The Effect of Vitamin "E" On the Peripheral Neurotoxicity of Antimony in Adult Male Albino Rat

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ABSTRACT: The present work was planned with the aim to study the histological changes that might occur in the sciatic nerve of adult male albino rat following antimony trioxide exposure and to throw more light on the protective role of vitamin "E" on the peripheral neurotoxicity induced by this environmental toxin. Sixty adult male albino rats, weighing 183 – 235 grams, were utilized in this work. The animals were divided into 3 groups; each of 20 rats: animals of group I served as control, animals of group II received antimony trioxide daily for 12 successive weeks, animals of group III received antimony trioxide and vitamin "E" daily for the same duration. Antimony trioxide was given in a daily dose of 500 mg/ kg body weight which represents 1/40 of the known LD50 and vitamin "E" was administered in a daily dose of 300 mg/kg body weight. Both antimony trioxide and vitamin "E" were given to the animals by gastric intubation. This research revealed many histological changes in the sciatic nerve, following exposure to antimony trioxide, including Wallerian degeneration in most myelinated nerve fibers with pleomorphic destruction, fragmentation, loss of normal lamination and rupture of myelin sheaths. The axoplasm of these nerve fibers were irregular, degenerated and contained myelin fragments with loss of neurofibrils. Obvious increase in endoneurium was also observed. Concomitant administration of vitamin "E" with antimony trioxide resulted in marked improvement in the histological changes observed in the sciatic nerve.

Key words: Neurotoxicity, Antimony, vitamin "E", Anatomy, Histology

INTRODUCTION

Based on information identified in the scientific and technical literature, antimony trioxide is primarily used as a plastic catalyst in the manufacture of polyethylene terephthalate (PET) plastic as well as in combination with halogenated compounds as a synergist to enhance flame-inhibiting properties (Vijverberg, 2002). Polyvinyl chloride (PVC) is an inherently inflammable halogenated polymer to which antimony trioxide may be added to improve flame retardant properties, particularly when flammable plasticizers are added (Joy, 2004). Theophilidis et al. (2009) mentioned that antimony trioxide as a flame retardant may be added to PVC and non-PVC plastics, textiles and rubbers. Typically, these products find use in electrical equipment, wires, automotive parts, some building materials and packaging (Calore et al., 2010). Flame retardants containing antimony trioxide are used in commercial and household items, including furniture, carpets, mattress covers, draperies and textiles, paper and plastic (Sheets, 2010). In addition, antimony trioxide is used in enamels for ceramics, plastics, pigments in paint and stabilizers in glass, rubber and adhesives (Narahashi, 2012).

Consumption of foods, drinks or air containing antimony compound that pollute the environment has potential health hazards (Wouter and Van Den Bercken, 2008). Kale et al. (2009) added that antimony could be absorbed through the skin, respiratory and gastrointestinal tracts. Soderlund et al. (2012) reported that the lipophilia of antimony favored their distribution into lipid-rich internal tissues, including body fat and elements of the central and peripheral nervous systems.

Intoxication of mammals, including human, by antimony trioxide had been observed in many studies, in the form of inflammatory changes in the lungs and liver (Haschek and Rousseau, 2011), induction of insulin-dependent diabetes mellitus ( Muller and Hahn, 2005), chromosomal aberrations (Talts et al., 2008) and depression of fertility and reproductive power (Islamov et al., 2012). Ray et al. (2006) found that antimony
Antimony trioxide had toxic effects on the central and peripheral nervous systems of both insects and mammals, as transient paraesthesia, muscle fasciculations and convulsions which might lead to death. On the contrary, Wouter and VanDenBercken (2008) found no changes in the sciatic, sural and tibial nerves of rats fed with antimony trioxide for short periods.

Vitamin "E" is an important effective lipid soluble antioxidant protecting the polyunsaturated fatty acids in the cell membrane (Jaha et al., 2008). Slater (2004) mentioned that antimony trioxide induced oxidative stress in the nervous system. Antimony trioxide generated free reactive oxygen radicals which caused damage to the DNA, lipids, proteins and antioxidant defense systems of cells (Hossain et al., 2005). El Demerdash et al. (2013) observed that vitamin "E" administration had a beneficial role in preventing antimony trioxide induced oxidative stress and neurotoxicity. On the contrary, Johnson (2007) denied the presence of any preventive or protective effect for vitamin "E" in antimony trioxide induced neurotoxicity. In addition, Mubarak et al. (2006) recorded irreversible antimony trioxide induced histological damage in the peripheral nerves of albino rats and vitamin "E" administration could not prevent or improve these changes.

