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Electron Microscopic Study of the Liver of *Tilapia nilotica* Exposed to Neopybuthrin

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Pesticides are widely used all over the world to control insects, pests and disease vectors. They considered as a source of pollution of the aquatic environment through runoff from the fields and have been found to be highly toxic to fishes and to the organisms which contribute to the food of fishes (Anees 1975).

Extensive investigations were carried out by many authors to study the morphological changes occurred in the liver and other organs of different species of fishes after treatment with various insecticides (Sastry and Malik 1979; Sastry and Sharama 1979; Mandal and Kulshrestha 1980; Singh *et al.* 1984; El-Elaimy *et al.* 1991; and Sakr and Gabr 1992). The present work was conducted to throw light on the ultrastructural changes in the liver of freshwater fish *Tilapia nilotica* after exposure to various concentrations of the pyrethroid insecticide, neopybuthrin which is widely used for insect control near water resources in Egypt.

MATERIALS AND METHODS

Live *Tilapia nilotica* were collected from the High Dam Lake, each weighing 200-300 g. Fishes were kept in specially equipped aquaria of 24 L capacity which were continuously aerated by air pumps. These aquaria contained fresh Nile River water. The water criteria which were maintained during the study consisted of dissolved oxygen 65 mg / L, alkalinity 120 mg / L, hardness 41 - 44 mg / L as $CaCO_3$, pH 7.2 ± 0.1 and temperature 25 ± 2 °C. Fishes were provided with suitable food of earth worms. Pyrethroid insecticide neopybuthrin was used in the present investigation. It is obtained from Kafr El-Zayat Pesticide Company, Egypt as technical material of purity 70 % and was emulsified with water to give the desired concentration. The LC_{50} of this insecticide was found to be 2.1 mg / L as obtained from lethal mortality curves constructed for this purpose in a previous investigation performed by El-Elaimy *et al*. (1990). The present work was aimed to study the effect of multitreatments of equal sublethal concentrations of neopybuthrin. The aquaria were prepared for this purpose as follows :
Aquarium (a): contained fish individuals in fresh Nile water (control).
Aquarium (b): contained fish individuals exposed to 1/2 LC_{50} of neopybuthrin

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(1.05 mg / L). This concentration was added to the aquaria at 24 hr until it reached a concentration equivalent to $1^1/_2$ LC_{50} after 72 hr. Groups of 3 surviving fishes were decapitated at intervals of 24, 48 and 72 hr exposed to $^1/_2$ LC_{50}, LC_{50} and $1^1/_2$ LC_{50} respectively. For electron microscopic studies, pieces of liver of control and treated fishes were removed and were immersed in 2.5 % glutaraldhyde buffered at pH 7.4 with 0.1 M sodium cacodylate for 3 hr followed by 1% OSO_4 solution for 2 hr, then the samples were dehydrated with graded concentrations of ethanol followed by propylene oxide and embedded in Epon epoxy resin. Ultrathin sections were stained with uranyle acetate and lead citrate (Reynolds 1963), and examined in JEM - 100 C electron microscope.

RESULTS AND DISCUSSION

The normal liver of *Tilapia nilotica* is formed of hepatic cells which are hexagonal in shape with more or less centrally located nucleus and homogenous cytoplasm. There is no clear division of the hepatic tissue into lobules. In this fish, the pancreatic tissue is usually found within the liver tissue and is located around the blood vessels; therefore the liver is referred to as hepatopancreas. Under the electron microscope, the hepatocytes appear rich with mitochondria which are rounded or oval in shape. Endoplasmic reticulum, rough and smooth are usually found in close association with the mitochondria. Golgi bodies are located between the nucleus and the bile canaliculus, and consist of aggregated smooth surfaced vesicles and flattened sacs. Zymogen granules are found frequently in the hepatocytes and appear as relatively large, round dense structures with a limiting membrane. Most of the cytoplasm is occupied by glycogen rosettes. The nuclei appear rounded and the nucleolus, when present, is prominent. The nucleoplasm consists of a finely granular component in a rather homogeneous matrix. Many microvilli are present on the surfaces of the cells surrounding the hepatic sinusoid (Fig. 1).

Liver of fishes exposed to $^1/_2$ LC_{50} of neopybuthrin showed many pathological changes. The microvilli were found irregular in shape and were fragmented. The mitochondria were degenerated and their cristea are difficult to be seen. The endoplasmic reticulum showed fragmentation. The nuclei were of normal appearence, but they were poor in chromatin content. Many lipid droplets were present in the apical region of the cell and some of them contained vacuoles. The sinusoidal lumens were found filled with pathological blood cells (Figs. 2 & 3).

