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Ultrastructural Changes Induced by Diazinon and Neopybuthrin in Skeletal Muscles of *Tilapia nilotica*

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Hazards in connection with the use of pesticides continue to be one of the major occupational and environmental problems . While these pesticides destroy insects and other pests, they may have a direct action on other animals or even human beings . Most of pesticides ultimately find their way into rivers, lakes and pond, and have been found to be highly toxic not only to fishes (Anees 1975) but also to the organisms which contribute to the food of fishes.

The effects of pesticides on fishes are of great concern. In recent years, incidences of fish mortality due to pesticides, industrial effluents and sewage pollution have been reported a number of times (Coppage and Braidech 1979). In histological studies, insecticides have been shown to induce many pathological changes in the organs of various species of fishes (Dubale and Shah 1979; Sastry and Sharma 1979 ; Mandal and Kulshrestha 1980 ; Desai *et al.* 1984; El - Elaimy *et al.* 1990). In the present study, the fresh water frish Tilapia nilotica was used as an aquatic model widely distributed in High Dam lake in Egypt, to investigate the ultrastructural alterations in skeletal muscle following intoxication with diazinon or neopybuthrin.

MATERIALS AND METHODS

Living samples of Tilapia nilotica were collected from High Dam lake, each weighing 200 - 300 g. Fishes were kept in specially equipped aquaria which were continously aerated by air pumps; these aquaria contained fresh Nile water. The water temperature was 25 ± 2 °C, water hardness was 41 - 44 mg / L as $CaCO_3$ and its pH was 7 .2 ± O. 1. Fishes were provided twice a day with earthworms as food; they were acclimatized for 3 d in these aquaria before the experiment.

Diazinon, an organophosphate insecticide, and neopybuthrin, a pyrethroid insecticide, were used in the present work . The LC_{50}s of these insecticides were found to be 20 mg / L for diazinon and 2.1 mg / L for neopybuthrin as obtained from the lethal curves constructed for this purpose in a previous investigation performed by EI - Elaimy *et al* (1990).

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The present work aims to study the effect of multitreatments of equal sublethal concentrations of diazinon and 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 diazinon .
Aquarium (c) : contained fish individuals exposed to neopybuthrin .
The aquaria (b) and (c) were prepared so that each contained an intitial concentration of $^1/_2$ LC $_{50}$ of diazinon (10 mg / L) and neopybuthrin (1.05 mg / L) respectively . These concentrations were added to the corresponding aquaria at 24 hr . until it reached a concentration aquivalent to 1 $^1/_2$ LC_{50} . Groups of fishes from each aquarium were decapitated at intervals of 24, 48 and 72 hr. Thus we have three groups of fishes exposed to $^1/_2$ LC_{50} , 1 LC_{50} and 1 $^1/_2$ LC_{50} of each insecticide, respectively .

For electron microscopic studies, small fragments of truncal muscle tissue of control and treated fishes were removed and fixed in 2.5 % glutaraldhyde buffered at pH 7.4 with 0.1 M sodium cacodylate and postfixed in 1 % osmium tetroxide (Dalton 1955) . After dehydration in a gradual series of ethanol, the tissue was embedded in Epon 812. Polymerized blocks with tissue were cut into thin sections on a porter Blum microtome with a glass knife. The sections were stained with uranyl acetate and lead citrate (Reynolds 1963) and examined in philips EM 200 electron microscope.

RESULTS AND DISCUSSION

The structural details of normal skeletal striated muscle are seen in Figure 1. A variety of ultrastructural changes took place in muscle of fishes exposed to diazinon. The earliest changes in muscles of fishes exposed to $^1/_2$ LC_{50} of diazinon consisted of swelling of sarcoplasmic reticulum in many fibers (Fig. 2) and appearence of many cytoplasmic vacuoles of different sizes . In muscle of fishes exposed to LC_{50} of diazinon , fragmentation of the myofibrils occurred over the entire length of the sarcomere with involvement of both the thick myosin filament of the A - band and the thinner actin filaments of the I - band . In these zones residual myofilaments were arranged haphazardly (Fig. 3). In fishes exposed to 1 $^1/_2$ LC_{50} of diazinon, there was evidence of severe splitting and fragmentation of myofibrils, the Z - band being interrupted into short irregular segments . Phagocytic lysosomes of different sizes were abundant (Fig. 4). The mitochondria showed swelling and disorganization of cristae.

