http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (18): 854-862, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.854.862

Effect of Lead Nitrate on the Liver of the Cichlid Fish (*Oreochromis niloticus*): A Light Microscope Study

[1]Bothaina M. Khidr, [2]Imam A.A. Mekkawy, [3]Ahmed S.A. Harabawy and [4]Abdel Salam M.I. Ohaida
[1]Department of Zoology, Faculty of Science, Assiut University, Egypt
[2]Department of Biology, Faculty of Science, Taif University, Saudi Arabia
[3]Department of Biology, Faculty of Science, North Jeddah,
King Abdul Aziz University, Saudi Arabia
[4]Department of Zoology, Faculty of Science, 7 October Misurata University, Libya

Abstract: The adverse impacts of heavy metals on fish liver were evident with great variability among organs and species. The present study deals with the histological changes of the hepatocytes of the Nile tilapia, *Oreochromis niloticus*, following exposure to 2.5, 5, 10 ppm of lead nitrate for 1, 2, 3, 4 weeks. The present results revealed that lead nitrate exerts some histological effects on the hepatic tissue after exposure to the first concentration in the form of dilatation and congestion of the blood vessels, vacuolation of hepatic cells, proliferation of connective tissue and hepatic necrosis. Leucocyte aggregation-mostly lymphatic in nature-was seen infiltrating hepatic tissue. These alterations became more pronounced in liver of fishes exposed to second concentrations indicating more progressive signs of necrosis. The presence of eosinophilic oedematous areas surrounding some blood vessels was also observed. Finally, at the third concentration, in addition to the above alterations, melanomacrophages, which store lipofuscin at the site of necrosis, were observed. These histological results imply that the fish liver may serve as a target organ for the toxicity of sublethal concentrations of lead nitrate.

Key words: Lead, liver, histopathology, *Oreochromis niloticus*, toxicity

INTRODUCTION

Lead is widely included in different industrial products such as pipes, battery cases, paints as well as petrol additives in the form of tetra-ethyl lead. Toxicity of lead on fish was considered by many authors including Weir and Hine (1970), Aronson (1971), Davies *et al.* (1976), Hussein and Mekkawy (2001), Khidr and Mekkawy (2008), Khidr *et al.* (2008) and Taweel *et al.* (2012). All life stages of fish are sensitive to the toxic effects of Pb (Mekkawy and Lashein, 2003; Osman *et al.*, 2007a, b, 2008); however, embryos are more sensitive to Pb than are later juvenile stages (Davies *et al.*, 1976; Mekkawy and Lashein, 2003). Pb toxicity can be altered by water quality especially hardness (Weir and Hine, 1970; Davies *et al.*, 1976). Generally, it is postulated that lead (Pb) is one of the most important pollutants in our environment which accumulates in the body due to its low rate of elimination; its biological half-life in bones is about 27 years (Shibamoto and Bjeldanes, 1993).

In Egypt, the river Nile suffers from lead pollution in some areas along its course especially in Assiut Governorate. This contamination could be attributed to several factories: superphosphate, petroleum, cement, soap and oil factories and large number of garbage dump which situated along its course in Assiut (Abdel-Nasser *et al.*, 1996a). Abdel-Nasser *et al.* (1996b) reported that the lead concentrations in water at different areas of river Nile in Assiut Governorate ranged from 0.200 to 1.940 ppm a value above the permissible limit of WHO (0.10 ppm) or United States Environmental Protection Agency (0.05 ppm). In the same trend, the lead levels in water were 0.18 and 0.257 ppm in Tanta and Helwan areas, respectively (Rizk *et al.*, 1999; Ahmed, 1996).

Histological studies on different fish organs were carried out to evaluate the impacts of different types of stress including diet, heavy metals and pesticides stress (Arnold *et al.*, 2000; Segnini *et al.*, 2000; Jiraungkoorskul *et al.*, 2002; Yang and Chen, 2003; Mekkawy *et al.*, 2007, 2008, 2011a, 2012; Mobarak and Sharaf, 2011; Sayed *et al.*, 2011, 2012a, b; Taweel *et al.*, 2012; Bais and Lokhande, 2012). Most of these studies highlighted on the histopathology of the liver, kidney and other organs. Different degrees of impacts were recorded depending on the dose, age and fish species. According

Corresponding Author: Imam A.A. Mekkawy, Department of Biology, Faculty of Science, Taif University, Saudi Arabia

to the previous findings, the present work was suggested and aimed to studying the effect of lead on histological changes of the liver of the Nile fish, *Oreochromis niloticus*.

MATERIALS AND METHODS

Specimens collection: Forty specimens of the Nile tilapia, *Oreochromis niloticus* (36.38±0.77 g) in weight and 12.93±0.88 cm in length) were caught in September 2003 from Umm Al-Kossor hatchery about 35 km away from Assiut and transported immediately to the fish laboratory at the Zoology Department, Assiut University.

Experimental design: The experimental fishes were raised in aerated glass aquaria (75×30×30 cm), each of 150 L capacity and acclimatized for two weeks before being used in the experimental study. The experimental fish were fed pellets at a rate of 3% of wet weight twice daily. Feces and residual feed were aspirated regularly. Fishes were weighed and measured at the first day and classified randomly into 4 groups (10 fish each).

Stock solution (1000 ppm) of lead as lead nitrate Analar (BDH) (Pb $[NO_3]_2$) was prepared and stored in clean glass bottles and diluted to concentrations of 2.5, 5.0 and 10.0 ppm. Such low sublethal lead concentrations were chosen according to levels monitored by Abdel-Nasser *et al*. (1996a) and Abdel-Rahman (1997). Lead doses were prepared and added constantly to the aquarium for four weeks. The experimental period (4 weeks lead exposure) was chosen according to Lashein (1999), Rizk *et al*. (1999) and Hussein and Mekkawy (2001). The test water was replaced daily with the required amount of stock solution to prevent deterioration of water quality and replenish lead levels.