Although most of the available researches dealt with the biochemical, pharmacological and physiological effects of antimony on the central and peripheral nervous systems of mammals, morphological studies are scarce and there are few studies with detailed histological description of nerve lesions caused by antimony trioxide. In addition, there is only little information about the protective role of vitamin "E" on neurotoxicity of antimony trioxide and the knowledge available from the literature is controversial. Therefore, the aim of the current study is to throw more light on the peripheral neurotoxic effect of antimony trioxide and the protective role of vitamin "E" on the sciatic nerve of adult male albino rat.

**MATERIAL AND METHODS**

This study was carried out on 60 adult male albino rats of Sprague Dawley strain. Their weight ranged between 183 and 235 grams. They were housed individually in well-aerated metal cages under standard conditions and were fed on a balanced powdered laboratory chow and tap water ad libitum throughout the study.

Antimony trioxide was supplied by El-Nasr Chemical Company, Jeda, KSA. Antimony trioxide was given in a daily dose of 500 mg/kg body weight which represents 1/40 of the known LD50, dissolved in distilled water before intubation (Van Rossum et al., 2004). Vitamin "E" in oily form was obtained from the same company, was dissolved in paraffin oil before intubation and was given in a daily dose of 300 mg/kg body weight (Gwino et al., 2008). The antimony trioxide and vitamin "E" used in this study were administrated orally to the animals by gastric intubation. The animals were divided into three groups; each of 20 rats:

- Group I (control group), received no medication and lived in the same environment.
- Group II (Antimony trioxide treated group), received antimony trioxide for 12 successive weeks (Van Rossum et al., 2004).
- Group III (Antimony trioxide and vitamin "E" treated group), received antimony trioxide and vitamin "E" daily for 12 successive weeks (Gwino et al., 2008).

**Histological Study**

At the end of the experiment, the animals were sacrificed. The sciatic nerve of each animal was dissected, excised and divided into two fragments. One fragment of each nerve was washed through two changes of distilled water for five minutes each, placed in 0.05% osmic acid solution for 24 hours, then washed in the running tap water, dehydrated and embedded in paraffin (Drury and Wallington, 2000). Transverse sections of 5μm-thickness were cut and examined by light microscopy. The other fragment was fixed in fresh 3% glutaraldehyde at 4°C for four hours, washed in 0.1 mol/L phosphate buffer, pH 7.4, for two hours (two changes), postfixed in 1% osmium tetroxide for one hour at 4°C, dehydrated and embedded in epoxy resin. Serial semithin sections were cut at 1μm thickness by ultramicrotome, stained with 1% toluidine blue and examined by Zeiss Axiophot microscope to detect the areas of interest (Ross, 2013). For electron microscopy, ultrathin sections (50 nm thick) were prepared using the same ultramicrotome and stained with uranyl acetate and lead citrate according to the method of Paul et al. (2007). These ultrathin sections were examined by transmission electron microscope and photographed under different magnifications for detection of ultrastructural pathologic changes.

**I. Morphometric Study:**

Morphometric measurements were performed in all specimens using Leica Qwin 500 image analyzer computer system (England). The image analyzer consisted of a coloured video camera, coloured monitor, hard disc of IBM personal computer connected to the microscope and controlled by Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The number of degenerated myelinated nerve fibers was counted in ten fields in each specimen. Their mean value ± SD were calculated using the interactive measuring.
RESULTS

Group I: (Control group)

Light microscopic examination of the sciatic nerves of control rats demonstrated the typical pattern of normal peripheral nerves. The sciatic nerve contained a large number of myelinated and non-myelinated nerve fibers which were variable in diameter and were arranged in fascicles of variable sizes (Figs. 1-3). Each nerve fiber was surrounded by endoneurium and each fascicle was enveloped by perineurium (Figs. 1-3). The sciatic nerve, including all fascicles, was enclosed by epineurium (Fig. 1). Many blood vessels of variable sizes were noticed in the epineurium, perineurium and endoneurium (Figs. 1-3).

