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Biomarkers of Chlorfos toxicity in Common Carp *Cyprinus carpio*

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Abstract
This investigation was designed to evaluate the toxicity effect of Chlorfos on Gills, and liver of Common Carp (Cyprinus carpio) as histological biomarkers through acute and chronic exposure with different concentrations(0.05ppm, 0.1 ppm, 0.25 ppm), the results showed remarkable effect of chlorfos toxicity as compared to the control group , the histological markers of gills are partial lamellar deformation, abnormal lamellae, marginal dilation, hyperplasia of epithelial cells, and marked gill deformation especially at chronic exposure while liver appeared with multiple markers such as marked focal infiltration of lymphocytes, hepatocytes degeneration, increased sinusoids, and marked degeneration with necrosis and more pronounced after chronic exposure for different concentrations of chlorfos, and none of these morphological changes were found in Control fish.
Key words: *Histological Markers, Chlorfos, toxicity, Common Carp*

I. Introduction

With increasing of pesticides using in last decades, Special attention has been focused on the behavior of these pesticides and their effect on aquatic ecosystem, especially on the aquatic animals, Chlorfos is Organophosphorus insecticide with Chlorpyriphos as active gradient and chemical formula is C4H8Cl3O4P (2,2,2-trichloro-1- dimethoxyphosphoryl), Chlorfos also remains active in soil for several days(ACVM Act,1997) .The common carp (Cyprinus carpio) is a widespread freshwater fish of eutrophic waters in lakes and large rivers in Europe and Asia, and is in the family Cyprinidae (minnow and carp family). Cyprinus carpio is easily identified by two pairs of barbells on each side of the upper jaw(USGS 2013).(Tantawy *etal*., 2005) have studied the immunohistopathological effect of Fenthiontoxicityon common carp, and they concluded that this pesticides causes adverse effects on immunological and histopathological parameters of exposed fish through different concentrations.Acute toxicity effect of synthetic pyrethroid pesticide on common carp were detected by (Aydin etal., 2005), the results of the study suggest that low levels of this pesticides in the aquatic environment may have a significant effect on the reproduction and development of carp.Haematological, biochemical and histopathological parameters of common carp were investigated by (Velisek etal., 2009) to study the toxicity effect of bifenthrin, The 96-h LC50 value of Talstar EC 10 (active substance 100 g l−1 bifenthrin) was found to be 57.5 μg l−1,Histological examination showed teleangioectasiae of secondary gill lamellae and degeneration of hepatocytes. The bifenthrinpesticide was identified as a substance strongly toxic for fish.Acute and chronic toxicity effects of Diazinon pesticides were studied by (Adi etal., 2005) on *Liza abu* (Heckel,1843) with different concentrations and histopathological examination showed the hyperplasia , necrosis, epithelial separation and fusion of adjacent secondary lamellae, these changes according to pesticides concentration and exposure time. Many parameters effect on the bioaccumulation of pesticides infish, includingSolubility of water, ionization,Chemicals structure and Lipid content (Pazou etal., 2006).

II. Materials and Methods

Cyprinus carpio samples (Body length 9-13cm,weight 7-12g) have been collected from Alforat fishes farm and then transferred live to Fiber class tanks in Biology Dept, College of Science, Babylon University, these tanks contained 350L of well aerated dechlorinated tap water at (±20 C°), (pH ±7.8), Dissolved oxygen (±8.2 mg/L), the water were changed every three days to remove accumulated fecal materials and feeding was stopped before 24 hr. and during exposure experiment with continuous removal of dead fish.

For Acute exposure (96hr) fish group were exposed to different sublethal Concentrations of Chlorfos (0.05ppm, 0.1 ppm, 0.25 ppm).and the same concentrations for Chronic exposure (14 day) and plastic aquarium (30L) was used for this experiment with 10 fishes for each of aquarium with four replicate for each concentration included control treatment. Finally, Gills and liver were extracted from Fish at the end of experiment, gills and Liver were fixed in Davidson's fixative, and then tissue was dehydrated in a graded series of ethanol and processed for paraffin embedding and sections of taken6- 7μm were taken and cut in leitz 1512

microtome (Wilson and Gamble, 2002) .All slides read by Light microscope (Olympus) type under 40X provided with digital camera after calibration.

III. Results and discussion

The gill is used by common carp for gas exchange, waste discharge , and ionic regulation, pesticides have a great effect on Gills morphology as appeared in this study and according to LC50, these histological changes were considered as biomarkers for chlorfos toxicity effect , as figures below many changes were detected especial after chronic exposure such as partial lamellar deformation, abnormal lamellae, partial terminal attachment of the lamellae, marginal dilation, hyperplasia of epithelial cells, marked gill deformation, marked lamellar aneurysm, marked lamellar fusion with epithelial cells hyperplasia, and diffuse mass of the gill lamella.

