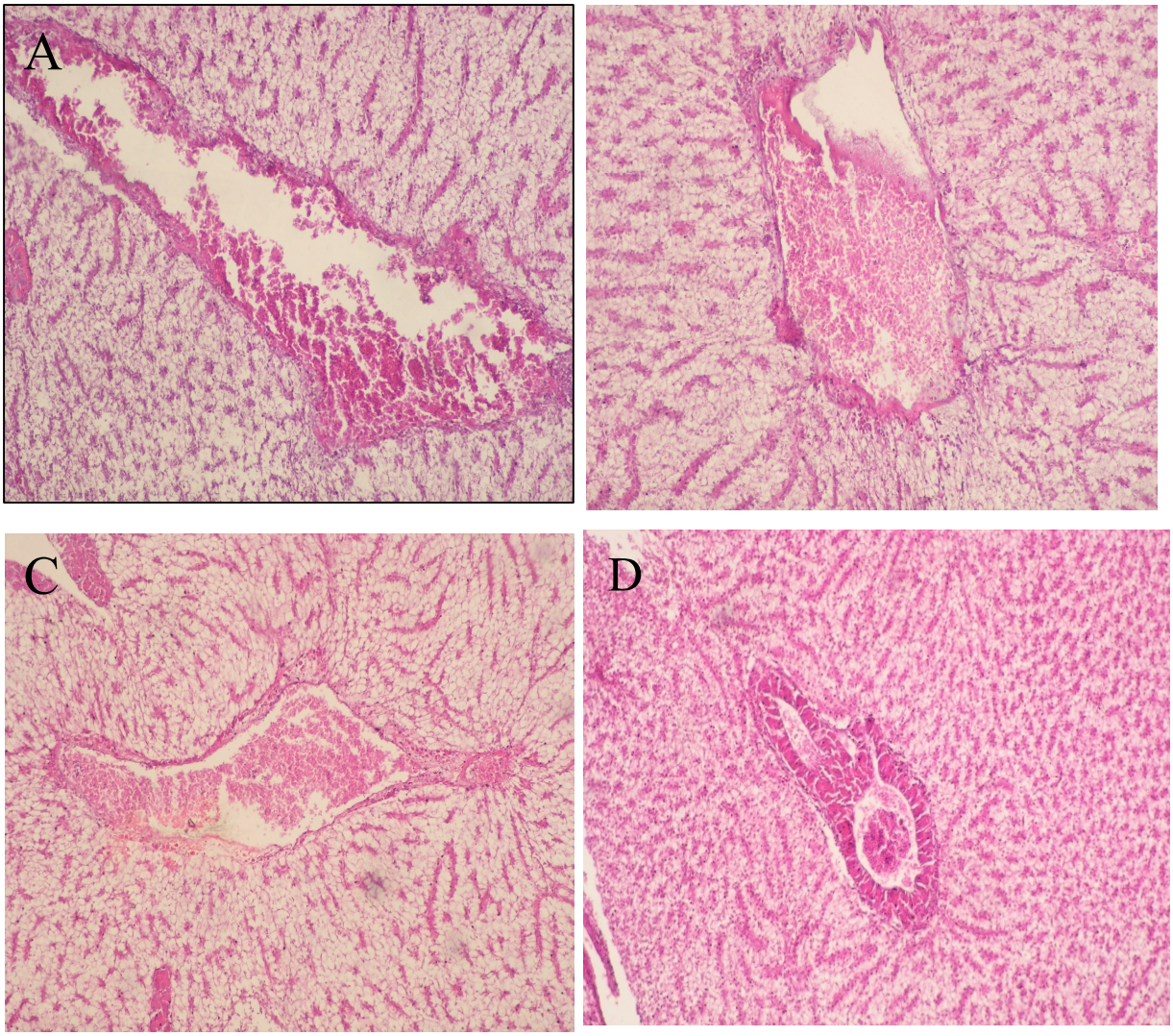

Fig. (7): Photomicrograph of liver section in fish. **(A)** Liver of fish exposed to Cd1/10LC_{50} at 22˚c for 21 days showing severe congestion with vacuolation and necrosis of hepatocytes (HE, x10) **(B)** Liver of fish exposed to Cd1/10LC_{50} at 26˚c for 42 days showing severe congestion with periportal necrosis (HE, x10) (**C**) liver of fish exposed to Cd1/20LC_{50} at 29˚c for 21 days showing severe congestion and necrosis of hepatocytes (HE, 10x). (**D**) Liver of fish exposed to Cd1/10LC_{50} at 29˚c for 21 days showing congestion with normal hepatopancrease (HE, x10).

