

Full Length Research Paper

Adverse effects of dimethyl disulphide on sciatic nerve of Swiss albino mice

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The plant-derived insecticides have introduced a new concept in insecticide research. In response to insect attack, some plants can release volatile compounds in the atmosphere, which are lethal for the generalist insects. In this study, changes in the ultrastructure and electrical activity of mice sciatic nerve were examined after acute exposure to dimethyl disulphide (DMDS), a sulfur compound released from *Allium porrum*. Animals were exposed to 1/4 LC₅₀ of DMDS (0.375 µl/l air) and tissue samples were evaluated by transmission electron microscopy. Degeneration in the myelin sheath and axon, decrease in the number of microtubules and neurofilaments, and evident damage in the mitochondria were observed. On the other hand, the effect of application of 1/4 LC₅₀ of DMDS on the electrical activity of the sciatic nerve in mice showed that the number and amplitude of most of the spikes were less than that recorded in the control mice. The observed mammalian toxicity requires serious attention regarding a possible use of DMDS for pest control.

Key words: Neuropathology, electrophysiology, dimethyl disulphide, sciatic nerve, mice.

INTRODUCTION

The use of botanical insecticide represents an exciting alternative way in the biological crop protection (Carlini and Grossi-de-sa, 2002). Some of the plants can produce, in response to insect attacks, volatile secondary compounds, also known as chemical defensives, which alter insect metabolism and nervous system activity (Rauscher, 1992). Moreover, volatiles emanating from different plant parts have been shown to reduce egg viability and post-embryonic development of different species of insects (Pathak and Krishna, 1992, 1993; Gurusubramanian and Krishna, 1996). Crude extracts and/or oil from the leaf, stem, root and seeds of various plant species have been reported to possess insecticidal, antifeedant and/or growth inhibitory properties (Ivbijaro and Agbaje, 1986; Panji et al., 1991; Arnason et al., 1992; Ewete et al., 1996).

Among plants producing volatile secondary compounds, *Allium* plant species, and particularly the leek *Allium porrum*, can release in the atmosphere, when they are damaged, sulfur volatile compounds such as thiosulfinates, which can lead to the formation of disulfides [example dimethyl disulfide (DMDS), tested in this study] (Auger et al., 1989). The secondary substances of *Allium* have been extensively studied for their pesticidal effects. Available data have shown that sulfur compounds in *Allium* can be classified not only as insecticides, acaricides, nematocides, herbicides, fungicides and bactericides, but also repellent against arthropods (Auger et al., 2004). Because the thiosulfinates are lethal (via a hypothetical neurotoxic activity) for many insect species, they could be used in plant protection and particularly in the seed storage systems as fumigant (Arthur, 1996; Dugravot et al., 2002).

In comparison with the available data on the high toxicity of DMDS against a range of insect pests including eggs, larvae and adults, information about its neurotoxic effect on mammals has been rarely tackled. Accordingly, this study was designed essentially to evaluate the toxic

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effects exerted by DMDS on the sciatic nerve of Swiss albino mice.

MATERIALS AND METHODS

Healthy, adult male Swiss albino mice (*Mus musculus*) obtained from the animal house of Medical Research Institute (Alexandria University) were used in this study. The experiments were carried out on 3 to 4 months old animals weighing 20 to 25 g each. The animals were housed on the usual type of metal cages that were cleaned daily. Animals were maintained under normal laboratory conditions and allowed free access to a standard balanced laboratory diet and tap water. Two separate experiments were conducted. In the first experiment, male albino mice were assigned into two groups with 30 animals in each group and treated as follows: Control group: Mice were exposed to confined air. DMDS treated group: Animals were exposed to 1/4 LC₅₀ of DMDS (0.375 µl/l air) (Dugravot et al., 2003). During exposure to DMDS, mice were placed in experimental chamber (5 mice in each). At first, mice were placed in a small plastic transparent box with water and food. Then, each box perforated on two sides was heightened in an experimental chamber to inhibit all CO₂ effects. In fact, CO₂ and H₂O were respectively captured with potassium hydroxide and calcium dichloride. The temperature was controlled with a thermometer and maintained at 21 ± 1°C. Just before airtight closing of the chamber, DMDS was quickly applied, on an absorbent paper (Whatman paper No.1; 2 × 5 cm) placed above a glass pot, for a swift and complete spraying of product. At the end of the exposure period (24 h), five mice from the control and experimental groups were sacrificed. Specimens of mice sciatic nerve were dissected out and immediately immersed in 4F₁G mixture for 2 h and then rinsed in 0.1 M phosphate buffer (pH 7.4). Fixed samples were postfixed in 1% OsO₄ for 2 h at 4°C and washed with phosphate buffer several times for 10 min, dehydrated in graded ethanols, treated with propylene oxide and embedded in Epon. Ultrathin sections were cut on the LKB ultramicrotome with a glass knife, then stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined by a Jeol 100 CX electron microscope of the Faculty of Science, Alexandria University.

