

Effects of Cypermethrin on Isolated Frog Sciatic Nerve: An Ultrastructural Study

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Aim: Cypermethrin is a potent representative of the type 2 pyrethroid insecticides. This study aimed to investigate the ultrastructural effects of cypermethrin on isolated frog sciatic nerve.

Materials and Methods: 28 *Rana ridibunda* frogs were chosen and randomly divided into four groups (n = 7). After decapitation, sciatic nerves were isolated. All nerves except the control group were exposed to 2.5 µM cypermethrin in Ringer's solution for 30 (group 1), 45 (group 2) and 60 (group 3) minutes, respectively. Tissue samples were evaluated by transmission electron microscope.

Results: Degeneration in the myelin sheath and axon, decrease in the number of microtubules and neurofilaments, and evident damage in mitochondria were observed in all treated groups.

Conclusions: Our findings suggest that cypermethrin affects both the myelin sheath and the axon in all groups, thereby impairing the nerve impulse conduction. In addition to these findings, notable degeneration of mitochondria and decrease in numbers of microtubules may also play an important role in this nerve conduction impairment.

Key Words: Cypermethrin, frog, sciatic nerve, ultrastructure

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Cypermethrinin Kurbağa İzole Siyatik Siniri Üzerine Etkileri: Ultrastrüktürel Bir Çalışma

Amaç: Cypermethrin tip 2 pyrethroid insektisitlerin önemli bir temsilcisidir. Bu çalışmada cypermethrinin izole kurbağa siyatik siniri ultrastrüktürü üzerine etkisinin incelenmesi amaçlanmıştır.

Yöntemler: 28 kurbağa seçilmiş ve rastgele dört gruba bölünmüştür (n = 7). Dekapitasyondan sonra siyatik sinirler izole edilmiştir. Kontrol grubu dışındaki bütün sinirlere 30 (grup 1), 45 (grup 2) ve 60 (grup 3) dakika 2.5 µM cypermethrin uygulanmıştır. Doku örnekleri transmisyon elektron mikroskopunda incelenmiştir. Bulgular: Cypermethrin uygulanan tüm gruplarda miyelin kılıfta dejenerasyon, axonal hasar, mikrotübül ve nörofilament sayısında azalma ve mitokondrilerde hasar gözlenmiştir.

Sonuç: Bulgularımız cypermethrinin tüm gruplarda miyelin kılıf ve aksonu etkileyerek sinir impuls iletiminde bozulmaya yol açtığını düşündürmektedir. Bu bulgulara ek olarak mitokondri dejenerasyonu ve mikrotübül sayısındaki azalmanın da sinir iletiminin bozulmasında önemli rol oynayabileceği düşünülmüştür.

Anahtar Sözcükler: Cypermethrin, kurbağa, siyatik sinir, ultra yapı

Introduction

Pyrethroids are a class of synthetic potent neurotoxic compounds that are known to have high insecticidal activity and low toxicity in mammals, and they leave little residue in the biosphere (1). They have been widely considered as ideal insecticides in agriculture, public health and veterinary applications on both farm animals and pets for the prevention and control of ectoparasites (2,3).

Pyrethroids may be classified into two large groups (4,5). Type I pyrethroids (e.g., allethrin, pyrethrin and permethrin) lack a cyano moiety. Type II pyrethroids (e.g., deltamethrin, fenvalerate and cypermethrin) have a cyano group in the α -position. Symptoms of poisoning in mammals differ for each type. Type I pyrethroids cause hyperexcitation, ataxia, convulsion, paralysis (6-8), and repetitive nerve firing (9,10). In contrast, type II pyrethroid poisoning is characterized by hypersensitivity, profuse salivation, choreoathetosis, tremor and paralysis (6,8), but no repetitive nerve firing in

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sensory nerves (11). Despite some differences in the symptoms of poisoning, the primary target for both types of pyrethroids is the sodium channels. All active pyrethroids interact with sodium channels in excitable tissue and prolong sodium current evoked by membrane depolarization. Type I pyrethroids hold sodium channels open for relatively short periods, but type II pyrethroids for longer periods (12,13).

