Journal of Applied Sciences Research 1(3): 263-267, 2005

Fenvalerate Induced Histopathological and Histochemical Changes in the Liver of the Catfish *Clarias Gariepinus*

[1]S.A.Sakr and [2]S.M. Jamal Al lail

[1]Department of Zoology, Faculty of Science, Menufiya University, Shebin El-kom, Egypt.
[2]Department of Biology, Faculty of Applied Sciences, Umm al-Qura University, Makka, Saudi Arabia.

Abstract: The hazardous effect of the pyrethroid insecticide, fenvalerate on the histology and histochemistry of the liver of the catfish (*Clarias gariepinus*) after exposure to 1/10LC_{50} for 5 and 10 days was investigated. The results showed that the histopathological changes induced in the liver were mainly represented by cytoplasmic vacuolization of the hepatocytes, blood vessel congestion , inflammatory leucocytic infiltration ,necrosis and fatty infiltrations. The histochemical observation revealed marked reduction in glycogen contents and total protein contents of the liver cells as compared with the control fish. These changes were time-dependent.

Key words: Pollution, fenvalerate,catfish, liver, histopathology, histochemistry

INTRODUCTION

Pesticides have been widely used all over the world to control insects, pests and disease vectors. They ultimately find their way into aquatic habitats such as rivers, lakes and ponds, and have been found to be highly toxic not only to fish but also to organisms which contribute substantially to the food chain. Such pesticides are also known to have affinity for residing in animal tissues, especially in the fatty ones[2]. Fenvalerate (RS) -alpha-cyano- 3- phenoxybenzyl(RS)-2-(4-chlorophenyl)-3-methylbutyrate is a pyrethroid insecticide widely used for insect control in different countries. It is highly toxic for many fish[16].

There is considerable informations indicating that pesticides are responsible for many adverse effects in fishes and other animals from the histopathological and histochemical points of view[1,18,13]. The present work was conducted to study the effect of the pyrethroid insecticide, fenvalerate on the liver of the catfish "*Clarias gariepinus*".

MATERIALS AND METHODS

Living samples of *Clarias gariepinus* were collected from the Bahr Shebin canal, Shebin El-kom, Egypt, each weighing 500-750 g. The fish samples were transported in well-aerated containers to large tanks in the laboratory. These tanks contain Nile water and were continuously aerated using air pumps. The fish samples were kept for at least 48 hours in these tanks before experimentation. The fishes were provided with suitable food of algae and grass.

Fenvalerate, a pyrethroid insecticide was used in the present study. The LC_{50} of fenvalerate at 48 hours was found to be 250 ug/L as obtained from the lethal curve. The experimental fishes were divided into four groups.

The 1st group: 10 fishes were exposed to 1/10 LC_{50} of fenvalerate for 5 days exposure period in specially equipped aquaria (80 x 50 x 50 cm). During this period, the fishes that die were immediately removed.

The 2nd group: 15 fishes were exposed to 1/10 LC_{50} of fenvalerate for 10 days.

The 3th group: 10 fishes were used as a control and kept in Nile water without any treatment.

At the end of each exposure time, fishes were decapitated and were dissected. Liver was removed and small pieces were fixed in 10% formalin and Carnoy's fluid. The fixed samples were dehydrated in ascending series of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Sections of 6 microns thickness were cut, mounted and stained with different stains according to the target of investigation. For histopathological investigations, 10% formalin - fixed sections were stained

Corresponding Author: S.A.Sakr, Zoology Department, Faculty of Science, Menufiya University, Shebin El-kom, Egypt.
E-mail: sabsak@yahoo.com

with haematoxylin and eosin. For histochemical investigations, materials fixed in Carnoy's fluid were stained with periodic acid Schiff's (PAS) technique[5] for demonstration of polysaccharides (liver glycogen). Total proteins were demonstrated by the mercury bromophenol blue method[8].

RESULTS AND DISCUSSIONS

Histopathological results: In liver sections of normal fish the hepatocytes form a rather cord-like pattern. These cords are arranged around tributaries of the hepatic vein. The liver cells are large in size, polygonal in shape with homogenous eosinophilic cytoplasm and centrally located nuclei. A large number of blood sinusoids were observed and separates the hepatic cords one from another (Fig.1). Exposure of *Clarias gariepinus* to 1/10 LC_{50} of fenvalerate for 5 days induced obvious histopathological changes in the liver. The hepatocytes have lost their normal architecture and a large number of these cells appeared with pyknotic nuclei. The intrahepatic blood vessels were dilated and congested with blood, and inflammatory leucocytic infiltrations were observed (Fig.2).Numerous hepatocytes showed marked cytoplasmic vacuolization (Fig.3).