In the second experiment, male mice (n = 5/group) were initially anesthetized by ether inhalation as induction anesthesia for approximately 5 min and then mounted in plasticine, ventral side up, with four legs immobilized by hooks in plasticine. Extracellular activity of the sciatic nerve was recorded with suction electrode using the extracellular recording technique (Andrew, 1972). The suction electrode containing the saline (0.9% NaCl) was placed over the sciatic nerve and drops of DMDS (1/4 LC₅₀) were applied onto the nerve. The signals of the nerve were amplified by an AC amplifier, filtered, then displayed on a Tektronic 502 oscilloscope and recorded on a magnetic tape. The recorded signals were digitized and stored on a compact disk for further computer analysis. The same subject groups were observed 4 times using the repeated-measures design. Data were expressed as mean ± SD, and the data between two groups were subject to Student's t-test. Statistical differences at P < 0.05 were considered significant.

RESULTS

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

group (Figure 2A and B). In mice treated with DMDS, the myelin appeared irregular in profile. Thus, many areas appeared with lot of invaginations in myelin sheath and wide separation of myelin lamellae. These degenerative changes were not homogeneously distributed, with some myelin sheaths affected more than others. In addition to these findings, in comparison with the control group, the neurotubules and neurofilaments decreased in number and were fragmented. The fragmented filaments aggregated in a somewhat reticulated pattern. Occasionally, the cytoskeletal elements of the axons were broken down to clumped granular debris. In the axoplasm, evident mitochondrial degeneration was also observed in treated group. Mitochondrial swelling and broken cristae (cristolysis) were noticed, and mitochondrial matrix was almost completely lost (Figure 2A). In addition, a general decrease in mitochondrial number was detected in the axoplasm. Moreover, Schwann cells showed signs of intoxication including marginal heterochromatin aggregation in the nucleus, dilatations in the nuclear envelope, vacuolated cytoplasm and poorly developed cell organelles (Figure 2B). Non-myelinated nerve fibers were observed to be intact and the arrangement and composition of organelles within these fibers did not appear to be affected by DMDS.

As shown in Figures 3 and 4, extracellular recordings from sciatic nerve of mice showed a clear variance in the firing activity of sciatic nerve treated with 1/4LC₅₀ DMDS as compared to the control that was treated with mammalian saline. Spike discharges and the amplitude of most of spikes were less than that recorded in control mice.

DISCUSSION

In this study, DMDS produced nerve damage. The nerve lesions were observed in all treated animals 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 (Griffin, 2000). Myelin is a highly specialized multilamellar membrane that results from the elaboration of plasma membranes of oligodendrocytes in the central nervous system, or Schwann cells in the peripheral nervous system. Myelination, one of the important developmental events in the nervous system, is essential for rapid propagation of action potentials and for normal neurological function (Waxman, 1980). This process is associated with increase in axonal caliber (Windebank et al., 1985), and is a major determinant of neuronal conduction velocity (Arbuthnott et al., 1980). Thus, the findings of defective myelination in this study provide an anatomical basis for the impaired nerve function observed in DMDS treated animals. Our ultrastructural findings showed that DMDS affected both the myelin sheaths and the axons. Similar results were reported in