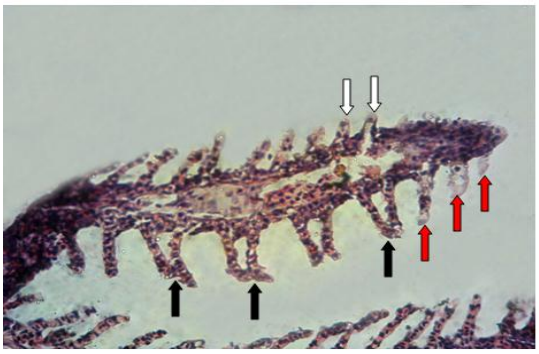

Figure 1: Photomicrograph of gills of the group treated with (acute 0.05ppm) showing partial lamellar deformation (white arrows), abnormal lamellae (red arrows), and partial terminal attachment of the lamellae (black arrows). 40x

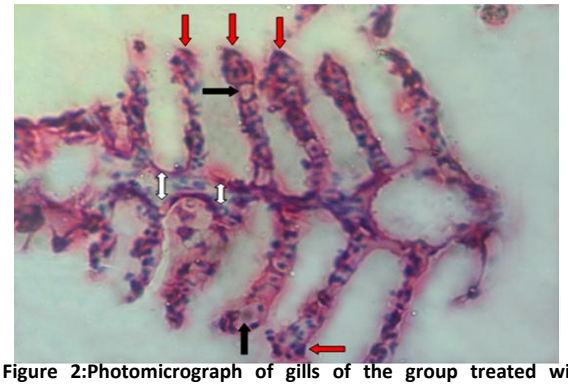

Figure 2:Photomicrograph of gills of the group treated with (Chronic 0.05ppm) showing marked decrease in size (white arrows),marginal dilation (black arrows), and hyperplasia of epithelial cells (red arrows).40x

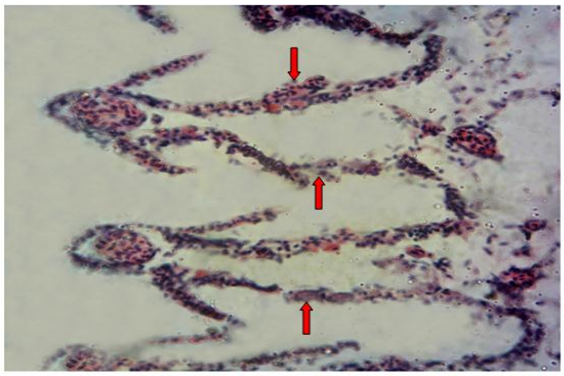

Figure 3: Photomicrograph of gills of the group treated with (chronic 0.1 ppm) showing marked gill deformation (sharp decrease of the number of lamellae) and degeneration. 40x

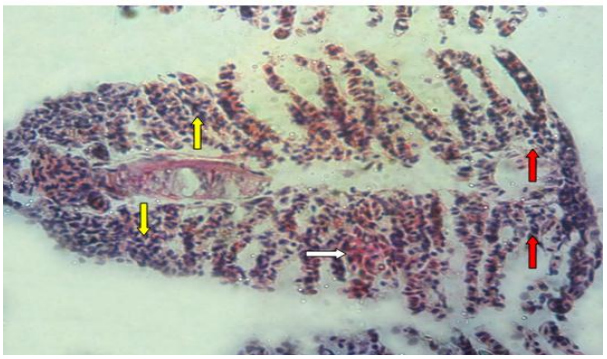

Figure 4: Photomicrograph of gills of the group treated with (acute 0.1ppm), showing marked lamellar aneurysm (white arrows), disintegration of epithelial cells (red arrows), and marked lamellar fusion with epithelial cells hyperplasia (yellow arrows). 40x

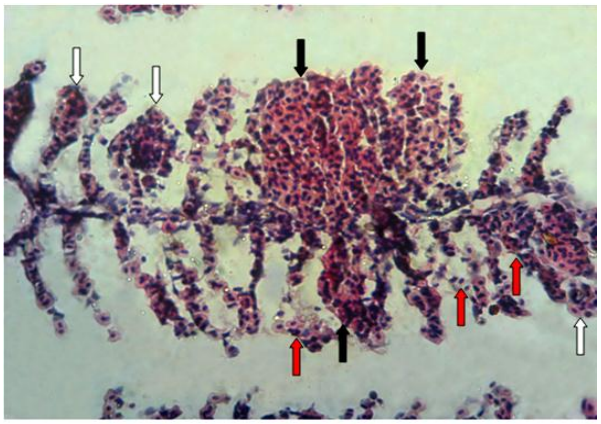

Figure 5:Photomicrograph of gills of the group treated with (Chronic 0.25 ppm) showing marked lamellar aneurysm(black arrows),disintegration of epithelial cells (red arrows), and marked lamellar fusion with epithelial cells hyperplasia (yellow arrows). 40x