Ultrastructural examination of muscles of fishes exposed to $^1/_2$ LC_{50} of neopybuthrin showed the same structures previously described in control fishes. In fishes exposed to LC_{50} of neopybuthrin, the muscles showed consequent separation of the bundles of myofibrils and more sarcoplasmic reticulum (Fig. 5). The nuclei are of normal appearence , but they were poor in chromatin content. The mitochondria were abundant and showed swelling. Fishes exposed to 1 $^1/_2$ LC_{50} of neopybuthrin showed marked degeneration of muscles. The contractile elements in many fibers were replaced by granular homogenously dense material

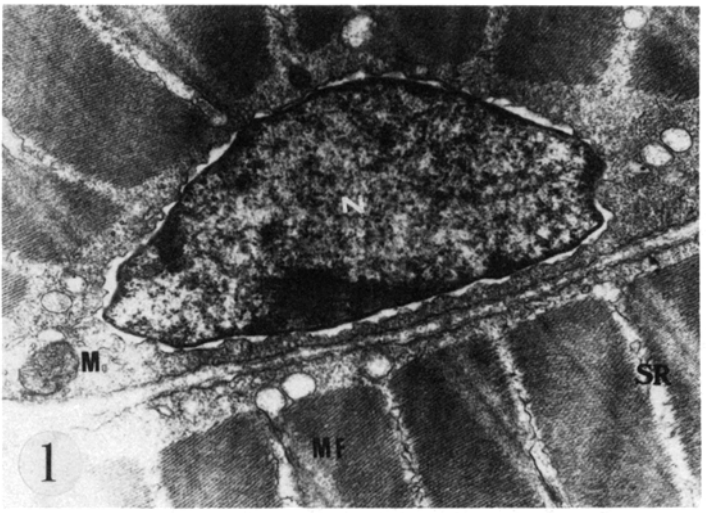

Figure 1.Electron micrograph of skeletal muscle of a control fish.The space between the fibrils (MF) is filled with sarcoplasmic reticulum (SR).The nucleus (N) is elongated, M:Mitochondria, (X 12000).

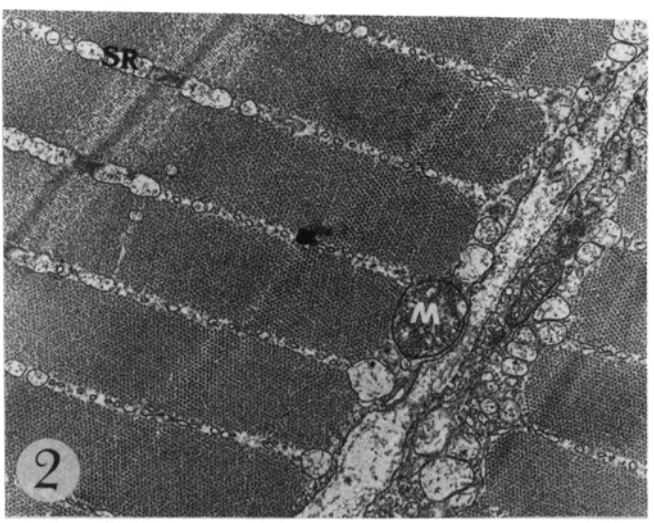

Figure 2.Electron micrograph of muscle of a fish exposed to ½ LC_{50} of diazinon showing swelling of sarcoplasmic reticulum (SR), (X 10000).