Many alterations were induced in the liver of fishes exposed to one LC_{50} of neopybuthrin. The microvilli became irregular in shape and collapse. The space of disse becames very narrow and disappeared at several regions. Fragmentation of the endoplasmic reticulum in both rough and smooth surfaced was observed. The mitochondria showed swelling, ring-like formation and degeneration of their cristae. Some nuclei showed rupture of the nuclear membrane at some regions and others have more or less normal appearance. Lipid droplets increased in number and size. Zymogen granules were present. The sinusoidal lumen was found filled with necrotic cells with degenerated cell organelles (Figs. 4 & 5).

Liver of fishes exposed to $1^1/_2$ LC_{50} of neopybuthrin revealed that the parenchymal

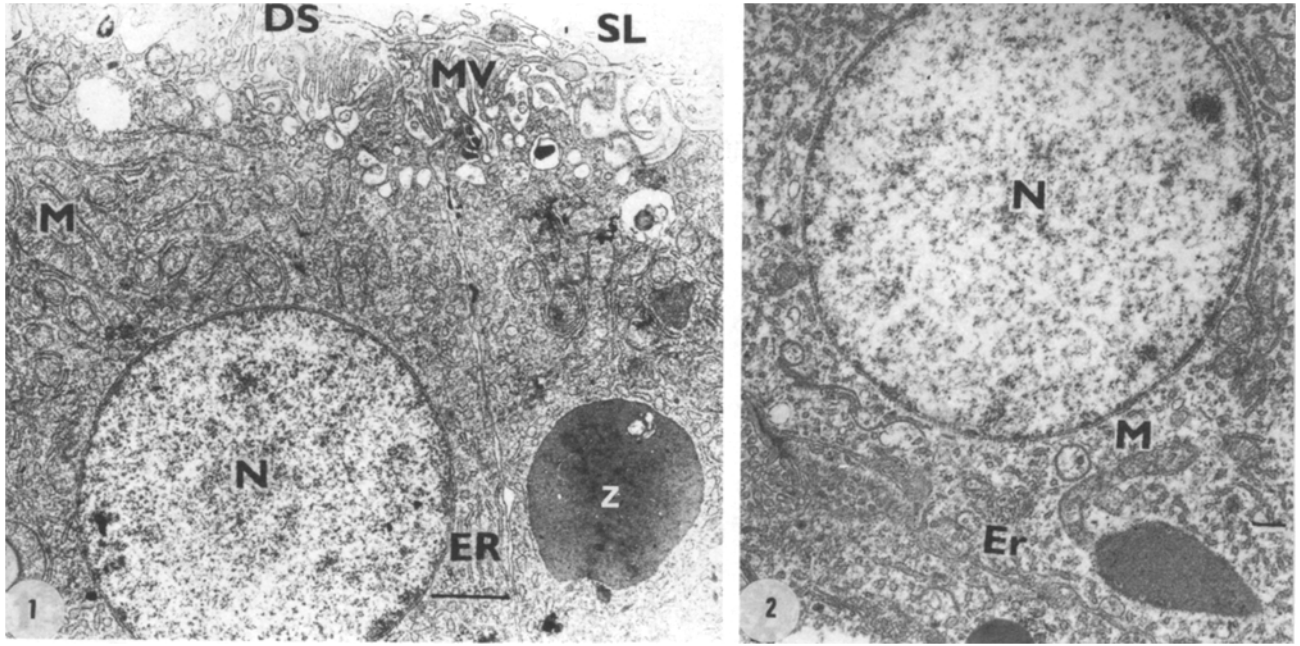
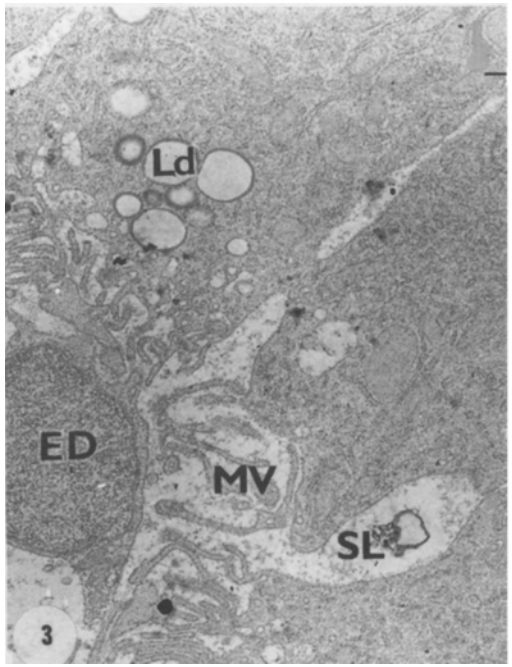
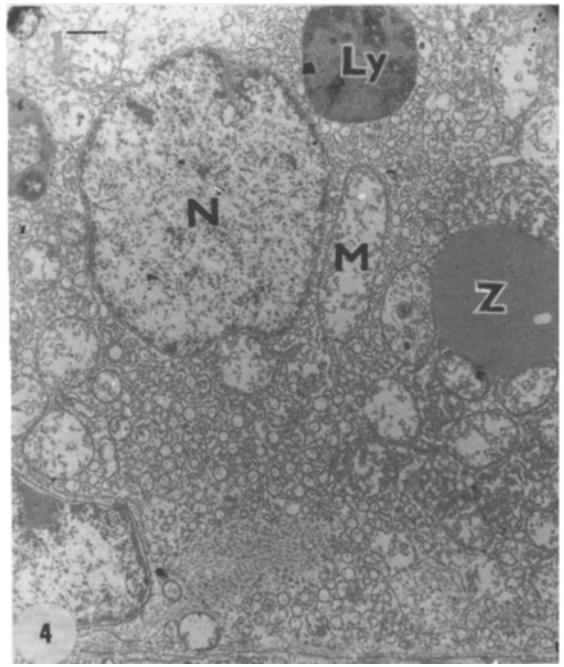