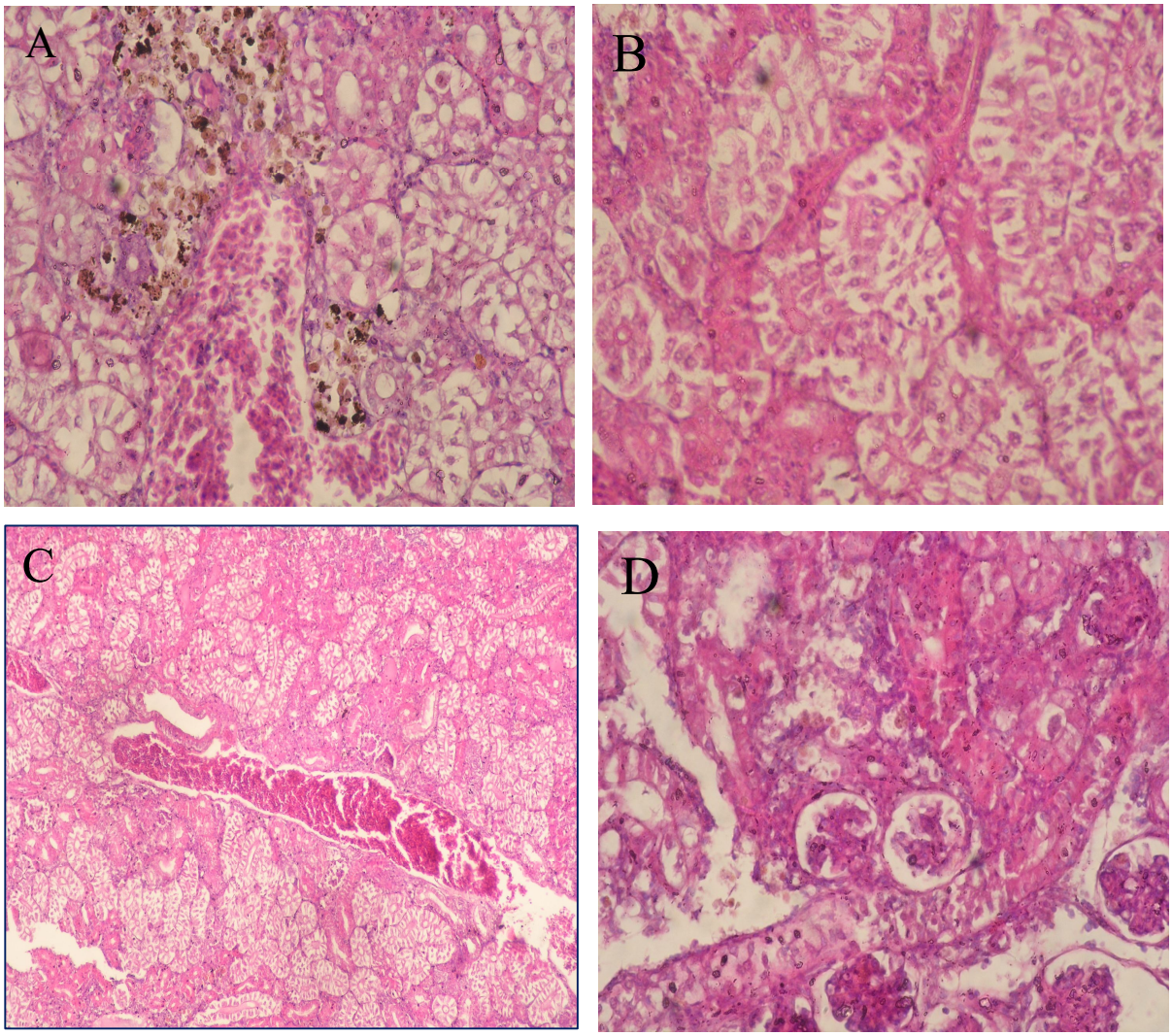

Fig. (8): Photomicrograph of kidney section in fish. **(A)** Kidney of fish exposed to Cd1/10LC_{50} at 22˚C for 21 days shows brown to black pigment deposition, congestion, vacuolation and necrosis of renal tubules (HE, x10) **(B)** Kidney of fish exposed to Cd1/20LC_{50} at 26˚C for 42 days shows necrosis and dissociation of tubular epithelium (HE, x10) (**C**) Kidney of fish exposed to Cd1/10LC_{50} at 26˚C for 21 days shows hemorrhage and coagulative necrosis in renal tubules(HE, x10) (**D**) Kidney of fish exposed to Cd1/10LC_{50} at 29˚C for 21 days shows necrosis of renal tubules and dissolution in renal glomeruli (HE, x10).