The histopathological changes of the liver were more pronounced after 10 days exposure period. The liver cells were degenerated with necrosis which appeared as focal areas with lymphocytic infiltration (Fig.4).A large number of cells were suffered from fatty degeneration (Fig.5).

Histochemical results:

Glycogen content: The PAS preparations of the normal liver of *Clarias gariepinus*, revealed that glycogen was observed in the cytoplasm of the hepatocytes as indicated by large number of reddish fine granules of different sizes. However, cells of the same specimen exhibit different intensities with PAS reaction (Fig. 6). After exposure of fishes to 1/10 LC_{50} of fenvalerate for 5 days, glycogen content of the liver cells decreased. This diminution was quite evidenced in the amount and stainability (Fig. 7). This reduction of glycogen inclusions became more pronounced after 10 days exposure period .In these specimens glycogen content displayed faint stainability and became hardly detectable (Fig.8).

Total proteins: In the hepatocytes of normal *Clarias gariepinus*, total proteins appeared as intensely dark blue coloured inclusions in the cytoplasm. Chromatin bodies and nucleoli exhibited a deep colouration with bromophenol blue (Fig. 9). Total proteins were found to exhibit a notcieable decrease in cytoplasm and nucleus of the liver cells of *Clarias gariepinus* exposed to

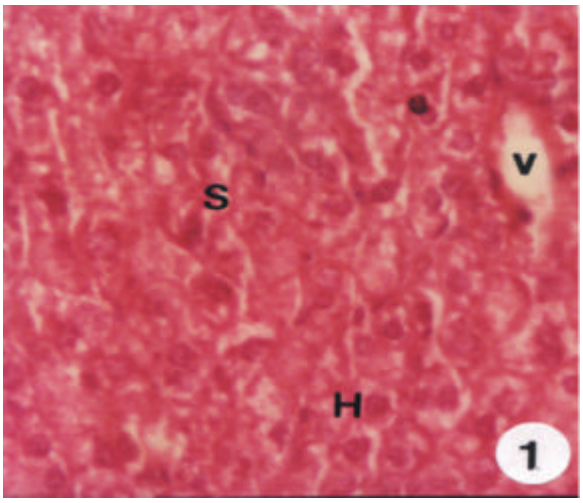

Fig.1: Section in the liver of a control fish, S: sinusoidal lumen, H: hepatocytes,V: vein X320.

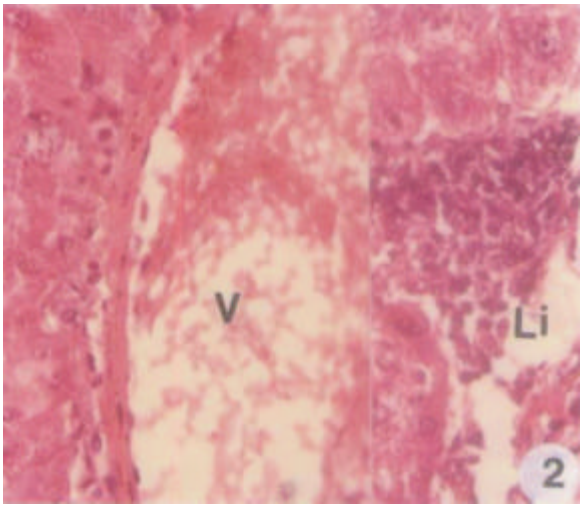

Fig. 2: Section in the liver of a fish exposed to 1/10LC_{50} of fenvalerate for 5 days showing a congested vein (V) and inflammatory leucocytic infiltration (Li) X320.

fenvalerate for 5 days (Fig. 10). The hepatocytes of fishes exposed to fenvalerate for 10 days showed an obvious reduction in the protein contents and their remnants were mainly located at the peripheries of the hepatic cells which showed sever cytoplasmic vacuolation (Fig. 11).