Histopathological preparations: At weekly intervals, representative samples of liver from control and experimental fishes groups (4 surviving fish of each group) were fixed in 10% neutral buffered formalin or aqueous Bouin's. Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 3μ and stained with hematoxylin and eosin (H and E) (Bancroft and Stevens, 1982). For the demonstration of lipofuscin pigment long Ziehl-Neelsen technique was used (Pearse, 1960).

PAS technique (McManus, 1948) was used for demonstration of 1:2 glycol linkage of carbohydrates. Control sections were incubated in human saliva at 37°C for one hour prior to PAS staining. The absence of stained material from such section was taken as an evidence for the presence of glycogen.

For semithin sections, proper sized samples (1 mm^3) from the previous tissues were immediately fixed by immersion in 4% glutaraldehyde in 0.1 M cacodylate buffer for 24 h at 4°C and then rinsed in 0.1 M cacodylate buffer. Tissues were post fixed in 1% osmium tetroxide for 1-2 h at 4 °C; the specimens were washed with cacodylate buffer for few minutes several times and subsequently dehydrated in upgraded ethanol series. Embedding of the processed tissues was carried out in Epon 812. Tissues were sectioned at 1 μm (semithin sections) and stained with toluidine blue.

RESULTS

Control liver: As described in most fish species, lobular organization of hepatic tissue is not conspicuous in the liver of *Oreochromis niloticus*. It rather consists of cord-like structures with hepatic plates emintly visible along blood sinusoids (Fig. 1). The endothelial cells that line these sinusoids as well as their nuclei are flattened and elongated. The blood sinusoids drain into a central vein. Hepatocytes of *Oreochromis niloticus* appear polygonal in shape; each hepatocyte contains a single spherical nucleus. The nuclei are mostly centrally located within the hepatocytes. Mononuclear leucocytes were sometimes observed in some control specimens. These structures were however, few in number if present. Exocrine pancreatic tissue (hepatopancreas) is a pronounced feature in the liver of *Oreochromis niloticus* and is clearly visible as darkly stained tissue around the hepatic portal veins. Macrophage centers are found in the normal liver of *Oreochromis niloticus*, mainly in the vicinity of hepatopancreatic tissue (Fig. 2). The content of these macrophage centers varies in size.

Treatment with 2.5 ppm lead nitrate: Examination of semithin sections of the liver after exposure to 2.5 ppm lead nitrate for 7 to 15 days showed dilatation and wall thickening of some blood vessels, which were congested with blood cells (Fig. 3). Moreover, a considerable number of hepatocytes showed clear cytoplasmic vacuolation with deeply stained nuclei (Fig. 4), while other areas were either still intact or slightly affected.

After 21 days of exposure to the same dose of lead nitrate, besides the proceeding changes, marked proliferation of the connective tissue was seen (Fig. 5). Sometimes patches of leucocytic aggregation, mostly lymphocytic in nature were seen infiltrating hepatic tissue (Fig. 6). By the 28th day of the experiment, areas of hepatic necrosis started to appear in some regions. Other regions showed large patches of haemorrhagic lesions (Fig. 7). Examination of the semithin sections revealed

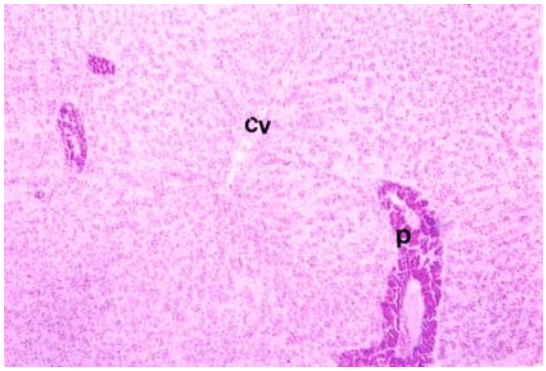
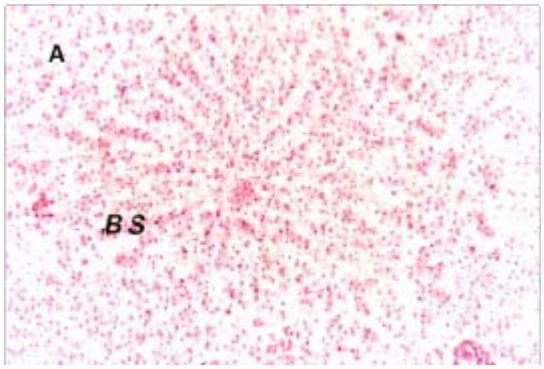


Fig. 1: Photomicrograph of a section of control fish's liver showing CV: central vein, the P: general structure and arrangement of the hepatocytes, diffused exocrine pancreas surrounding the hepatic portal vein. (H and E. X. 100)



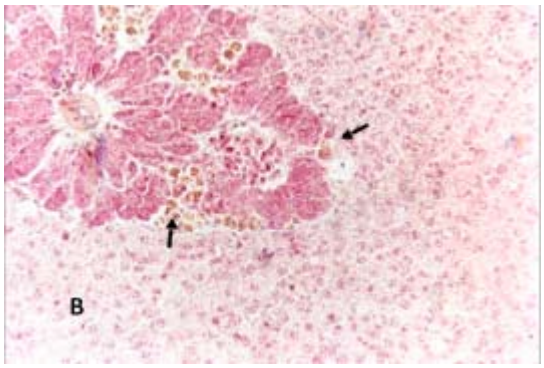


Fig. 2: Two photomicrographs of control fish's liver sections (a) BS: General structure of the liver and the blood sinusoids (BS). (H and E. X. 200) and (b) Exocrine pancreatic tissue (hepatopancreas) surrounding the hepatic portal vein, ↑: Macrophage centers were in the vicinity of hepatopancreatic tissue (H and E. X. 400)