DISCUSSION

The plasma protein level can be considered an index of fluid volume disturbances (**Goss and Wood, 1988**). The decrease in proteins level may be due to formation of lipoproteins, which are utilized for repair of damaged cell and tissue organelles or direct utilization by cells for energy requirements (**Ghosh and Chatterjee 1989**) or by disturbance in liver protein metabolism. The present results revealed significant decrease in TP after Cd exposure after 21 days and more decline after 42 days which agree with **Remyla et al. (2008)** results in fresh water fish Catle Catle which be due to reduced protein synthesis or increased protein excretion caused by nephrosis or by cirrhosis. These results can be confirmed by severe congestion, vacuolation and necrosis histopathologically after 21 days.

ALT and AST are frequently used in the diagnosis of damage caused by pollutants in various tissues, such as liver, muscle, and gills (**De la Tore et al. 2000**). The present results shows an increase in plasma AST and ALT activities in fish exposed to Cd after 21 days and their activities increased with increased time to 42 days which caused mainly by leakage of these enzymes from liver cytosol into the bloodstream as a result of liver damage caused by metal exposure. These results are in accordance with the finding of **Almeida et al. (2002)** and **Kaoud et al. (2011)** and attributed the increase in their activities to the hepatocellular damage and impairment of liver parenchyma.

Alkaline phosphatases are intrinsic plasma membrane enzymes found on the membranes of all animal cells which catalyses the dephosphorylation of phosphorylated organic compounds and also involves in bone formation and in membrane transport activities **(Molina et al., 2005).** The measurement of AP activity is carried out in ecotoxicological studies as it serves as an indicator of intoxication because of its sensitivity to metallic salts (**Boge´ et al., 1988**). The present results shows marked elevation of ALP activity after 21 days and its activity increased with increased exposure period to 42 days which agrees with **Atli and Canli (2007)** results which may be attributed to the enhancement of this enzyme can be observed as a signal of severe tissue damage (**Bernet et al. 2001).** A histopathological screening of the liver in the present study revealed severe congestion with vacuolation and necrosis of hepatocytes after exposure to Cd1/10LC_{50} for 3 and 6 weeks which agree with **Guardiola et al., 2013** who reported the same results in Gilthead seabream. The vacuolation of hepatocytes may indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the systemic circulation (**Gingerich,**

1982). Previous studies concluded that Cd, at relatively low, sublethal concentrations, can target vascular endothelial cells at a variety of molecular levels, including cell adhesion molecules, metal ion transporters and protein kinase signalling pathways (**Prozialeck et al., 2006**).

There are several ways in which temperature can influence metal toxicity. Firstly, temperature can affect the general behavior, physiology and activity (**Wolf, 1998**). Secondly, temperature directly and positively affects the metabolic rate (**Vergauwen et al., 2013**), thereby affecting energy metabolism and many other processes. Combined with the energetic cost associated with metal exposure, this could alter toxicity. Thirdly, the metal uptake rate as well as reaction rates can increase with increasing exposure temperature (**Bervoets et al., 1996**). Finally, temperature itself may become a stressor, especially near the thermal tolerance limits (**Heugens et al., 2003**).Results revealed that exposure to high temperatures increase cadmium toxicity, significant increase in activities of ALT, AST and ALP in Cd1/10LC_{50} at 26 and 29˚c than at 22˚c in addition to significant increase in their activities in Cd1/20LC_{50} at 29˚c than at 26˚c after 21 days. Moreover, ALT., AST and ALP levels increased in Cd1/10LC_{50} at 26 than at 22˚c after 42 days. Also, TP level shows significant increase with increased temperature and Cd after 21 days while after 42 days, its level markedly decreased. In other temperature effect studies in fish in which the metal was supplied as waterborne Cd (**Douben, 1989; Kőck et al., 1996**) the temperature dependent increase of metal content was explained by a greater increase in uptake rate than the increase found in the elimination rate (**Douben, 1989**). The increased bioaccumulation in liver with high temperature may explain the increased toxic effects of Cd on liver which reflects on elevated enzyme activities, TP level and histopathological findings. Temperature influences the incidence and severity of histological changes in liver. Severe congestion, vacuolation and necrosis in Cd1/20LC_{50} at 26˚c exposed group increased with rise of temperature to 29˚c which have an agreement with **(Hallare, 2005)** results in Zebrafish emberyo after exposure to $Cdcl_2$ 2mg/L at (21, 26 and 33 ˚C) for 48h. These results agree also with **Salazar-Lugo, 2011** who illustrate the liver histology in fish paraquat exposed showed as initial necrosis in paraquat /29 ◦C group, and generalized necrosis in paraquat /35 ◦C group suggesting that 35 ◦C temperature produced a synergistic effect on paraquat -mediated liver damage.