Studies of the toxicity of pyrethroid insecticides in the central and peripheral nervous system of non-target organisms are generally concerned with the biochemical, pharmacological, and physiological effects of these insecticides (9,14). Morphological studies are scarce and observed changes are discrete. Husain et al. (15) described dendritic degeneration of Purkinje neurons of the cerebellar region of rats treated with deltamethrin. With respect to the peripheral nervous system, Dick et al. (16) found no changes in sural and tibial nerves of permethrin-fed rats for long periods. Peripheral nerve damage was described in teasing of individual nerve fibers of rats intoxicated with high-dose permethrin (17). Calore et al. (18) investigated sciatic and tibial nerves of rats submitted to acute intoxication with the cyanopyrethroid deltamethrin by transmission electron microscopy and observed axonal damage and myelin degeneration.

Cypermethrin (+ Cyano-3-Phenoxybenzyl (+ Cis, trans) 3-(2,2-Dichlorovinyl)-2,2-dimethyl cyclopropone carboxylate) is a potent representative of the type II pyrethroid insecticides. This pyrethroid is used for control of insects on animals, in agriculture, house and garden. However, as with the other insecticides, cypermethrin affects non-target organisms in addition to the targets and causes various toxic effects (19-25). There is no study about the effect of cypermethrin on peripheral nerve ultrastructure. The aim of the present study was to investigate the effect of cypermethrin on isolated frog sciatic nerve with different exposure times by using electron microscopic methods. Although the frog myelinated sciatic nerve is not a mammalian nerve, it has physiologic and morphologic properties much like those of mammalian peripheral nerves (26,27).

Materials and Methods

In the present research, the subject organism was the adult frog *Rana ridibunda*. The frogs were collected from

Mersin Muftu stream. For this study, 28 frogs weighing 45-50 g were used. The study was approved by the research and ethical committee at the University of Mersin. The animals were randomly divided into four groups of seven frogs each. The frogs were stored in a tank filled with river water at 20-24 °C at 12 h photoperiods. These animals were kept in the laboratory to give them adaptation for two weeks prior to experiments. After decapitation, the isolated sciatic nerves were removed and placed in Petri dishes containing Ringer's solution (NaCl, 111.87 mM; KCl, 2.47 mM; CaCl₂, 1.08 mM; and NaHCO₃, 2.38 mM, pH 7.2). All experiments were carried out at room temperature, 20-24 °C.

All isolated sciatic nerves except the control were treated with 2.5 µM of cypermethrin (Arrivo 200g/L pure cypermethrin; LD₅₀, orally 4030 mg/kg to rats, Hektas, Turkey) for 30 min (group 1, n=7), 45 min (group 2, n=7), and 60 min (group 3, n=7). Seven isolated sciatic nerves were used as the control group. The treatment of nerves with cypermethrin in Ringer solution was undertaken in Petri dishes. Preliminary experiments were carried out to determine effective cypermethrin concentration. Cypermethrin concentrations between 0.1-5 µM were tested. The 2.5 µM concentration was chosen for further testing because it produced significant changes in sciatic nerve ultrastructure.

For electron microscopic examination, tissue samples were transferred and fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide, dehydrated in a series of increasing concentrations of ethanol solutions, cleared in propylene oxide and embedded in a resin kit (Electron Microscopy Sciences, Cat No: 14300, USA) in all groups. 70 nm ultrathin sections, cut by ultramicrotome (Leica UCT-125), were contrasted with uranyl acetate and lead citrate and examined and photographed by JEOL-JEM 1011 electron microscope.

Results

The structure of the sciatic nerve was examined at ultrastructural level. Normal peripheral nerve ultrastructure was observed in the control group (Figure 1), while degeneration in the myelin sheaths and shrinkage in the axoplasm were determined in all cypermethrin-treated groups in increasing degrees