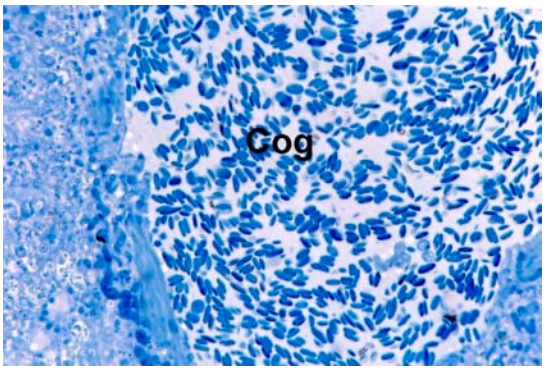


Fig. 3: Photomicrograph of a semithin section of fish's liver exposed to 2.5 ppm of lead nitrate for 7 days showing congested (Cog) and thickened wall of blood vessel. (Toluidine blue. X. 400)

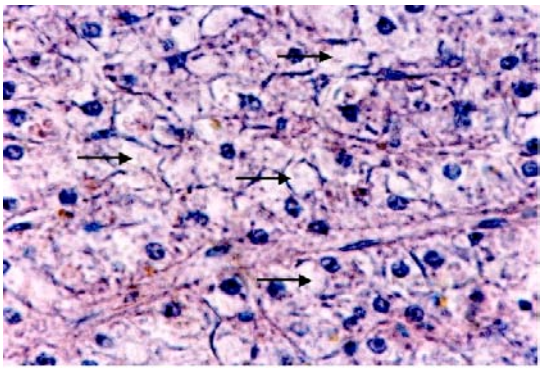


Fig. 4: Photomicrograph of a section of fish's liver exposed to 2.5 ppm of lead nitrate for 7 days ↑: deeply stained nuclei and the vacuolated cytoplasm (arrows). (H and E. X. 1000)

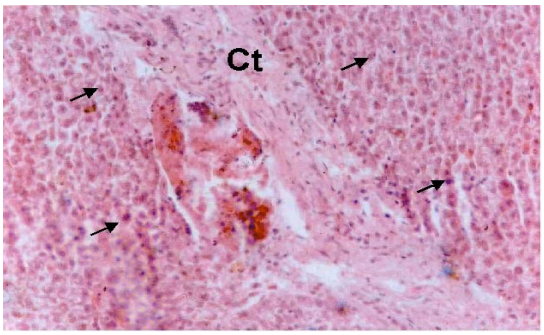


Fig. 5: Photomicrograph of a section of fish's liver exposed to 2.5 ppm of lead nitrate for 21 days CT: The proliferation of intrahepatic connective tissue and ↑: pyknotic nuclei. (H and E. X. 400)

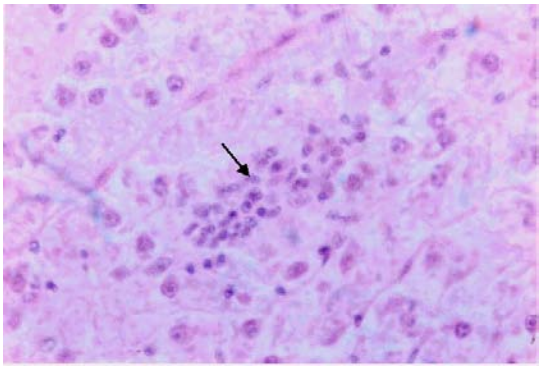


Fig. 6: Photomicrograph of a section of fish's liver exposed to 2.5 ppm of lead nitrate for 21 days showing inflammatory leucocytic infiltration (arrow). (H and E. X. 1000)

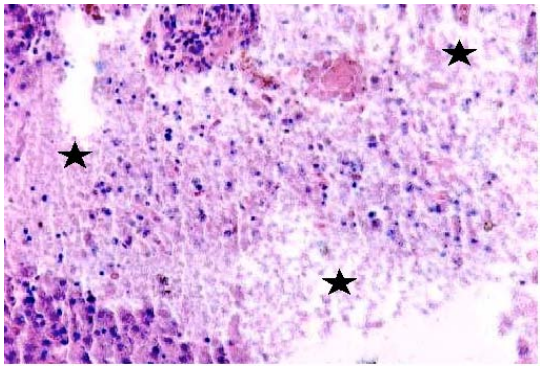


Fig. 9: A photomicrograph of a section of fish's liver exposed to 5 ppm of lead nitrate for 7 days showing necrotic areas (★). (H and E. X. 400)

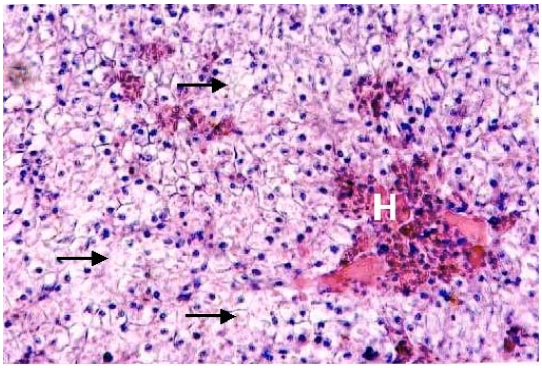


Fig. 7: Photomicrograph of a section of fish's liver exposed to 2.5 ppm of lead nitrate for 28 days ↑: degeneration of hepatic cells with patches of haemorrhages (H). (H and E. X. 400)