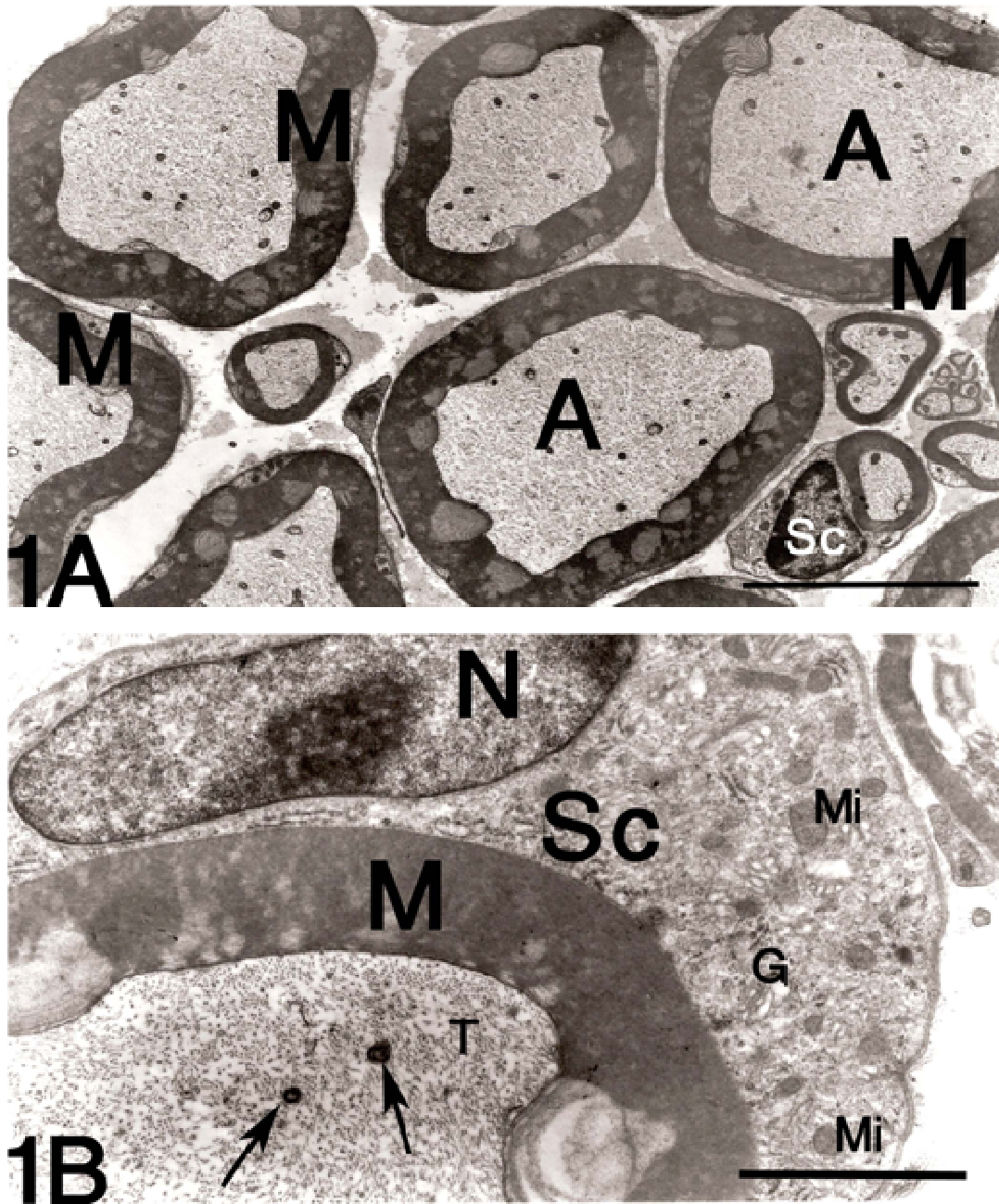


Figure 1. Electron micrographs of a cross section of sciatic nerve of control mice. (1A) Normal myelin sheaths (M) and axoplasm (A) are seen. Sc: Schwann cell. Scale bar: 5 μ m. (1B) Myelinated (M) nerve axon at the level of the nucleus (N) of an ensheathing Schwann cell (Sc), Mi: mitochondria, G: Golgi body. The axoplasm has mitochondria with normal appearance (arrows) and many neurotubules (T). Scale bar: 2 μ m. Uranyl acetate-lead citrate stain.