The present results showed that fenvalerate induced many histopathologiocal changes in the liver of catfish *Clarias gariepinus* . These lesions included cytoplasmic vacuolization of the hepatic cells, inflammatory leucocytic infiltrations ,congestion of blood vessels ,necrosis and fatty infiltrations. Similarly, Teh *et al*[15] found that exposing 7-day-old larvae of the fish Sarcamento splittail to sublethal

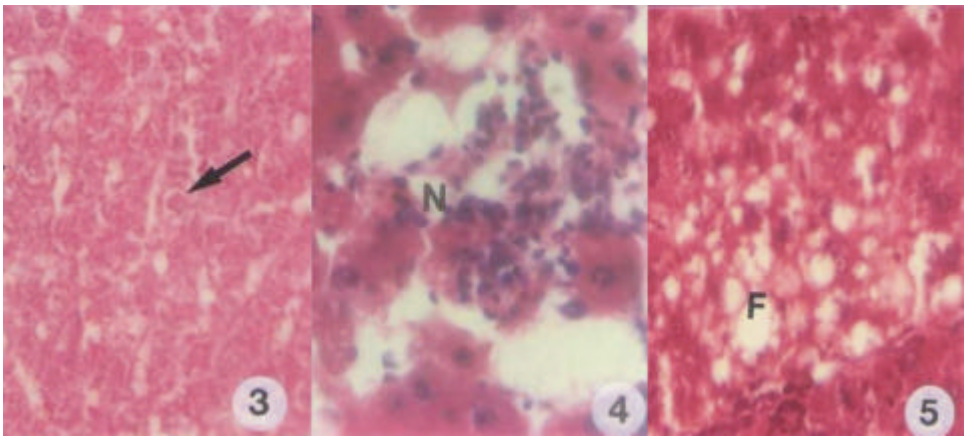

Fig. 3: Section in the liver of a treated fish showing cytoplasmic vacuolization of the hepatocytes (arrow) X320.
Fig. 4: Section in the liver of a fish 10 days post-exposure to fenvalerate showing necrotic area (N) with leucocytic infiltrations X320.
Fig. 5: Section in the liver of a treated fish showing fatty infiltration (F) X320.

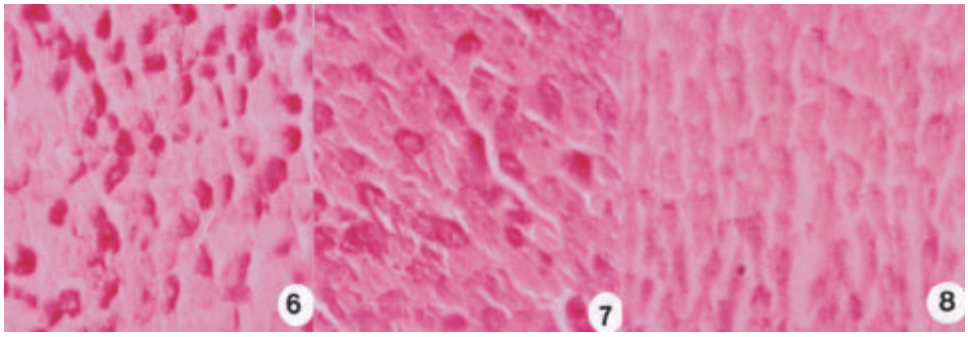

Fig. 6: Sections in the liver of a control fish stained with PAS technique showing distribution of glycogen in the hepatocytes,X320.
Fig. 7: Hepatocytes with reduction of glycogen after 5 days-exposure to fenvalerate, X320.
Fig. 8: Marked reduction of glycogen after 10 days exposure period, X320.

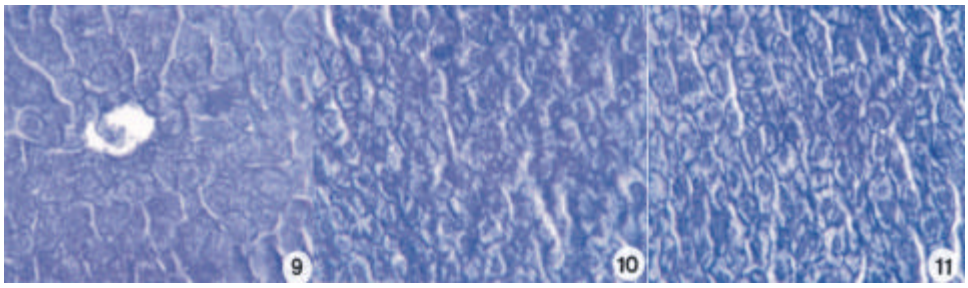

Fig. 9: Sections in the liver of control fish stained with mercury bromophenol blue for total proteins, X320.
Fig. 10: Total proteins in liver of a fish exposed to fenvalerate for 5 days, X320.
Fig. 11: Reduction of total proteins in hepatocytes after 10 days exposure period to fenvalerate, X320.

concentrations of esfenvalerate for one week induced vacuolar degeneration and cell necrosis in the liver.