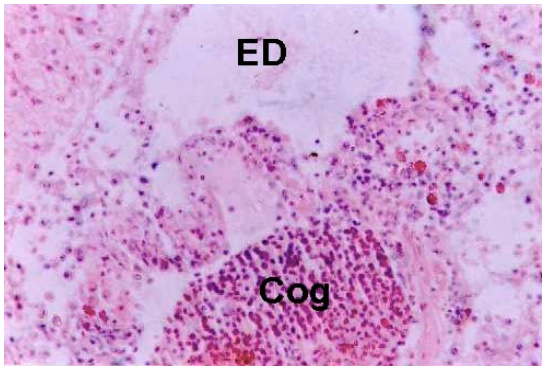


Fig. 10: Photomicrograph of a section of fish's liver exposed to 5 ppm of lead nitrate for 14 days ED: Faintly stained eosinophilic oedematous area Cog: surrounding congested blood vessel. (H and E. X. 400)

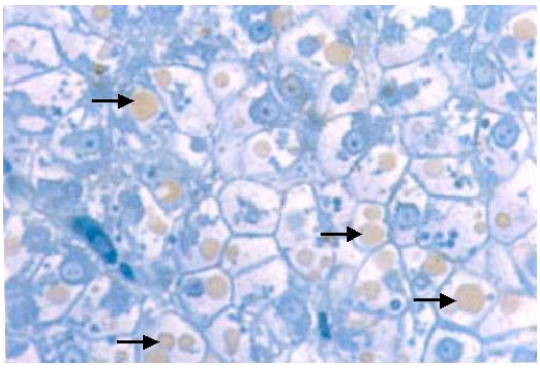


Fig. 8: Photomicrograph of a semithin section of fish's liver exposed to 2.5 ppm of lead nitrate for 28 days ↑: lipid droplets in the cytoplasm and a dark areas around the nucleus against lighter stained cytoplasm. (Toluidine blue, X.1000)

numerous lipid droplets in the cytoplasm, which also contained clumped cellular organelles (Fig. 8). The latter appeared as dark areas around the nucleus against the lighter staining cytoplasm.

Treatment with 5 ppm lead nitrate: Examination of liver sections of fish after 7 to 14 days of 5 ppm lead nitrate exposure displayed more progressive signs of necrosis compared to those of the previous group (2.5 ppm lead nitrate) (Fig. 9). The hepatocytes membranes were ruptured with dispersion of cellular contents and loss of stainability (Fig. 9). Other signs of degeneration were manifested including the presence of faintly stained eosinophilic oedematous areas surrounding some blood vessels (Fig. 10). Moreover, the vessels were severely congested with blood and some inflammatory cells.

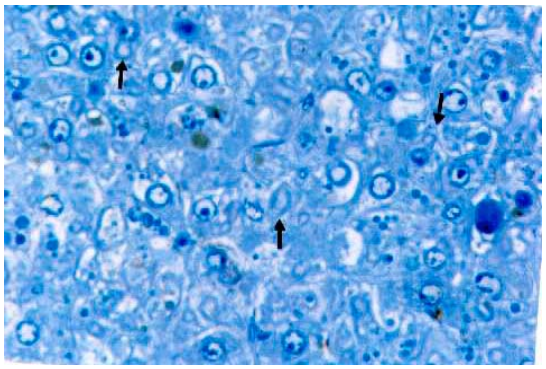


Fig. 11: Photomicrograph of a semithin section of fish's liver exposed to 5 ppm of lead nitrate for 14 days ↑: circular basophilic profiles in the cytoplasm. (Toluidine blue, X. 1000)

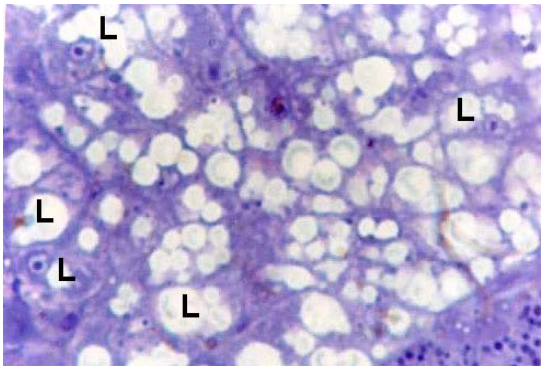


Fig. 12: A photomicrograph of a semithin section of fish's liver exposed to 10 ppm of lead nitrate for 14 days L: Accumulation of lipid droplets in the cytoplasm of hepatocytes. (Toluidine blue, X. 1000)

Examination of the semithin sections revealed basophilic circular profiles around the nuclei and in the cytoplasm in addition to lipid droplets (Fig. 11). After 21 to 28 days of exposure to the same dose of lead nitrate, a progressive increase in the number of the circular profiles was observed.

Treatment with 10 ppm lead nitrate: Exposure of fish to 10 ppm lead nitrate for 7 to 14 days showed histopathological changes in the liver. Examination of the semithin sections showed numerous lipid droplets in the cytoplasm (Fig. 12). Other hepatocytes showed basophilic granules. After 21 to 28 days of lead exposure, necrotic changes associated with lymphocytic infiltration were detected. Moreover, melanomacrophages, which store lipofuscin at the site of necrosis were also observed in addition to dissociation of hepatic tissue (Fig. 13, 14).