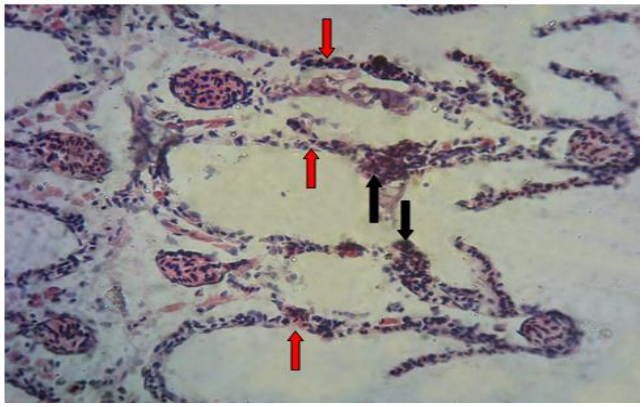

Figure 6:Photomicrograph of gills of the group treated with (Chronic 0.25 ppm) showing marked gill deformation (sharp decrease of the number of lamellae) and degeneration (red arrows), and hyperplasia of epithelial lining leading to diffuse mass of the gill lamella (black arrows). 40x

The gills histopathology considered as a common biomarker of pesticides toxicity, the same histological alterations in gills were found by many studies such as (Capkin etal.,2010) when they found gills were found to be the most seriously affected organ compared to liver, and these changes in gills due to the gills are the main entrance of dissolved pesticides to fish's body, and gills are the first sites of direct contact with Pollutants So gills are the first site to reflect structural and functional responses(Ba-Omar etal.,2011) whose they concluded that pesticides toxicity have important role in gills histological changes and environmental factors have a great effect on histological alteration. In addition the increasing of lesions in gills with concentrations increasing due to the loss of ability to maintain homeostasis and that because injury of hematopoietic tissue with decreasing in oxygen uptake due to the pesticides toxicity effect especially after chronic exposure (Barbieri &Alves Ferreira, 2011) The liver is the primary organ for metabolism, detoxification of pollutants like pesticides, and discharge of harmful substances (Capkin etal.,2010), The major functions of the liver involve protein, lipid and carbohydrate metabolism, as well as detoxification of pollutants, However, increased concentrations of these pollutants can vanquish hepatic detoxification, which could lead to histological damage (DaCunã etal.,2011) as appeared in this study such as marked focal infiltration of lymphocytes, hepatocytes degeneration, increased sinusoids, marked degeneration and necrosis represented by large degenerated area especially after chronic exposure (Figure 1-12)

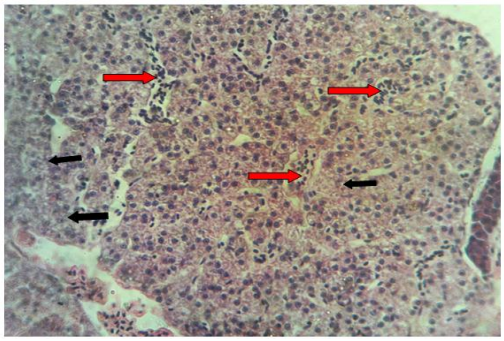

Figure 7: Photomicrograph of liver of the group treated with (acute 0.05ppm), showing marked focal infiltration of lymphocytes (red arrows), and hepatocytes degeneration (black arrows). 40x.

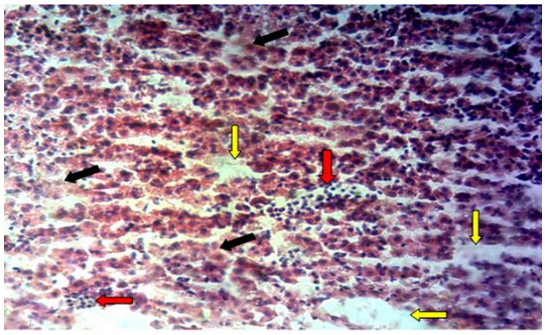

Figure 8: Photomicrograph of liver the group treated with (Chronic 0.05ppm), showing infiltration of lymphocytes (red arrows), increased sinusoids (yellow arrows), and marked degeneration and necrosis (black arrow).40x