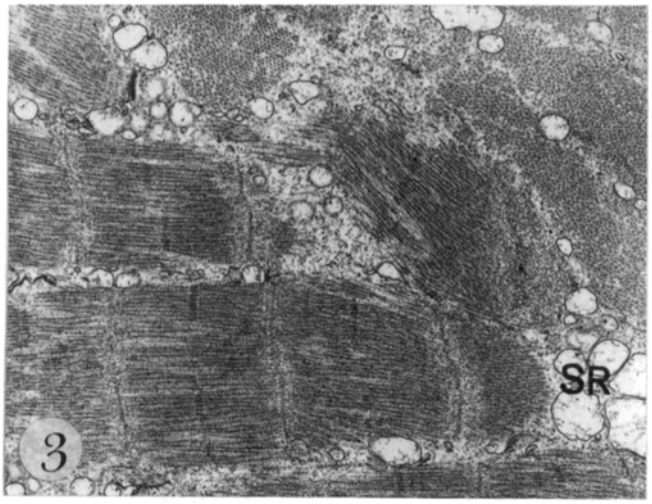

Figure 3.Electron micrograph of muscle of a fish exposed to one LC_{50} of diazinon.There is focal loss of myofibrils extending over the entire length of the sarcomere. Vesicles of sarcoplasmic reticulum (SR) are seen, (X 12000).

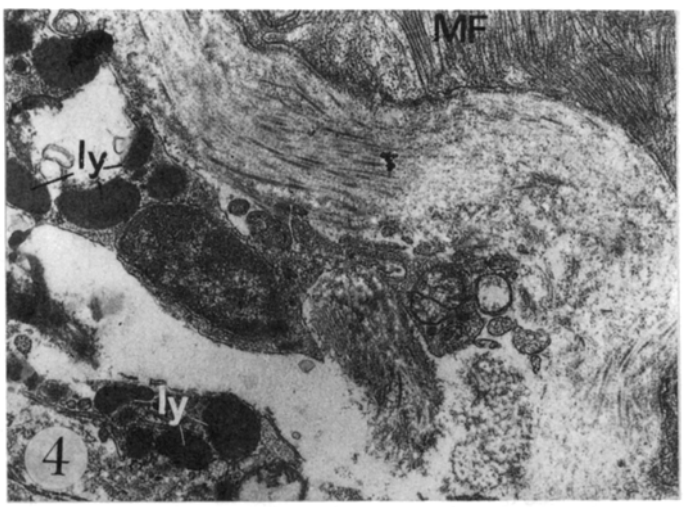

Figure 4.Electron micrograph of muscle of a fish exposed to 1 ½ LC_{50} of diazinon showing destruction of the muscle structure. Lysosomes (ly) are abundant,MF:Myofibrils, (X 12000).

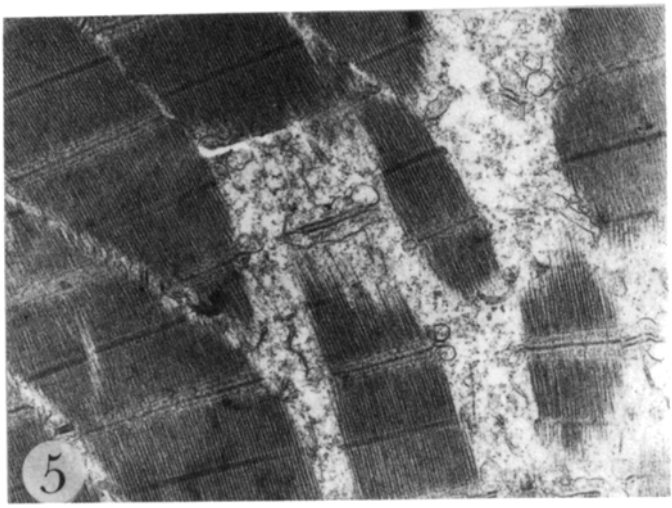

Figure 5.Electron micrograph of muscle of a fish exposed to LC_{50} of neopybuthrin showing fragmentation of myofibrils, (X 12000).