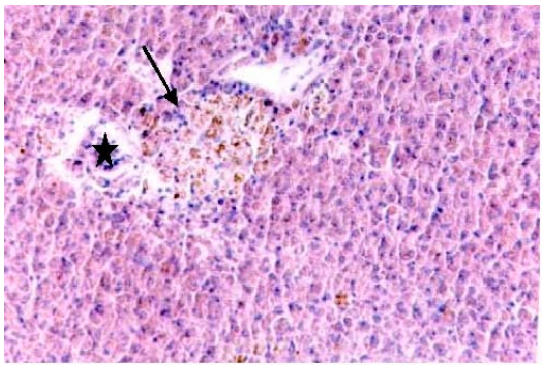


Fig. 13: Photomicrograph of a section of fish's liver exposed to 10 ppm of lead nitrate for 21 days showing necrotic area (★) associated with the presence of lymphocytes and melanomacrophages. (H and E. X. 200)

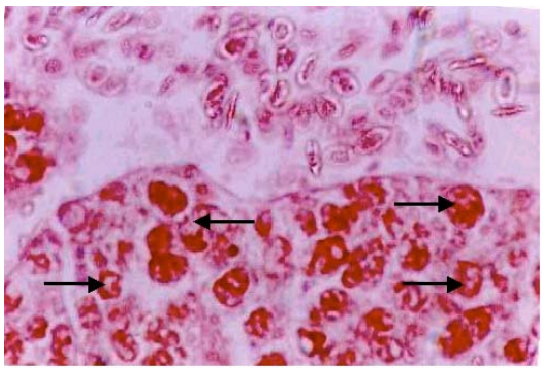


Fig. 14: Photomicrograph of a section of fish's liver exposed to 10 ppm of lead nitrate for 21 days showing the ↑: Melanomacrophage storing lipofuscin pigments. (Long Ziehl. Neelsen technique, X. 1000)

Histochemical studies: In the hepatocytes of control animals, a considerable amount of polysaccharide material was observed in the cytoplasm as shown by their strong PAS positive reaction (Fig. 15). All these positively stained materials have been proved to be glycogen as verified by PAS staining with and without previous treatment with diastase. In all experimental groups, the hepatocytes displayed a remarkable depletion in the glycogen content. The cytoplasm of different cells exhibited a faint colouration with PAS (Fig. 16).

DISCUSSION

In the present investigation, a remarkable collection of inflammatory invasion of the parenchymal cells of the

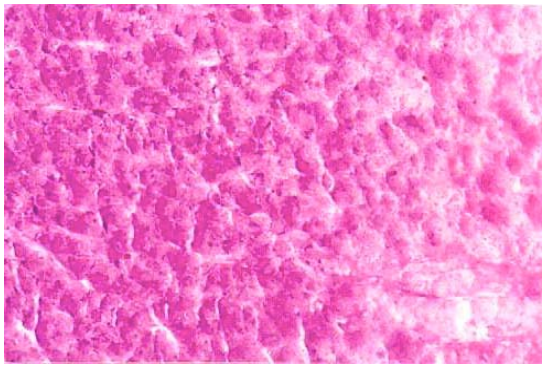


Fig. 15: Photomicrograph of a section of control fish's liver showing glycogen content in the hepatocytes. (PAS. X. 400)

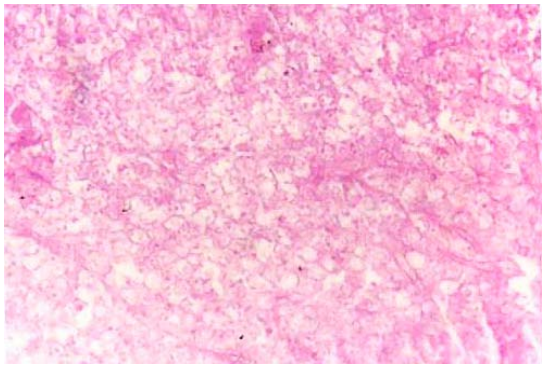


Fig. 16: Photomicrograph of a section of fish's liver exposed to 2.5 ppm of lead nitrate for 7 days showing a remarkable depletion in glycogen. (PAS. X. 400)

liver were observed after exposure to lead nitrate. Walter and Israel (1974) suggested that such cellular infiltration may due to the presence of necrotic cells which act as an irritant substance attracting the inflammatory cells. Moreover, El-Banhawy *et al.* (1993) suggested that abundance of leucocytes -in general- and lymphocytes -in particular- are a prominent response of body tissues facing any injurious impact.

In the present study, lipofuscin pigment in melanomacrophage was detected. Melanomacrophages are known to accumulate antigenic materials (Roberts, 1975), store products difficult to eliminate lipofuscin and ceroids (Wood and Yasutake, 1956) and store metals i.e., iron as haemosiderin (Agius, 1979). The number, size and contents of melanomacrophages are highly variable, not only between species but also related to the health status of fish (Matthiessen and Roberts, 1982; Bunton *et al.*, 1987). Infiltration of the necrosed hepatic cells by migrating melanomacrophages in the present study support the suggestion that these cells infiltrate the damaged tissue to engulf lipofuscin pigments. On the other hand, Cheville (1983) suggested that lipofuscin pigments are found in lysosomes of cells undergoing progressive and prolonged oxidation of unsaturated lipids and are a pathognomonic sign of free radical and associated peroxidative injury to polyunsaturated lipids of subcellular membranes (Robbins and Kumar, 1987).

A drastic decrease in glycogen amount in *Oreochromis niloticus* exposed to lead nitrate was observed in the present investigation. In addition, other studies showed a reduction in hepatic glycogen following exposure to xenobiotic compounds (Gluth and Hanke, 1985, Braunbeck, 1992, Mekkawy *et al.*, 2012) which can be explained by increased glycolytic activity to meet the energy demands imposed by enhanced metabolic activity (Hanke *et al.*, 1983; Gluth and Hanke, 1985). Such activity was confirmed biochemically by Mekkawy *et al.* (2011b). Moreover, lead acetate intoxication stimulated the adrenals in rats (Wright *et al.*, 1975) via inducing the release of adrenal catecholamines causing glycogenolysis which was also observed in the fish *Anabas scandens* (Chandravathy and Reddy, 1996). Thus it can be concluded that lead would disturb the carbohydrate metabolism. Other physiological processes such as sexual maturation (Yamamoto and Egami, 1974), or nonchemical stresses, such as temperature (Braunbeck *et al.*, 1987) and hypoxia (De Zwaan and Zandee, 1972; Yuness, 2005) are associated with glycogen depletion; hence, hepatocyte glycogen is non-specific parameter indicating stress of the orgamism (Sylvie *et al.*, 1996). On the other hand, long-term exposure to contaminants lead to accumulation of glycogen in the fish liver (Kranz and Peters, 1985).