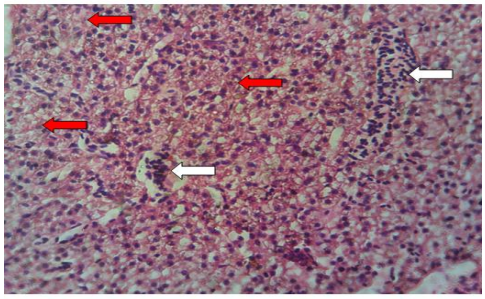

Figure 9: Photomicrograph of liver of the group treated with (acute 0. 1ppm), showing focal infiltration of lymphocytes (white arrows), and degeneration and necrosis (red arrows).40x

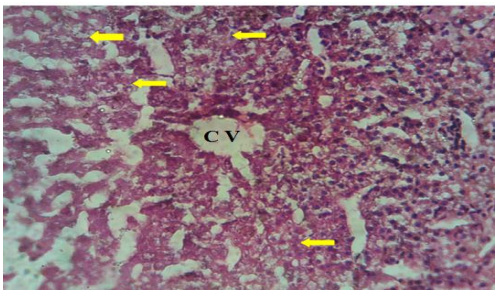

Figure 10: Photomicrograph of liver of the group treated with (Chronic 0. 1ppm), showing marked central and paracentral degeneration and necrosis (yellow arrows).40x

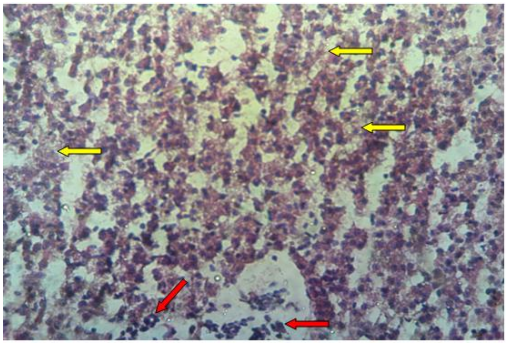

Figure 11:Photomicrograph of liver of the group treated with (Acute 0.25 ppm), showing infiltration of lymphocytes from the central vein (red arrows), and degeneration and necrosis (yellow arrows).40x

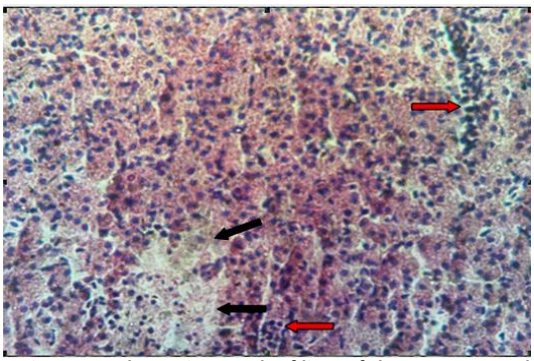

Figure 12: Photomicrograph of liver of the group treated with (Chronic 0.25 ppm), showing infiltration of lymphocytes (red arrows), and degeneration and necrosis represented by large degenerated area (black arrow).40x

The previous histological alteration in Liver due to the chlorfos toxicity and carp liver is more capable of detain the pollutants compared to gills and kidney (DeSmet etal.,2001).Also the protein and carbohydrate metabolism in the liver and muscle tissue is obstruct according to pesticides exposure(Begum,2004), and this leads to Histological changes. Histological changes in liver were found in many studies and these changes so associate with hepatosomatic index and histological alteration is more appeared with increasing of exposure concentrations (Bukhar etal.,2012).In addition, the production of free radicals, lipid perioxidation, and changing in antioxidant status are vital factors in the toxic effects of pesticides on the liver (Sevgiler etal.,2004).During chronic exposure of pesticides , the liver function will be effected leads to reduction in protein concentration(Saravanan etal.,2011) and that influence on liver tissue , also exposure to pesticides leads to lipid peroxidation overproduction in various tissue as well as liver and causes histological alterations, and reactive oxygen species induce peroxidative damage in liver and this may be one of the molecular mechanism of Chlorfos toxicity(Velisek etal.,2011).Moreover the physiological changes in Fish depends on inferences between species , types of pesticides, pesticides concentration, and exposure period(Oruç&Üner,2000), and the most effective histological changes occur when increase in cellular and nuclear volume with cytoplasmic and nuclear disruption with bile dysfunction with extremist metabolic activity of the hepatocytes (Maduenho& Martinez,2008).

IV. Conclusion

Acute and Chronic chlorfos exposure leads to disrupts Morphology of gills and liver of common carp and morphological alteration can be consider as good biomarkers for the pesticides toxicity effect. Also common carp is a reliable indicator of pesticides toxicity.

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