sciatic and tibial nerves of Wistar rat after acute intoxication with the cyanopyrethroid deltamethrin (Calore et al., 2000), in sural nerve biopsy of a patient poisoned with organophosphate insecticide (Chuang et al., 2002), and in isolated frog sciatic nerves exposed to cypermethrin (Yilmaz et al., 2008). Electron microscopic study of peripheral nerve after DMDS intoxication provided further evidence that specific injury to the myelin-

sustaining Schwann cells is the primary pathologic event in this neuropathy. Schwann cell damage and cytoplasmic disintegration were detectable. In addition to myelin collapsed, we observed progressive dissolution of the cytoskeletal elements in the axoplasm. Microtubules are important determinants of cell architecture. They play key roles in intracellular transport, are a primary determinant of cell morphology, are structural correlates

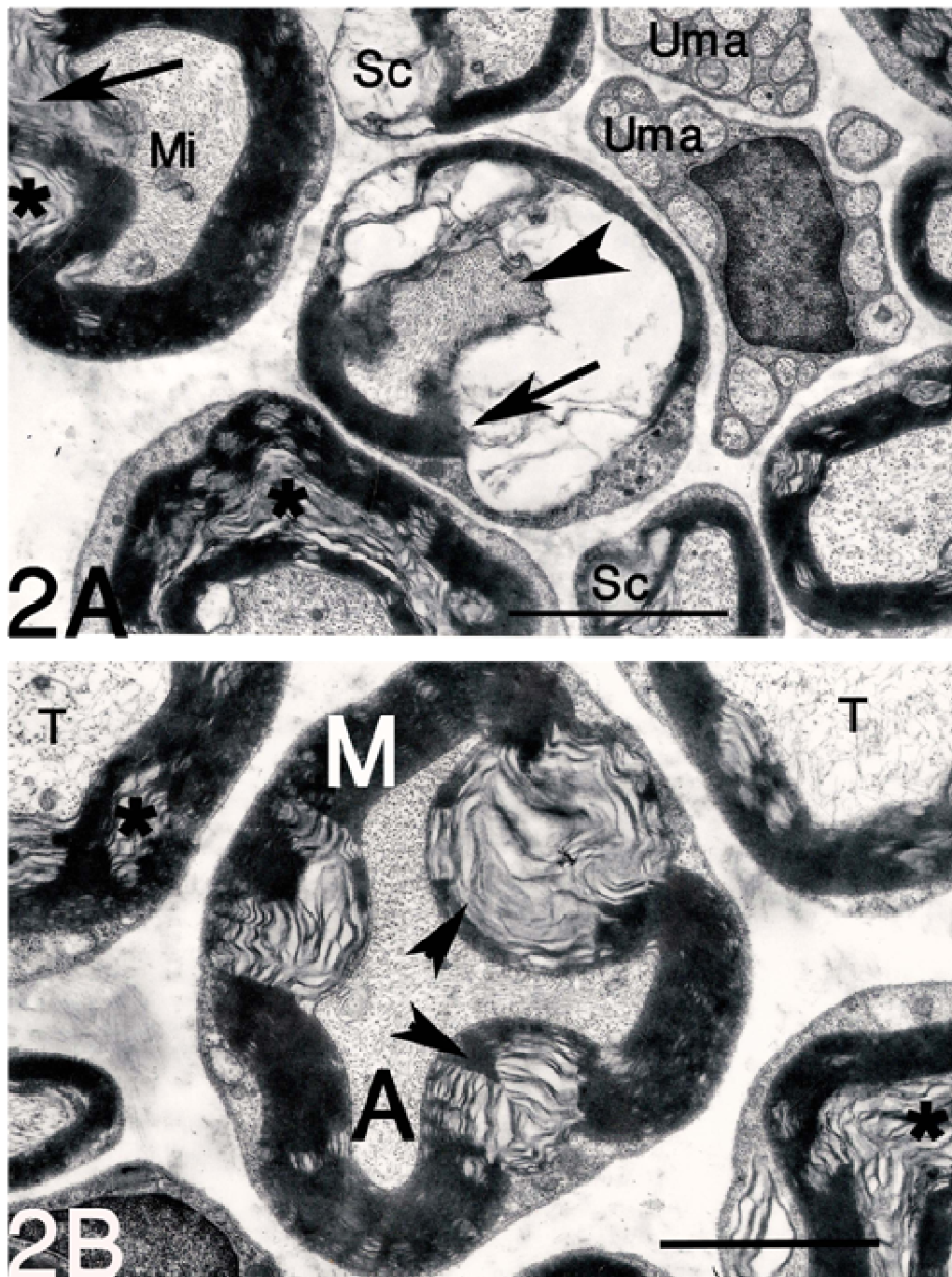


Figure 2. Electron micrographs of a cross section of sciatic nerve of mice treated with DMDS. (2A) Extensive degenerations of myelin sheath (arrows) and separations (asterisk). Shrinkages of axoplasm (arrowhead), swelling of axonal mitochondria (Mi), vacuolated Schwann cell (Sc) cytoplasm. Uma: unmyelinated axons. Scale bar: 2.68 μm . (2B) Around the axon (A), myelin sheaths initially become disrupted and form a lot of invaginations (arrowheads). Separation between myelin lamellae (asterisk) is clearly noted. Markedly decreased cytoskeletal components (neurotubules; T) is also discernible in some axons. Scale bar: 2 μm . Uranyl acetate-lead citrate stain.