Figure 1. Electron micrograph of a control hepatic cell showing microvilli (MV) in the space of disse (DS). ER, endoplasmic reticulum; SL, sinusoidal lumen; Z, zymogen granules. X 14000.

Figure 2. Electron micrograph of hepatic cell of a fish exposed to $^1/_2$ LC_{50} of neopybuthrin showing fragmentation of endoplasmic reticulum (ER). M, mitochondria; N, nucleus. X 19000.





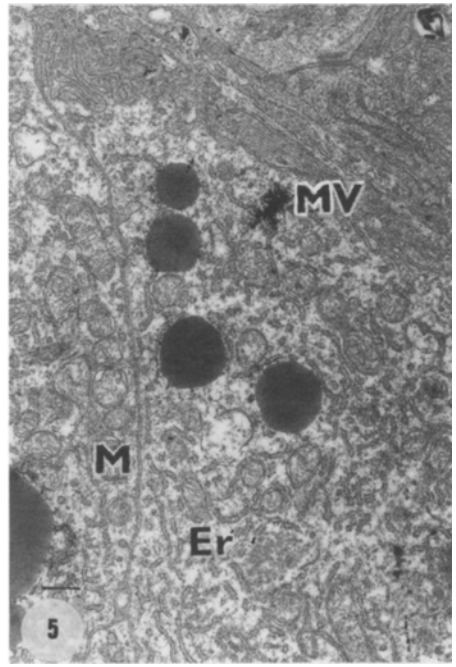


Figure 3. Hepatic cell of a fish exposed to $^1/_2$ LC_{50} showing fragmentation of the microvilli (Mv), lipid droplets (Ld) and endothelial cell (ED). X 14000.

Figure 4. Hepatic cell of a fish exposed to LC_{50}. Ly, lysosomes; M, mitochondria with degenerated cristae; N, nucleus; Z, zymogen granules. X 18000

Figure 5. Electron micrograph of liver of a fish exposed to LC_{50} of neopybuthrin showing collapsed micovilli (MV), Er, endoplasmic reticulum; M, mitochondria X 18000