Creatinine and uric acid levels are indicators of kidney function, urea is the major nitrogen-containing metabolic product of protein metabolism and the elevation in blood urea is known to be correlated with an increased protein catabolism and/or the conversion of

ammonia to urea as a result of increased synthesis of arginase enzyme involved in urea production **(Harper et al., 1979).** This study showed a significant increase in creatinine and urea in fish exposed to Cd for 21 and 42 days which agree with **Abdel-Tawwab and Wafeek, 2010** results which may be due to the action of heavy metal on glomeruli filtration rate (**El-Bagori 2001; Abbass et al. 2002**) and/or Cd may cause pathological changes to the kidney resulting in dysfunction. The latter explanation can be proved with the histological changes in kidney architecture in form of hemosiderosis, congestion, vacuolation and tubular necrosis after 21 days and increased after 42 days. The present study revealed that exposure to high temperatures and Cd lead to significant increase in urea and creatinine after 21and 42 days except urea value after 42 days because the excretion of divalent ions is a major function of renal tubular epithelium and Cd is primarily nephrotoxic metal **(Gingerich, 1982).** The histopathological alterations occurred in the kidney were hemosiderosis, congestion, vacuolation and necrosis of renal tubules after exposure to Cd1/10 LC_{50} for 3 weeks which agree with **Omer et al., 2012**. There was a steady accumulation of Cd in both kidney and liver and an increase in renal metallothionine levels correlated with the accumulation of Cd in the kidney of rainbow trout (**Olsson et al., 1996**). It has been reported that when MT becomes saturated with Cd, it may lead to tubular epithelial cell necrosis (**Chan and Rennert, 1981**). Cd is found in the cell bound to MT association of this avid metal binding protein may be a reason for the low excretion rate of this metal in fish (**Harrison and Klaverkamp, 1989**). The Cd bound to MTs can be removed by exposure to low pH (**Roesijadi, 1992**). Cd inhibited the activity of Na+/H+ exchange that was present on the brush border of the eel kidney tubular cells and was able to alter the passive H+ permeability. The interference of Cd might inhibit the transporter and impair the overall osmoregulatory process (**Vilella et al., 1991**). The cytoarchitecture of kidney showed mild hemorrhage with hemosiderosis in Cd1/10LC_{50} at 22˚c exposed group which developed to severe hemorrhage and coagulative necrosis with rise temperature to 26˚c. In Cd/29˚c exposed group, renal tubules showed necrosis and hemorrhage with dissolution in renal glomeruli. **Salazar-Lugo, 2011** detected that exposure to paraquat at paraquat/29 ◦C lead to intense leukocyte infiltration, principally of basophil cells, as well as hyperplasia of MMC. These lesions were enhanced in paraquat/35 ◦C besides hyperplasia of endothelium with rupture of vascular membrane.

Overall, the present study validates that elevated temperatures increase hepato-renal toxicity induced by cadmium in *Oreochromis niloticus* fish.

REFERENCES

Abbass H. H., Zaghloul K. H. and Mousa M. A. A. (2002): Effect of some heavy metal pollutants on some biochemical and histopathological changes in blue tilapia; *Oreochromis niloticus*. Egyptian Journal of Agricultural Research 80:1385–1411.