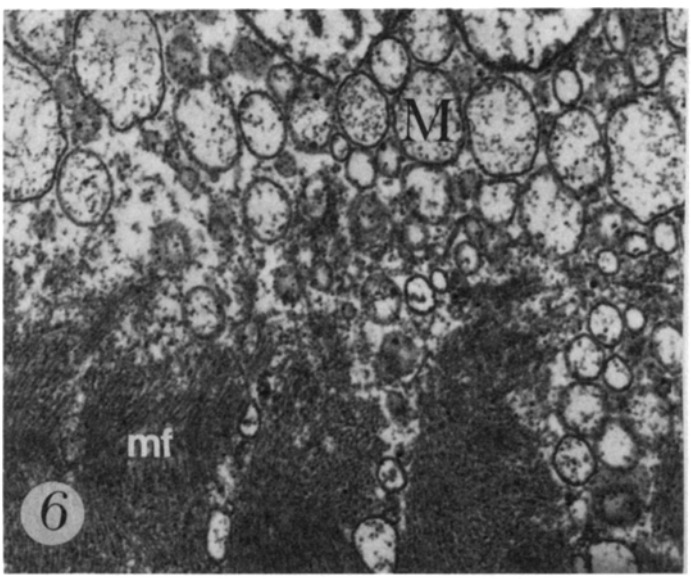

Figure 6.Electron micrograph of muscle of a fish exposed to ½ LC_{50} of neopybuthrin.There is replacement of the myofibrils (mf) by granular homogeniously dense materials arranged in bands.Many mitochondria (M) are swollen with degeneration of its cristae,(X 14000).

arranged in bands (Fig. 6). In the nuclei , the chromatin material aggregated in the periphery near the nuclear membrane leaving the center free of chromatin. The mitochondria showed marked swelling with disorganization of the cristae (Fig. 6) Numerous phagocytic lysosomes were noticed and appeared as vacuoles bounded by a membrane which contain homogenous materials.

The results of the present work have demonstrated that the organophosphate insecticide, diazinon, and the pyrethroid insecticide, neopybuthrin , when present in sufficently high concentrations in the medium, led to histopathological alterations in the muscle of Tilapia nilotica.

Many studies have dealt with the histopathological effects of different insecticides in fishes. Eller (1971) found that there were cellular changes in the liver of Salmo clarki following food or water exposure of 0.01 mg / kg body wt or 0.01 ppm of the insecticide endrin. Malathion was found to induce histopathological lesions in the liver of Channa punctatus (Dubale and Shah 1979). Mandal and Kulshrestha (1990) studied the effects of sublethal concentration of sumithion on the liver, kidney and intestine of Clarias batrachus. They observed liver necrosis, vacuolation of the epithelial cells of the urineferous tubules and lesion formation in the villi of the intestine. Desai *et al.* (1984) investigated histological changes in the liver of Tilapia mossambica after exposure to monocrotophos, an organophosphate, characterized by necrosis at the intial stage of intoxication. They suggested that this could be either due to a direct effect of the toxicant on the cell or to an accumulation of acetylcholine in the tissues. El-Elaimy *et al.* (1990) reported that exposure of Tilapia nilotica to increasing concentrations of diazinon or neopybuthrin induced numerous ultrastructural alterations in the intestine. Kibler (1973) found that injection of the organophosphate insecticid parathion in rats for 2 wk induced skeletal muscle necrosis. Fenichal et al . (1972) demonstrated that the chronic administration of paraoxon, the active metabolite of parathion, in doses of about two-thirds the LD_{50} produced a progressive myopathy. The myopathy was prevented by denervation and modified by hemicholinium and was thought to be the result of excessive acetylcholine stimulation at the neuromuscular junction.

The most important mode of action of insecticides in general is the inhibition of acetylcholinesterase (AchE) which would bring about the inabillity of body muscles to perform properly (Coppage and Braidech 1976). Acute and chronic exposure to different organophosphates resulted in marked decrease in AchE activity in different fishes. George and Robert (1974) found that sublethal concentration of malathion reduced the AchE activity in three salmonid species. The inhibition of brain AchE under toxicity stress of malathion was observed by Mukhopodhyay and Dehodrai (1980) in Clarias bartachus. AchE inhibition was found in brain of cod Gadus callorias exposed to paraoxon (Alsen *et al.* 1963). Gabr (1986) found that AchE activity was inhibited in the brain and liver of Tilapia nilotica subjected to diazinon.

Pyrethroid insecticides were found also to be cholinergic inhibitors. It was reported that the destructive changes due to the attachment of such insecticides at the ends of cholinergic nerve fibers leads to excessive production of acetylcholine and then inhibition of AchE (Narahashi 1971).

In the present work , exposure to diazinon , as well as neopybuthrin, at different concentration levels induced many pathological changes in the muscle cell organelles of Tilapia nilotica. It is speculated that these effects may be attributed to the anti - cholinesterase activity of these insecticides.

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