The effect of insecticides on the liver of different fish species were studied by many investigators. Mandal and Kulshrestha[7] studied the effects of sublethal concentration of sumithion on liver, kidneys and intestine of *Clarias batachus*. They observed liver necrosis, vacuolization and breakdown of the cell boundaries. They also observed vacuolization of epithelial cell of uriniferous tubules and degeneration of the glomeruli in the kidney,

while in the intestine, they noticed lesion formation in the villi and enlargement of mucous cells. Histological changes in the liver of *Tilapia mossambica* after exposure to the organophosphate monocrotophos were reported by Desai *et al*.,[3]. At the initial stage of intoxication, necrosis and vacuolization of hepatocytes were recorded, while fatty degeneration was observed later on. Treatment with endrin produced acute pathological changes in the liver of *Channa punctatus*[12]. Elezabi *et al*.[4] studied the effect of malthion on the fish *Oreochromis niloticus* and their results showed that this insecticide induced many histopathological changes in the liver and gills of the fishes. These changes were hemorrhage, necrosis and destruction of lamellae of the lungs, and necrosis and lipidosis in the liver. Sakr *et al*[10]. studied the effect of the organophosphorous insecticide (Hostathion) on the liver of the catfish (*Clarias gariepinus*). Their results showed that this insecticide produced histopathological changes in the liver represented by liver cord disarray, cytoplasmic vacuolization of the hepatocytes, damage of blood sinusoids, blood vessel congestion and inflammatory leucocytic infiltrations.

It was found that fenvalerate induced marked reduction in glycogen content in the hepatocytes of *Clarias gariepinus*. This result is in agreement with those of *Reddy et al*[9] who reported that fenvalerate altered glycogen metabolism in liver and muscles of *Cyprinus carpio*. Singh and Srivastava[14] mentioned that carbohydrates decreased as a result of exposure to a sublethal concentration of a mixture of aldrin and formothion. These insecticides induced marked diminution in the glycogen content of the liver and muscles of this fish. Exposure of freshwater fish *Mystus vittatus* to sublethal concentrations of the two pesticides thiotox and dichloruos for one month was found to induce marked depletion in both liver and muscle glycogen[18]. Sublethal concentrations of quinalphos resulted in reduction of glycogen in the liver of *Channa punctatus*[13]. The activity levels of succinate dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G6PD) were assessed in various tissues of *Cyprinus carpio* exposed to lethal concentrations of different pyrethroids including fenvalerate for a period of 72 h. The results indicated a steady decrease in SDH activity with a concomitant increase in G6PD activity. The decreased SDH activity indicated inhibition of SDH at mitochondrial level and the increased G6PD activity an enhancement of an alternative pathway of carbohydrate metabolism, viz the hexose monophosphate shunt (HMP) or pentose phosphate pathway as a biochemical adaptation to overcome the toxic stress[6].

Total protein contents of the liver of *Clarias gariepinus* showed a noticeable decrease after exposure to fenvalerate. These results agree with those of Tripathi and Verma[17] who reported that exposing fish *Clarias batrachus* to fenvalerate induced a highly significant decrease of protein contents of the liver, brain and skeletal muscle. Reddy *et al*[9] found that total, structural and soluble proteins were decreased, whereas the free amino acids and the activities of protease, aspartate aminotransferase and alanine aminotransferase significantly increased in fenvalerate exposed fish *Cyprinus carpio*. Hepatic total protein content significantly decreased after treating freshwater fish *Labeo rohita* with chlordane[1]. Sancho *et al*[11] reported that total proteins had decreased in liver of the European eel, *Anguilla anguilla* after fenitrothin exposure for different time intervals up to 96hrs. Reduction in protein content in liver of fenvalerate-exposed fish might be due to either arrested metabolism in the liver or to use it to build up new cells or enzymes to reduce the stress.