Electron microscopy of the sciatic nerves of the control rats revealed the normal structure of the non-myelinated and myelinated nerve fibers as well as Schwann cells. The non-myelinated nerve fibers were arranged in groups; their axoplasm contained mitochondria and neurofibrils (Fig. 4). One or more non-myelinated nerve fibers were invaginated into the cytoplasm of a Schwann cell so that each fiber was embedded in the Schwann cell cytoplasm and surrounded by a double layers of Schwann cell plasma membrane called mesaxon (Fig. 4). Schwann cells supporting the non-myelinated axons were surrounded by dense endoneurium (Fig. 4). The myelinated nerve fibers consisted of central axoplasm, contained mitochondria and numerous neurofibrils, and surrounded by compact thick myelin sheath which had laminated onion-peat appearance (Fig. 5). Schwann cells supporting the myelinated axons were surrounded by loose endoneurium (Fig. 5). Schwann cells had large nuclei and their cytoplasm appeared surrounding the myelinated and non-myelinated axons (Figs. 4 & 5).

Morphometric study of sciatic nerves of the animals of this group revealed no degenerated myelinated nerve fibers (Table and Graph).

Group II: (Antimony trioxide treated group)

In this group of antimony trioxide–exposed rats, light microscopy showed Wallerian degeneration in most of the myelinated nerve fibers (Figs. 6 & 7). These degenerated nerve fibers appeared deeply stained, swollen and irregular with myelin fragments inside their axoplasm (Figs. 6 & 7). Few normal myelinated fibers could be detected in some fascicles (Fig. 6). On the other hand, the non-myelinated nerve fibers appeared healthy (Figs. 6 & 7). In all fascicles, there was obvious increase in endoneurium surrounding the nerve fibers (Figs. 6 & 7).

Electron microscopic examination of the sciatic nerves belonging to this group revealed severe degeneration of the myelinated nerve fibers (Fig. 8). The axoplasm of these fibers were irregular, degenerated with loss of their neurofibrils and contained myelin fragments (Fig. 8). There were pleomorphic destruction, fragmentation, loss of normal lamination and rupture of myelin sheaths (Fig. 8). The myelin sheath was absent around some nerve fibers and its fragments replaced the degenerated axoplasm (Fig. 9). Complete destruction of some myelinated nerve fibers with appearance of pleomorphic vacuoles in their axoplasm and myelin sheaths were also observed (Fig. 9). The non-myelinated nerve fibers appeared similar to those of the control group (Fig. 8). The endoneurium was irregular, distorted and contained tissue debris (Figs. 8 & 9).

Morphometric study of sciatic nerves of the animals of this group showed prominent appearance of degenerated myelinated nerve fibers. The mean number of these degenerated myelinated nerve fibers was 18 and P value proved to be statistically very highly significant (Table & Graph).

Group III

On light microscopy, examination of sciatic nerves of rats in group III revealed mild changes in most fascicles. Most myelinated nerve fibers were either control-like or regenerating with beaded myelin sheaths (Figs. 10 & 11). Wallarian degeneration appeared in few myelinated nerve fibers (Figs. 10 & 11). There were no obvious changes in the non-myelinated fibers (Figs. 10 & 11). Many blood vessels appeared in the endoneurium (Fig. 10).

Electron microscopic investigation of the sciatic nerves in this group demonstrated intact control-like large Schwann cells which appeared surrounding mildly degenerated myelinated nerve fibers (Fig. 12). There were small pleomorphic defects in the myelin sheaths of the degenerated fibers, while their axoplasm were healthy (Fig. 12). Complete regeneration with normal myelin sheaths and axoplasm were noticed in most myelinated nerve fibers (Figs. 12 & 13). Few nerve fibers showed moderate degeneration affecting the myelin sheath only with pleomorphic vacuoles while their axoplasm were intact (Fig. 20). The non-myelinated nerve fibers appeared normal (Fig. 13). Normal loose fibers of endoneurium were observed around the myelinated nerve fibers and Schwann cells (Figs. 12 & 13). On the other hand, dense endoneurium was detected around the non-myelinated nerve fibers (Fig. 13).
Morphometric study of the sciatic nerves in rats of this group showed that the mean number of degenerated myelinated nerve fibers was 2 with a P value statistically insignificant.

| Table 1. Mean values of the number of degenerated myelinated nerve fibers in the sciatic nerve of the control and experimental groups. |
|-----------------+-----------------+-----------------|
|                | Group I          | Group II         |
| Mean ± S.D     | 0 ± 0           | 18 ± 7           |
| Range          | 0 – 0           | 26 – 12          |
| t-test         | -               | 6.735            |
| P value        | < 0.001         | 0.09             |

S.D.: Standard deviation.