Our results showed that the liver of *Oreochromis niloticus* exposed to lead nitrate, displayed an increase in the amount of hepatocellular lipid deposits. Fatty infiltration in the liver has been experimentally induced in cat, fish and Atlantic cod which was fed Polychlorinated Biphenyl's (PCBs) (Hinton *et al.*, 1978; Freeman *et al.*, 1982). A similar increase in lipid droplets was also found in liver cells of *Pseudopleuronects americanus* after copper poisoning (Baker, 1969) and in *Carassius auratus* after lead exposure (Franchini *et al.*, 1991). The increase in cytoplasmic lipids may be related to the hypoxic condition induced by lead as observed in other tissues (Fantin *et al.*, 1985). In conclusion, the present study suggested that lead nitrate exerts some toxic effects of the liver of *Oreochromis niloticus* with regards to the histological changes. Under lead sublethal dose of

45 mg L^{-1}, blood congestion in sinusoids, vaculation of hepatocytes and necroses in the liver of *Oreochromis niloticus* were observed (Lamchumchang *et al*., 2007). These authors and Kosai *et al*. (2011) reported that dietary Ca^{2+} will be protective in reducing Pb burdens in fish exposed to environments contaminated with waterborne Pb. In a similar way, Hussein and Mekkawy referred to the clay as protective agent in reducing Pb-toxicity. Mekkawy *et al*. (2011b, 2012) evaluate the dietary tomato paste and vitamin E as a protective agent against toxicity of cadmium.

CONCLUSION

Finally, one can conclude that lead toxicity on fishes could be evaluated by histological characteristics of liver. Such lead-induced histological changes are function of heavy metal doses, time of exposure and fish species. These pathologic alterations reflect the stress-induced biochemical and physiological changes. Further studies are required to evaluate the diet supplementary protective natural antioxidants in counteracting the lead adverse impacts on fishes.

REFERENCES

Abdel-Nasser, M., A.A. Shaaban, S.M. Aly and M.M. Sayed, 1996a. Lead, copper, Mercury and Cadmium levels in river Nile waters at some assiut regions. Egypt. Assiut Vet. Med. J., 34: 85-94.

Abdel-Nasser, M., A.A. Shaaban, S.M. Aly and M.M. Sayed, 1996b. Levels of some heavy metals in fish caught from River Nile at Assiut Governorate, Egypt. Assiut Vet. Med. J., 34: 68-84.

Abdel-Rahman, M.A., 1997. Toxicological studies of heavy metals on *Siganus rivulatus*. M.Sc. Thesis, Alexandria University, Alexandria, Egypt.

Agius, C., 1979. The role of melano-macrophage centers in iron storage in normal and diseased fish. J. Fish Dis., 2: 337-343.

Ahmed, M.A., 1996. Environmental studies on the effect of some heavy metals pollution on tilapia fish in Egypt. M.V.Sc. Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt.

Arnold, B.S., C.H. Jagoe, T.S. Gross, E.W. Howerth and R.E. Reinert, 2000. Effects of low levels of methyl mercury on the development of gonads in the Nile Tilapia, *Oreochromis niloticus*. Proceedings of the 20th Annual Meeting of the Society of Environmental Toxicology and Chemistry, November 14-18, 1999, Philadelphia, PA., USA.

Aronson, A.L., 1971. Biologic effects of lead in fish. J. Washington Acad. Sci., 61: 124-128.

Bais, U.E. and M.V. Lokhande, 2012. Effect of cadmium chloride on histopathological changes in the freshwater fish *Ophiocephalus striatus* (*Channa*). Int. J. Zool. Res., 8: 23-32.

Baker, J.T.P., 1969. Histopathology and electron microscopical observations on copper poisoning in the winter flounder (*Pseudopleuronectes americanus*). J. Fish. Res. Board Canada, 26: 2785-2793.

Bancroft, D. and A. Stevens, 1982. Theory and Practice of Histological Techniques. 2nd Edn., Churchill Livingstone, Edinburgh, London, Melaborne, Pages: 262.

Braunbeck, T., 1992. Detection of environmentally relevant concentrations of toxic organic compounds using histological and cytological parameters: Substance-specificity in the reaction of rainbow trout liver. Proceedings of the EIFAC Symposium on Sublethal and Chronic Effects of Pollutants on Freshwater Fish, May 19-22, 1992, Lugano Switzerland.

Braunbeck, T., K. Gorgas, V. Storch and A. Volki, 1987. Ultrastructure of hepatocytes in golden ide (*Leuciscus idus melanotus* L.: Cyprinidae: Teleostei) during thermal adaptation. Anat. Embryol., 175: 303-313.

Bunton, T.E., S.M. Baksi, S.G. George and J.M. Frazier, 1987. Abnormal hepatic copper storage in a teleost fish (*Morone americana*). Vet. Pathol., 24: 515-524.

Chandravathy, V.M. and S.L.V. Reddy, 1996. Lead nitrate exposure changes in carbohydrate of freshwater fish, *Anabas scandems*. J. Environ. Biol., 17: 75-79.

Cheville, N.F., 1983. Cell Degeneration. In: Cell Pathology, Cheville, N.F. (Ed.). 2nd Edn. Iowa State University Press, Ames, IA., USA., pp: 75-129.