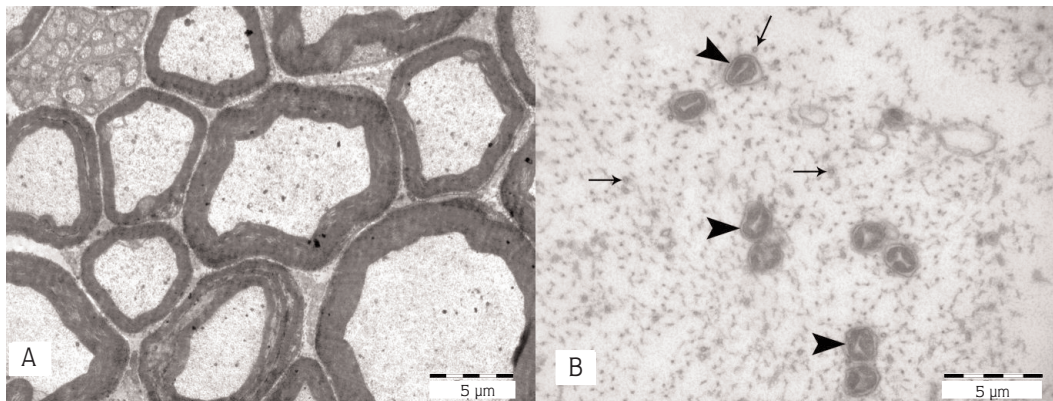


Figure 1. A. Control group. Normal myelin sheaths and axoplasm (X4000). B. Mitochondria with normal appearance (arrowhead) and many neurotubules (thin arrow) (X50000).

(Figures 2-4). These degenerative changes were not homogeneously distributed, with some myelin sheaths affected more than others. In addition to these findings, comparing to the control group, the number of neurotubules and density of the neurofilaments were markedly decreased in all treated groups, especially in groups 3 and 4 (Figures 3B, 4B). Evident mitochondrial degeneration was also observed in all treated groups. While slightly degenerated cristae were observed in group 1 (Figure 2B), severe damaged cristae were determined in groups 2 and 3 (Figures 3B, 4B). In all treated groups, mitochondrial matrix was almost completely lost (Figures 2A, 3A, 4A).

Discussion

In the present study, 2.5 µM dose of cypermethrin administered for 30 min, 45 min and 60 min produced

nerve damage. These lesions were observed in all of the animals except the controls and characterized by axonal and myelin sheath degeneration. Normal function of myelinated nerve fibers depends on the integrity of both the axon and its myelin sheath (28). Myelin provides the electrical insulation of neuronal processes and its absence leads to slowing of conduction (29). Our histopathological findings suggest that cypermethrin affects both the myelin sheaths and the axons in all treated groups. The results of our study are consistent with those of Calore et al. (18).

In addition to these findings, we observed decrease in the number of neurotubules and neurofilaments and degeneration of mitochondria in axoplasm. To our knowledge, decrease in neurotubule and neurofilament numbers was demonstrated for the first time with this study. Microtubules are important determinants of cell

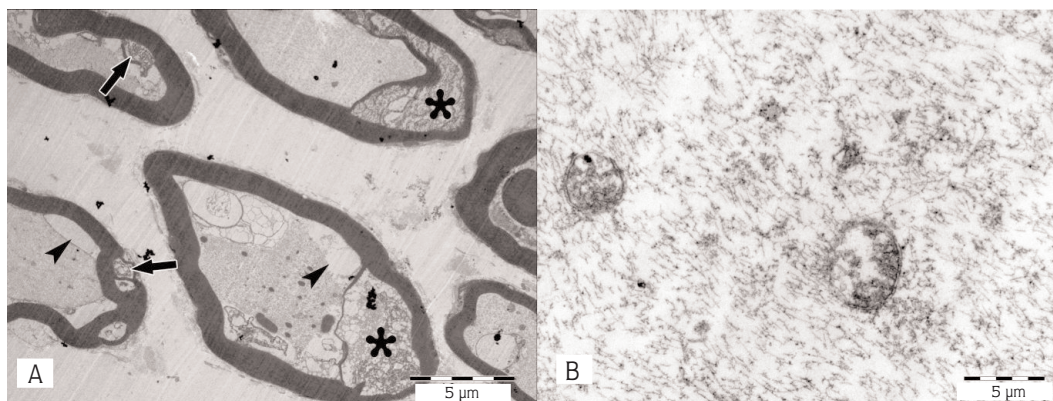


Figure 2. A. Group 1. Myelin sheath degenerations (arrow) and separations (asterisk). Shrinkages of axoplasm (arrowhead) (X2500). B. Slight crista degeneration and matrix loss (X40000).