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

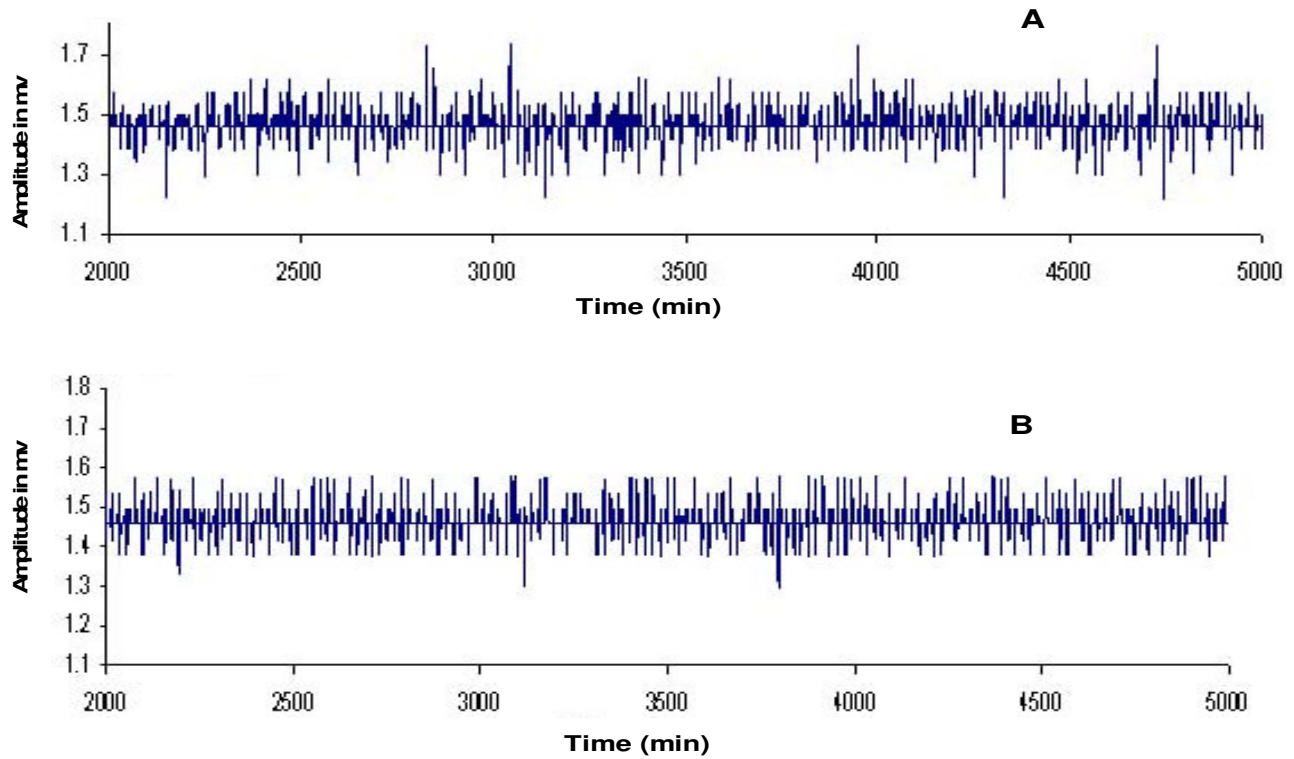


Figure 3. Spike discharges in sciatic nerve of mice in response to mammalian saline (0.9% NaCl) (A) and 1/4 LC₅₀ DMDS (B).

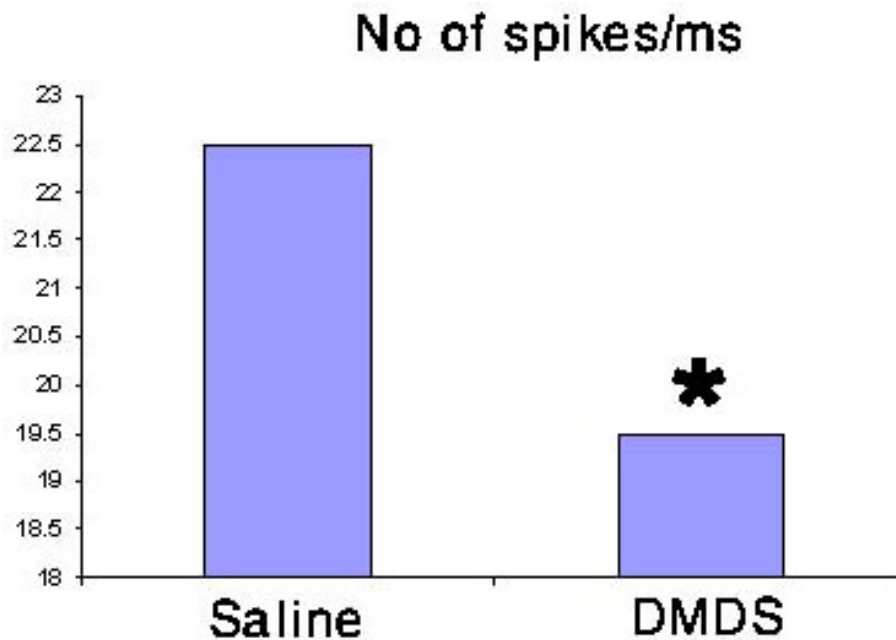


Figure 4. Effect of 1/4 LC₅₀ DMDS on the spike frequencies recorded from sciatic nerve of mice. *p<0.05 as compared to control (saline treated).

believed that DMDS treatment may be associated with significant damage in the cytoskeletal structure in mice sciatic nerves.

The obtained findings suggest that DMDS caused notable degeneration of mitochondria, and this may be one of the reasons for reduced conduction of electrical

impulses. The mitochondria were swollen or absent in the degenerated axons. 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 (Brady et al., 1999). DMDS has been reported to decrease ATP production via an inhibition of the mitochondrial respiratory chain complex IV (cytochrome oxidase) (Dugravot et al., 2003). Complex IV inhibition and its potential downstream consequences like K_{ATP} activation could also lead to membrane hyperpolarization and reduction of neuronal activity (Dugravot et al., 2003).

In this study, DMDS affected both the myelin sheath and the axon, thereby impairing the nerve impulse conduction. In addition, notable degeneration of mitochondria and decrease in numbers of microtubules may also play an important role in the nerve conduction impairment. Our findings may add another dimension to the toxicity of this agent, and there is a need for detection of such toxicity in health surveillance studies of workers exposed to DMDS.

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