Davies, P.H., J.P. Goettl Jr., J.R. Sinley and N.F. Smith, 1976. Acute and chronic toxicity of lead to rainbow trout, *Salmo gairdneri* in hard and soft water. Water Res., 10: 199-206.

De Zwaan, A. and D.I. Zandee, 1972. The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis* L. Comp. Biochem. Physiol. Part B: Comp. Biochem., 43: 47-54.

El-Banhawy, M.A., S.M. Sanad, S.A. Sakr, I.A. El-Elaimy and H.A. Mahran, 1993. Histopathological studies on the effect of anticoagulant rodenticide Brodi facoum on the liver of rat. J. Egypt. Ger. Soc. Zool., 17: 369-393.

Fantin, A.M., A. Franchini, E. Ottaviani and L. Benedetti, 1985. Effect of pollution on some freshwater species. II. Bioaccumulation and toxic effects of experimental lead pollution on the ganglia in *Viviparus ater* (Mollusca, Gastropoda). Basic Applied Histochem., 29: 377-387.

Franchini, A., E. Barbanti and A.M. Fantin, 1991. Effects of lead on hepatocyte ultrastructure in *Carassius carassius* (L.) Var auratus. Tissue Cell, 23: 893-901.

Freeman, H.C., G. Sangalang and B. Fleming, 1982. The sublethal effects of a polychlorinated biphenyl (Aroclor 1254) diet on the Atlantic cod (*Gadus morhua*). Sci. Total Environ., 24: 1-11.

Gluth, G. and W. Hanke, 1985. A comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. Ecotoxicol. Environ. Safety, 9: 179-188.

Hanke, W., G. Gluth, H. Bubel and R. Muller, 1983. Physiological changes in carps induced by pollution. Ecotoxicol. Environ. Safety, 7: 229-241.

Hinton, D.E., J.E. Klaunig and M.M. Lipsky, 1978. PCB-induced alterations in teleost liver: A model for environmental disease in fish. Mar. Fish. Rev., 40: 47-50.

Hussein, S.Y. and I.A.A. Mekkawy, 2001. The effects of lead-exposure and lead-clay interaction on the growth performance, biochemical and physiological characteristics and histopathology of *Tilapia zillii*. Bull. Fac. Sci., Assiut Univ., 30: 65-97.

Jiraungkoorskul, W., E.S. Upatham, M. Kruatrachue, S. Sahaphong, S. Vichasri-Grams and P. Pokethitiyook, 2002. Histopathological effects of roundup, a glyphosate herbicide, to Nile tilapia (*Oreochromis niloticus*). ScienceAsia, 28: 121-127.

Khidr, B.M. and I.A.A. Mekkawy, 2008. Lead nitrate induced ultrastructural changes of renal tubules of the kidney of the Nile Tilapia fish (*Oreochromis niloticus*). J. Egypt. Ger. Soc. Zool., 56: 247-269.

Khidr, B.M., I.A.A. Mekkawy, A.S. Harabawy and A.M. Ohaida, 2008. Effect of separate and combined lead and selenium on the liver of the Cichlid fish *Oreochromis niloticus*: Ultrastructural study. Egypt. J. Zool., 50: 89-119.

Kosai, P., W. Jiraungkoorskul, A. Synsatayakul and K. Jiraungkoorskul, 2011. Efficacy of calcium reducing lead toxicity in hematology of *Oreochromis niloticus*. J. Fish. Aquat. Sci., 6: 346-355.

Kranz, H. and N. Peters, 1985. Pathological conditions in the liver of ruffe, *Gynmocephalus cernua* (L.), from the Elbe estuary. J. Fish Dis., 8: 13-24.

Lamchumchang, K., W. Jiraungkoorskul, S. Sahaphong, T. Tansatit, P. Kosai and R. Madarasmi, 2007. Dietary calcium reducing effects of waterborne lead uptake in Nile Tilapia (*Oreochromis niloticus*). AJAVA., 2: 104-114.

Lashein, F.E., 1999. Effect of cadmium and lead on ontogeny and function of chloride cells in the larval stages of the grass carp, *Cteuopharyngodon idella* (Cuvier and Valenciennes, 1844). Egypt. J. Zool., 32: 125-140.

Matthiessen, P. and R.J. Roberts, 1982. Histopathological changes in the liver and brain of fish exposed to endosulfan insecticide during tsetse fly control operations in Botswana. J. Fish Dis., 5: 153-159.

McManus, J.F.A., 1948. Histological and histochemical uses of periodic acid. Biotech. Histochem., 23: 99-108.

Mekkawy, I.A.A. and F.E. Lashein, 2003. The effect of lead and cadmium on LDH and G-6-PDH isozyme patterns exhibited during the early embryonic development of the teleost fish, *Ctenopharyngodon idellus* with emphasis on the corresponding morphological variations. Proceeding of the 26th Annual Larval Fish Conference, July 22-26, 2002, Bergen, Norway.

Mekkawy, I.A.A., U.M. Mahmoud and A.T. Ibrahim, 2007. Heavy metal distribution and the corresponding damage in some organs of *Bagrus bajad* (Forsskal, 1775) collected from three localities at Assiut, Egypt. J. Egypt. Ger. Soc. Zool., 54: 119-137.

Mekkawy, I.A.A., U.M Mahmoud and A.T.A. Ibrahim, 2008. Heavy metal distribution in some organs of *Oreochromis niloticus* (Linnaeus, 1758) collected from three localities at Assiut, Egypt and the corresponding tissue damage. J. Egypt. Ger. Soc. Zool., 55: 97-127.

Mekkawy, I.A., U.M. Mahmoud and A.E.D.H. Sayed, 2011a. Effects of 4-nonylphenol on blood cells of the African catfish *Clarias gariepinus* (Burchell, 1822). Tissue Cell, 43: 223-229.