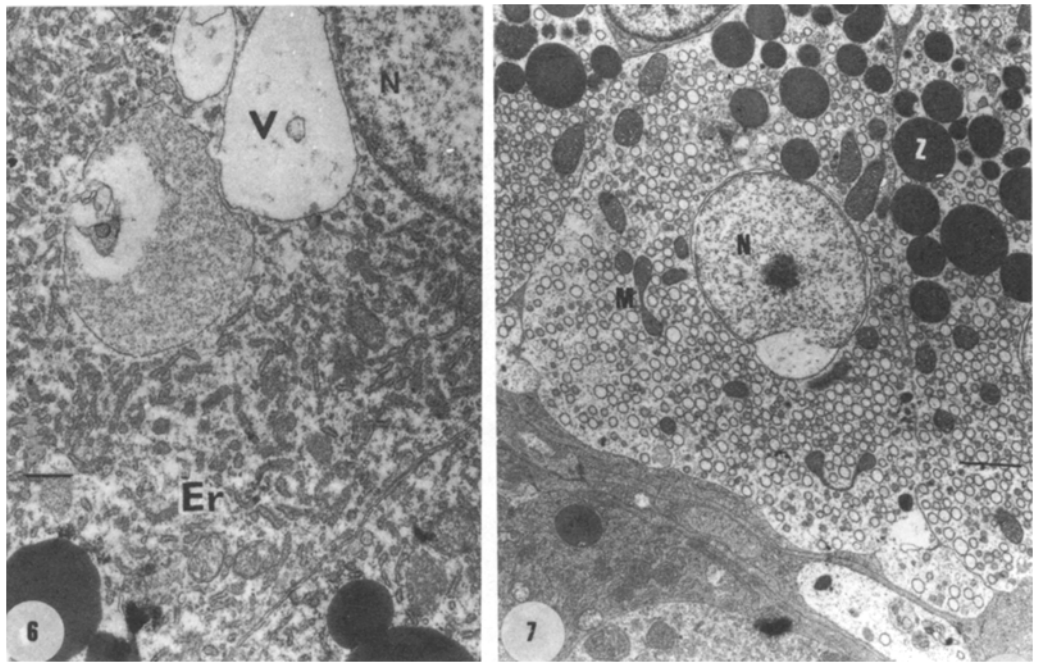


Figure 6. Hepatic cell of a fish exposed to $1^1/_2$ LC_{50}. Er, endoplasmic reticulum; N, nucleus; V, vacuole. X 14000.

Figure 7. Hepatic cell of a fish exposed to $1^1/_2$ LC_{50}. M, mitochondria; N, nucleus; Z, zymogen granules.x 10000.

cell organelles showed the same pathological changes as those in fishes exposed to one LC_{50} of neopybuthrin especially the endoplasmic reticulum fragmentation and degeneration of mitochondrial cristae. The zymogen granules were increased in number and concentrated around the bile canaliculi. The nuclei showed degenerative changes, and the nuclear membrane of some of them were found protruded at one or more regions. In some cells, the cytoplasm was found filled with lipid droplets which were vacuolated and small in size. Necrotic tissues filled all the sinusoidal lumen, and the microvilli disappeared at some regions (Figs. 6 & 7).

The present study demonstrates that mitochondria, endoplasmic reticulum and the microvilli of the liver were affected by exposing the fish *Tilapia nilotica* to the pyrethroid insecticide neopybuthrin. The lipid droplets increased in number and accumulated around the sinusoidal border of the hepatocytes. There were many necrotic cells characterized by irregularity in shape and few cytoplasmic organelles, and pyknotic nucleus.

The present observations in *Tilapia nilotica* on exposing to the pyrethroid insecticide are in agreement with those of other investigators working on organophosphate (Mandal and kulshrestha 1980) and organochlorines (Sastry and Sharma 1979). Sastry and Malik (1979) reported that the mucosal epithelium of the stomach, intestine and pyloric coeca of fresh water fish *Channa punctatus* was ruptured and degenerated after exposure to a sublethal concentration of dimecron. The authors added that the liver cell membranes as well as connective tissue were markedly damaged. Similar alterations were also observed in the liver of the same fish under the effect of the organochlorinate insecticide, endrin (Sastry and Sharma 1979). Histopathological changes by a sublethal dose of the organophosphorus sumithion insecticide in liver, kidney and intestine of *Claris batrachus* (Linn) were studied by Mandal and Kulshrestha (1980). Histological changes in the liver of *Tilapia mossambica* after exposure to the organophosphate monocrotophos were characterized also by necrosis and vacuolation of hepatocytes at the initial stage of intoxication, while fatty degeneration observed later (Desai *et al.* 1984). Rojik *et al.* 1983, have investigated ultrastructural changes in the liver and kidney of silver crap *Hypophthalmichthys molitrix* induced by paraquat treatment. They observed that the intracellular bile canaliculi were swollen and the microvilli were shortened.

In the present work, mitochondria in the hepatic cells of pyrethroid - treated fish were noticed to exhibit remarkable pathological alterations similar to those noticed in mammalian animals under the effect of different insecticides (Saxena and Sarin 1979 and Manring and Moreland 1981). Pyrethroid insecticides, apart from inhibiting acetyl cholinestrase activity (Narahashi 1971), induce disturbances of other metabolic processes (Elliott *et al.* 1972). Toxicity and pharmacokinetic studies suggest that the action of pyrethroid is mediated by sterospecific binding to an unidentified pyrethroid receptor in the nerve membrane (Jacques *et al.*, 1980). It seems that the pyrethroid insecticide, neopybuthrin is capable of binding to the mitochondrial membrane and consequently may alter mitochondrial functions. The increase in lipid droplets noticed in the liver of treated fishes may be due to reduced fatty acid oxidation resulting from impairment of mitochondrial function (Diazani 1957).

From the results of the present study we can conclude that neopybuthrin produces multiorganalle lesions and the severity of such lesions is to large extent dependent on the concentration level of the insecticide in water.

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