In conclusion, the present study proved that fenvalerate affected the structure and histochemical contents of the liver of *Clarias gariepinus* and this effect was time-dependent.

REFERENCES

1. Bansal, S.K., S.R. Verma, A.K. Gupta, R.C. Dalela, 1979. Physiological dysfunction of the haemopoietic system in a fresh water teleost *Labeo rohita* following chronic chlordane exposure. Part II. Alterations in certain organic components and serum electrolytes. Bull Environm Contam Toxicol. 22: 674-680
2. Deichmann, W.B., W.E. MacDounald, D.A. Cubit, 1975. Dieldrin and DDT in the tissues of mice feed endrine and DDT for seven generation. Arch. Toxicol. 34: 173-182
3. Desai, A.K., U.M. Joshi, P.M. Ambadka, 1984. Histological observations on the liver of *Tilapia mossambica* after exposure to monocrotophos, an organophosphorus insecticide. Toxicol. Lett. 21: 325-331
4. Elezaby, M.M., S. El-Serafy, R. Heckmann, Kh Sharf Eldeen, M.M. Seddek, 2001. Effect of some toxicants on the fresh water fish *Oreochromis niloticus*. J. Egypt Ger. Soc. Zool. 36: 407-434
5. Hotchkiss, R.D., 1948. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem. 16: 131
6. Kamalaveni, K., V. Gopal, U. Sampson, D. Aruna, 2001. Effect of pyrethroids on carbohydrate metabolic pathways in common carp,*Cyprinus carpio*. Pest. Manag. Sci. 57 (12): 1151-1154.

7. Mandel, P.K., A.K. Kulshrestha, 1980. Histopathological changes induced by the sublethal sumithion in *Clarias batrachus* (Linn). Ind. J. Exp. Biol. 18: 547-552
8. Mazia, D., P.A. Brewer, M. Alfert, 1953. The cytochemical staining and measurement of protein with mercuric bromophenol. blue. J. Biol. Bull. 104: 57-67
9. Reddy, P.M., G.H. Philip, M. Bashamohideen, 1991. Fenvalerate induced biochemical changes in the selected tissues of freshwater fish, *Cyprinus carpio*. Biochem. Int., 23(6):1087-1096
10. Sakr, S.A., S.M. Hanafy, N.E. El-Desouky, 2001. Histopathological,histochemical and physiological studies on the effect of the insecticide ,hostathion,on the liver of the catfish *Clarias gariepinus*. Egypt. J. Aquatic Biol. Fish 6(2):103-124
11. Sancho, E., M.D. Ferrando, C. Fernandez, E. Andreu, 1998. Liver energy metabolism of *Anguilla anguilla* after exposure to fenitrothion. Ecotoxicol. Environm. Saf. 41(2): 168-175
12. Sastry, K.V., K.S. Sharma, 1979. The effect of endrin on the histopathological changes in the liver of *Channa punctatus*. Bull. Environ. Contam. Toxicol. 20(5): 674-677
13. Sastry, K.V., A.A. Siddique, 1984. Some hematological, biochemical and enzymological parameters of a fresh water teleost fish *Channa punctatus* exposed to sublethal concentrations of quinalphos pesticide. Bioch. Physiol. 22: 8-13.
14. Singh, N.N., A.K. Srivastava, 1981. Effect of a paired mixture of aldrin and formation on carbohydrate metabolism in a fish *Heteropneustes fossilis*. Pestic. Biochem. Physiol. 15(3): 257-261.
15. Teh, S., S. Hung, F. Teh, D. Deng, I. Werner, 2005. Sublethal toxicity of orchard stormwater runoff in Sacramento splittail (*Pogonichthys macrolepidotus*) larvae. Mar. Environ. Res. 59(3): 203-216 .
16. Tilak, K.S., K. Veeraiah, T.A. Susan, K. Yacoub, 2001. Toxicity and residue studies of fenvalerate to some selected freshwater fishes. J. Environ. Biol. 22 (3): 177-180
17. Tripathi, G., P. Verma, 2004. Fenvalerate-induced changes in a catfish, *Clarias batrachus*: metabolic enzymes, RNA and protein. Comp. Biochem. Physiol. 138(1): 75-79
18. Verma, S.R., R. Sarita, I.P. Tonk, R.C. Dalela, 1983. Pesticide induced dysfunction in carbohydrate metabolism in fresh water fishes. Environm. Res. 32(1) : 127-133.