Abdel-Tawwab M. and Wafeek M. (2010): Response of Nile tilapia, *Oreochromis niloticus* (L.) to environmental cadmium toxicity during organic selenium supplementation. Journal of the World Aquaculture Society. (41), 1:106-114.

Almeida J.A., Diniz Y.S., Marques S.F.G., Faine L.A., Ribas B.O., Burneiko R.C. and Novelli E.L.B. (2002): The use of the oxidative stress responses as biomarkers in Nile tilapia *(Oreochromis niloticus)* exposed to *in vivo* cadmium contamination. Environment International 27: 673–679.

Atli G. and Canli M. (2007): Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. Comparative Biochemistry and Physiology, Part C 145: 282–287.

Bancroft J.P., Stevenes A., Turner D.R. (1990): Theory and practice of histological techniques, third edition. Clurechill Livingston, Edinburgh, London.

Bernet D., Schmidt H., Wahli T. and Burkhardt-Holm P. (2001): Effluent from a sewage treatment works causes changes in serum chemistry of brown trout (Salmo trutta L.). Ecotoxicol. Environ. Saf. 48: 140–147.

Bervoets L., Blust R. and Verheyen R. (1996): Effect of temperature on cadmium and zinc uptake by the midge larva *Chironomus riparius*. Arch. Environ. Contam. Toxicol. 31: 502–511.

Boge´ G., Bussiere D. and Peres G. (1988): Effect du chrome hexavalent sur les activities enzymatiques et de transport de l'intestin de la truite arc en ciel (*Salmo gairdneri*). Water. Res. 22: 441–447.

Chan C.D. and Rennert O.M. (1981): Cadmium nephropathy. Ann. Clin. Lab. Sci. 11:229-238.

Chowdhury M. J., McDonald D. G., and Wood C. M. (2004): Gastrointestinal uptake and fate of cadmium in rainbow trout acclimated to sublethal dietary cadmium. Aquat. Toxicol. 69: 149–163.

De la Tore FR, Salibian A, Ferrari L (2000): Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. Environ Pollut. 109:227–278.

Douben P.E.T. (1989): Uptake and elimination of waterborne cadmium by the fish *Noemacheilus barbatulus L*. (Stone Loach). Arch. Environ. Con. Tox. 18: 576–586.

El-Bagori H. M. (2001): Pathological studies on some environmental pollution on some freshwater fish in Sharkia Governorate. M.V.Sc. Thesis, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

Ghosh T.K. and Chatterjee S.K. (1989): Influence of nuvan on the organic reserves of Indian freshwater murrel *Channa punctatus*. J Environ Biol 10:93–99.

Gingerich W.H. (1982): Hepatic Toxicology of Fishes. In: Aquatic Toxicology, Weber, L.J. (Ed.). Raven Press, New York, pp: 55-105.

Goss G.G. and Wood C.M. (1988): The effects of acid and acid/ aluminium exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout, *Salmo gairdneri*. J Fish Biol 32:63–76.

Gravato C, Teles M, Oliveira M, Santos MA (2006): Oxidative stress, liver biotransformation and genotoxic effects induced by copper in *Anguilla anguilla L.* – the influence of pre-exposure to bnaphthoflavone. Chemosphere 65:1821–1830.

Guardiola F.A., Cuesta A., Meseguer J., Martínez S., Martínez-Sánchez M.J., Pérez-Sirvent C. and Esteban M.A. (2013): Accumulation, histopathology and immunotoxicological effects of waterborne cadmium on gilthead seabream *(Sparus aurata)*. Fish and Shellfish Immunology 35: 792-800.

Hallare A.V., Schirling M., Luckenbach T., Kőhler H.R. and Triebskorn R. (2005): Combined effects of temperature and cadmium on developmental parameters and biomarker responses in zebrafish *(Danio rerio)* embryos. Journal of Thermal Biology 30: 7–17.

Harper H.A., Rodwell V.W., Mayes P.A., Cochrum K.C., Grodsky G.M., Martin D.W., Tyler D.D. and Wallin J.D. (1979): Review of Physiological Chemistry, 17th ed. Lange Medical Publications, Los Altos, California, USA Illus Paper, XV 702p.

Harrison S.E. and Klaverkamp J.F., (1989): Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri*) and lake whitefish (*Coregous clupeaformis*). Environ. Toxicol. Chem. 8: 87–97.