Mekkawy, I.A.A., U.M. Mahmoud, E.T. Wassif and M. Naguib, 2011b. Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. Fish Physiol. Biochem., 37: 71-84.

Mekkawy, I.A.A., U.M. Mahmoud, E.T. Wassif and M. Naguib, 2012. Protective roles of tomato paste and vitamine on cadmium-induced histological and histochemical changes of liver of *oreochromis niloticus* (linnaeus, 1758). J. Fish. Aquat. Sci., 7: 240-265.

Mobarak, Y.M.S. and M.M. Sharaf, 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.

Osman, A.G.M., I.A. Mekkawy, J. Verreth, S. Wuertz, W. Kloas and F. Kirschbaum, 2008. Monitoring of DNA breakage in embryonic stages of the African catfish *Clarias gariepinus* (Burchell, 1822) after exposure to lead nitrate using alkaline comet assay. Environ. Toxicol., 23: 679-687.

Osman, A., I. Mekkawy, J. Verreth and F. Kirschbaum, 2007a. Effects of lead nitrate on the activity of metabolic enzymes during early developmental stages of the African catfish *Clarias gariepinus* (Burchell, 1822). Fish Physiol. Biochem., 33: 1-13.

Osman, A.G.M., S. Wuertz, I.A. Mekkawy, H.J. Exner and F. Kirschbaum, 2007b. Lead induced malformations in embryos of the African catfish *Clarias gariepinus* (Burchell, 1822). Environ. Toxicol., 22: 375-389.

Pearse, A.G.E., 1960. Histochemistry, Theoretical and Applied. 2nd Edn., Churchill Ltd., London, UK.

Rizk, T., B.E. El-Sayed-Badawy and O.S. Sallam, 1999. Bioaccummulation and toxicity of some heavy metal pollutants in *Biomphalaria alexabdria* (Gastropoda, Pulmonata). Egypt. J. Zool., 32: 319-337.

Robbins, S.L. and V. Kumar, 1987. Cell Injury and Adaptation. In: Basic Pathology, Manke, D. (Ed.). 4th Edn., W. B. Saunders and Co., Philadelphia, PA., USA., pp: 21-22.

Roberts, R.J., 1975. Melanin Containing Cells of Teleost Fish and their Relation to Disease. In: The Pathology of Fishes, Ribelin, W.E. and G. Migaki (Eds.). Wisconsin University Press, Madison, WI., USA., pp: 399-422.

Sayed, A.E.H., I.A.A. Mekkawy and U.M. Mahmoud, 2011. Effects of 4-Nonylphenol on African Catfish *Clarias gariepinus*: Haematological, Biochemical, Histological and Molecular Characteristics of *Clarias gariepinus* Exposed to 4-Nonylphenol. In: LAP Lambert Academic Publishing, Germany, ISBN-13: 978-3845444673, Pages: 240.

Sayed, A.E., U.M. Mahmoud and I.A.A. Mekkawy, 2012a. Reproductive biomarkers to identify endocrine disruption in *Clarias gariepinus* exposed to 4-nonylphenol. Ecotoxicol. Environ. Saf., 78: 310-319.

Sayed, A.E.H., I.A.A. Mekkawy and U.M. Mahmoud, 2012b. Histopathological Alterations in some Body Organs of Adult *Clarias gariepinus* (Burchell, 1822) Exposed to 4-Nonylphenol. In: Zoology, Garcia, M.D. (Ed.). InTech Publisher, Rijeka, Croatia, ISBN: 978-953-51-0360-8, pp: 163-164.

Segnini, B.M.I., H.G. Finol, J. Mediana, A. Boada-Sucre, S. Marcano and B. Bello, 2000. Kidney ultrastructural alterations in the tropical fish *Colossoma macropomum* (Cuvier, 1818) treated with a herbicide. Proceedings of the International Congress on the Biology of Fish, July 23-27, 2000, Aberden, Scotland, pp: 133-144.

Shibamoto, T. and L.F. Bjeldanes, 1993. Introduction to Food Toxicology. Academic Press Inc., New York, USA., ISBN-13: 9780126400250, pp: 126-132.

Sylvie, B.R., C. Pairault, G. Vernet and H. Boulekbache, 1996. Effect of lindane on the ultrastructure of the liver of the rainbow trout, *Oncorhynchus mykiss*, sac-fry. Chemosphere, 33: 2065-2079.

Taweel, A.K.A., M. Shuhaimi-Othman and A.K. Ahmad, 2012. Analysis of heavy metal concentrations in Tilapia fish (*Oreochromis niloticus*) from four selected markets in Selangor, Peninsular Malaysia. J. Biol. Sci., 12: 138-145.

Walter, J.B. and M.S. Israel, 1974. General Pathology. 4th Edn., Churchill Livingstone, Edinburgh, London, New York.

Weir, P.A. and C.H. Hine, 1970. Effects of various metals on behavior of conditioned goldfish. Arch. Environ. Health, 20: 45-51.

Wood, E.M. and B.A. Yasutake, 1956. Ceroid in fish. Am. J. Pathol., 32: 591-603.

Wright, G.L., M.A. Lessler and S.G. Iams, 1975. Effect of lead ingestion on *in vitro* steroid synthesis of the rat adrenal gland. Ohio J. Sci., 75: 155-171.

Yamamoto, M. and N. Egami, 1974. Sextual differences and age changes in the fine structure of hepatocytes in the medaka, *Oryzias latipes*. J. Fac. Sci. Univ. Tokio, 13: 199-210.

Yang, J.L. and H.C. Chen, 2003. Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. Zool. Stud., 42: 455-461.

Yuness, H.A.M., 2005. Role of Melatonin in reducing hypoxia-induced oxidative stress. M.Sc. Thesis, Assiut University, Egypt.