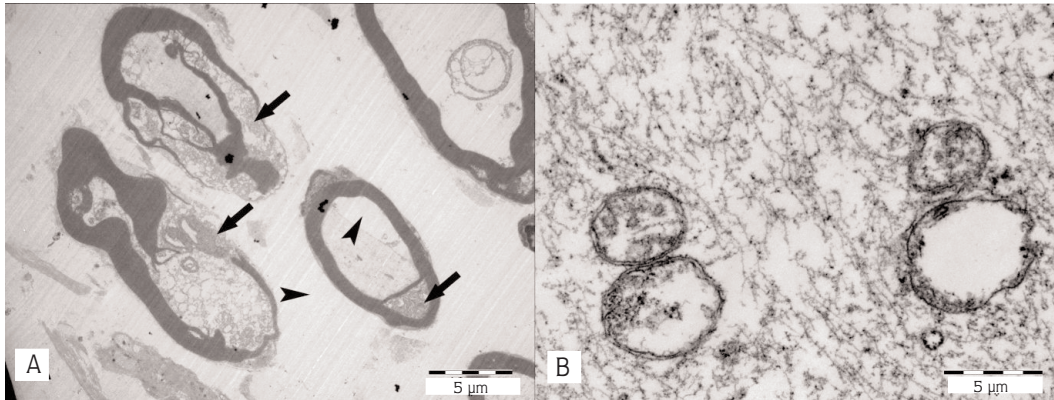


Figure 3. A. Group 2. Degenerated myelin sheaths (arrow) and axoplasm shrinkages (arrowhead) (X2000). B. Marked crista breakdown and matrix loss in mitochondria. Slightly decreased cytoskeletal components (X50000).

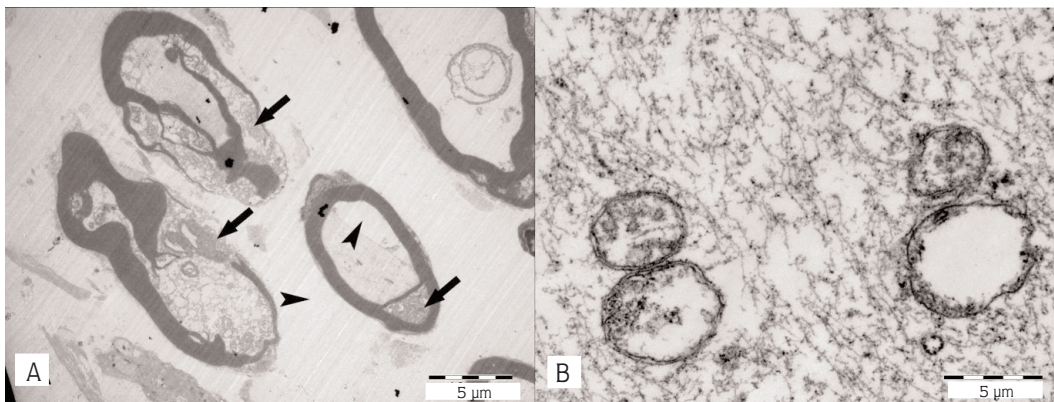


Figure 4. A. Group 3. Evident myelin sheath degenerations. Some axons almost entirely lost (asterisk) (X2500). B. Crista and matrix loss and membrane disintegrations of mitochondria. Markedly decreased cytoskeletal components (X50000).

architecture. They play key roles in intracellular transport, are a primary determinant of cell morphology, are structural correlates of the mitotic spindle, and form the functional core of cilia and flagella. The transport performed by neurotubules in axons is essential for axonal growth and maintenance. Besides the changes seen on neurotubules, decrease in the other cytoskeleton component density was also observed in axoplasm. In light of these findings, we believe that cypermethrin treatment may be associated with significant damage in the cytoskeletal structure in frog sciatic nerves.

The inner membrane of the mitochondrion is the site of oxidative phosphorylation in which the step-by-step transfer of electrons from oxygen intermediary

metabolites to molecular oxidation is coupled to proton transport and ATP synthesis. Thus, this organelle has an essential role in providing the large amount of ATP required for the electrical activity of the neurons (30). Our ultrastructural findings suggest that cypermethrin caused notable degeneration of mitochondria, and this may be one of the causes for reduced conduction of electrical impulses.

In the present study, ultrastructural degeneration of axon, myelin sheath and mitochondria and reducing numbers of neurotubules and neurofilaments were observed in isolated frog sciatic nerve. These results suggest that cypermethrin has neurotoxic effects that increase with exposure time.

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