Heath AG (1995) :Water pollution and fish physiology, 2nd ed. CRC Press, New York, pp, 359.

Henry R. J. (1974): Clinical Chemistry, Principles and Techniques (2nd Edition). Haper and Row Publishers. New York.

Heugens E.H.W., Jager T., Creyghton R., Kraak M.H.S., Hendriks A.J., Van Straalen N.M., Admiraal W. (2003): Temperature-dependent effects of cadmium on Daphnia magna: accumulation versus sensitivity. Environ. Sci. Technol. 37:2145–2151.

IPCC. (2001): Summary for policy makers. Third assessment report. Intergovernmental Panel of Climate Change. pp, 1–18.

Kaoud H.A., Zaki M.M., El-Dahshan A.R., Saeid S. and ElZorba H.Y. (2011): Amelioration the toxic effects of cadmium exposure in Nile tilapia (*Oreochromis Niloticus*) by using Lemna gibba L.Life Science Journal, 8(1):185-195.

Kingsley, G,R: Bio, chem..,131,197-200 (1939).

Kőck G., Triendl M. and 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.

Molina R., Moreno I., Pichardo S., Jos A., Moyano R., Monterde J.G. and Camean A. (2005): Acid and alkaline phosphatase activities and pathological changes induced in Tilapia fish (*Oreochromis sp.)* exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. Toxicon 46: 725–735.

Olsson P.E., Larsson A. and Haux C. (1996): Influence of seasonal changes in water temperature on cadmium inducibility of hepatic and renal metallothionein in rainbow trout. Mar. Environ. Res. 42: 41–44.

Omer S.A., Elobeid M.A., Fouad D., Daghestani M.H., Al-Olayan E.M., Elamin M.H., Virk P. and El-Mahassna A. (2012): Cadmium bioaccumulation and toxicity in Tilapia fish (*oreochromis niloticus*). Journal of Animal and veterinary advances11 (10):1601-1606.

Parvez S., Pandey S., Ali M. and Raisuddin S. (2006): Biomarkers of oxidative stress in Wallago attu (Bl. and Sch.) during and after a fish-kill episode at Panipat, India. Sci Total Environ 368:627–636.

Prosser C.L. (1961): Oxygen respiration and metabolism. In: Prosser CL, Brown FA Jr, editors. Comparative Animal Physiology. Philadelphia: WB Saunders. PP: 688.

Prozialeck W.C., Edwards J.R. and Woods J.M. (2006): The vascular endothelium as a target of cadmium toxicity. Life Sci; 79(16): 493-506.

Remyla S.R., Ramesh M., Sajwan K.S. and Kumar K.S. (2008): Influence of zinc on cadmium induced haematological and biochemical responses in a freshwater teleost fish Catla catla. Fish Physiol Biochem 34:169–174**.**

Roesijadi G., (1992): Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat. Toxicol. 22: 81–114.

Salazar-Lugo R., Mata C., Oliveros A., Rojas L.M., Lemus M. and Rojas-Villarroel E. (2011): Histopathological changes in gill, liver and kidney of neotropical fish *Colossoma macropomum* exposed to paraquat at different temperatures. Environmental toxicology and pharmacology 31: 490–495.

Sassi A., Annabi A., Kessabi K., Kerkeni A., Said K. and Messaoudi I., (2010): Influence of high temperature on cadmium-induced skeletal deformities in juvenile mosquitofish (*Gambusia affinis*). Fish Physiology and Biochemistry 36, 403–409.

Soares S.S., Martins H., Gutie´rrez-Merino C. and Aureliano M. (2008): Vanadium and cadmium in vivo effects in teleost cardiac muscle: metal accumulation and oxidative stress markers. Comp**.** Bochem Physiol (C): 147:168–178**.**

Thomann R. V., Shkreli F. and Harrison S. (1997): A pharmacokinetic model of cadmium in rainbow trout. Environ. Toxicol. Chem. 16, 2268–2274.

Tietz N.W. (1976): Fundamentals of Clinical Chemistry W.B. Saunders Co., Philadelphia.

USEPA. 2001. Update of ambient water quality criteria for cadmium. EPA-822-R-01 001. United States Environmental Protection Agency (USEPA). Washington, DC, USA.

Vassault, A.Ann.Biol. Clin., 44,686,(1986).

Vellinger C., Felten V., Sornom P., Rousselle P., Beisel J.N. and Usseglio-Polatera P. (2012): Behavioural and physiological responses of *gammarus pulex* exposed to

cadmium and arsenate at three temperatures: individual and combined effects. PLoS ONE 7(6): e39153.

Vergauwen L., Knapen D., Hagenaars A., De Boeck G. and Blust R. (2013): Assessing the impact of thermal acclimation on physiological condition in the zebrafish model. J. Comp. Physiol. B. 183: 109–121.

Vilella S., Zonno V., Cassano G., Maffia M. and Storelli C. (1991): Na+/H+ exchange in the kidney of eels (*Anguilla anguilla*) adapted to sea water or to freshwater environments; studies with brush border membrane vesicles. Comp. Biochem. Physiol. (2): 445–460.

Wolf G. (1998): Evaluation of the swimming activity of Daphnia magna by image analysis after administration of sublethal cadmium concentrations. Comp. Biochem. Physiol. 120: 99–105.

Young, D.S, et al.,Clin Chem.21(5),1972.

الملخـــص العـــربي

التأثير السمى للتعرض للكادميوم و الحرارة على الكبد والكلى فى اسماك البلطى النيلى

فتحى رضوان سليم ممدوح محمد ابو المجد علا على حبته

جامعة المنصورة- كلية الطب البيطرى- قسم الطب الشرعى والسموم

أجريت هذة التجربة لدراسة التأثير السمى للكادميوم والحرارة في اسماك البلطى النيلى من خلال دراسة التغيرات فى كلا من فحوصات بلازما الدم والدراسات الهستوباثولوجية للكبد والكلى.

اجريت الدراسة على ١٢٠ سمكة متوسط وزنها ٦٠-٧٠ جرام . بعد فترة اسبوعين من الملاحظة المعملية تم تقسيمهم الى ٦ مجموعات تحتوى كل مجموعة ٢٠ سمكة موزعة على حوضين(١٠ لكل حوض): المجموعة الاولى ضابطة وكانت درجة حرارة المياه بها ٢٢°م والثانية تم تعرضها لجرعة ٣,٤٤ مجم كادميوم /لتر وكانت درجة حرارة المياه بها ٢٢°م والثالثة تم تعرضها لجرعة ١,٧٧ مجم كادميوم/لتر وكانت درجة حرارة المياه بها ٢٦°م والرابعة تم تعرضها لجرعة ٣,٤٤ مجم كادميوم/لتر وكانت درجة حرارة المياه بها ٢٦°م والخامسة تم تعرضها لجرعة ١,٧٧ مجم كادميوم /لتر وكانت درجة حرارة المياه بها ٢٩°م والسادسة تم تعرضها لجرعة ٣,٤٤ مجم كادميوم /لتر وكانت درجة حرارة المياه بها ٢٩°م.و قد تم أخذ عينات الدم فى اليوم٢١ و٤٢من بداية التجربة و اجراء الصفة التشريحية وتم فصل بلازما الدم لمعرفة نشاط الانزيمات الخاصة بوظائف الكبد والبروتين الكلى واليوريا والكرياتينين.

واسفرت النتائج عن ان الكادميوم يسبب زيادة ملحوظة فى كلا من نشاط الانزيمات الخاصة بوظائف الكبد واليوريا والكرياتينين ونقص ملحوظ فى مستوى البروتين الكلى بعد ٢١ و٤٢ يوما من التجربة. والدراسات الهستوباثولوجية وجدنا فيها تغيرات مرضية فى كلا من الكبد والكلى.

وايضا وجود تاثير واضح للحرارة على التأثير السمى للكادميوم فقد وجد زيادة ملحوظه فى كلا من نشاط الانزيمات الخاصة بوظائف الكبد والبروتين الكلى والكرياتينين اليوريا بعد ٢١ يوم من التجربة اما بعد ٤٢ يوم من التجربة وجد نقص ملحوظ فى كلا من البروتين الكلى واليوريا مع استمرار زيادة باقى المؤشرات والدراسات الهستوباثولوجية وجدنا فيها تغيرات مرضية واضحة فى كلا من الكبد والكلى التى تزداد ايضا استجابة الى رفع درجة الحرارة.

و قد خلصت الدراسة الى ان ارتفاع الحرارة يؤدى الى زيادة التأثير السمى للكادميوم على الكبد والكلى فى اسماك البلطى النيلى