- P > 0.05: Insignificant
- P < 0.05: Significant
- P < 0.01: Highly significant
- P < 0.001: Very highly significant

DISCUSSION

Contamination of the environment with antimony and its compounds may negatively impact animal and human health (VanRossum et al., 2004). Moreover, sustained contamination results from the adsorption of antimony to small dust particles, various surfaces, air, water, and soil (Tipton and Singer, 2013). Antimony is very important as it is widely used in many industries and one of the most dangerous toxins that can insult the environment (Joy, 2004). Antimony neurotoxicity was chosen as the target for this study due to possible chronic exposure to small doses of antimony over long periods by millions of general population all over the world and its possible potential hazards to the human health. The current research is designed to evaluate the protective effect of vitamin “E” administration on the neurotoxicity of antimony trioxide on the sciatic nerve of adult male albino rat.

Adult male albino rats were used as a mammalian model for studying the possible neurotoxicity of antimony trioxide, as they are available and easy in handling (Gupta et al., 2009). In addition, albino rats were the experimental model of choice for many investigations of peripheral nerve injury as, excluding size and connective tissue density, the rat nerve was fundamentally indistinguishable from that of man (Tseng et al., 2012).

Vijverberg (2002) mentioned that there were age, sex, species and strain difference in antimony trioxide peripheral neurotoxicity, adult male albino rats were more sensitive to its toxicity than female or mice, guinea pigs and monkeys. Schulz and Beal (2011) recorded that large peripheral nerves were the major and the first site to be damaged by antimony trioxide neurotoxicity. These observations directed the decision to the sciatic nerve to be the target site for this study.

In this investigation, antimony trioxide was given in a daily low dose of 500 mg/kg body weight which represents 1/40 of the known LD 50 for 12 consecutive weeks. This low dose and duration of antimony trioxide administration were adjusted to avoid its acute intoxication and animal death (VanRossum et al., 2004). In
addition, this dose did not alter the body weight or induce growth retardation and malnutrition which might produce peripheral nerve damage (Hossain et al., 2005). Moreover, this dose of antimony trioxide was nearly similar to its environmental level in air, water and soil, in USA, as measured by Sheets (2010). The route of administration of antimony trioxide in this study was via gastric intubation because it is best absorbed through the gastrointestinal tract and the commonest way for antimony trioxide intoxication is through contaminated food and water (Kale et al., 2009). On the other hand, vitamin “E” was given in a daily dose of 300 mg/kg body weight, through the same route, which is the same dose and route of administration of vitamin “E”, as antioxidant, in adult male albino rats (Gwino et al., 2008).

Degenerative changes in the myelinated nerve fibers were observed in all sciatic nerves of experimental rats following antimony trioxide administration. Most myelinated nerve fibers were affected by severe Wallerian degeneration. This degeneration was more prominent in the myelin sheaths than in the axoplasm. The myelin sheaths revealed pleomorphic destruction, fragmentation, loss of their normal lamination, rupture, pleomorphic vacuolation or completely absent. In addition, the axoplasm were irregular and contained myelin fragments with loss of their neurofibrils. These observations are close to those recorded by Muller and Hahn (2005). On the other hand, the findings of the current study are in contrast with those of Lund and Narahashi (2003) who recorded no sciatic nerve or other peripheral nerve neurotoxicity in workers spraying antimony trioxide apart from cutaneous hyperesthesia with abnormal muscular and bony aches. The difference of these results from those of the present research may be due to the difference in the doses and duration of exposure to antimony trioxide.

Many mechanisms have been postulated to be the basis of antimony trioxide induced peripheral neurotoxicity. In 2009, Gupta et al. found changes in blood brain barrier permeability, biochemical changes, oxidative damage end-products and an increase in brain glutathione following antimony trioxide exposure. Tipton and Singer (2013) mentioned that peripheral nerve changes following antimony trioxide intoxication were secondary to central nervous system affection. Gautan et al. (2006) suggested that peripheral nerve degeneration could be due to the lack of signals received by some anterior horn cells resulting in degeneration of their peripheral fibers. Wouter and Van Den Bercken (2008) stated that antimony trioxide caused DNA fragmentation of brain cells and marked decrease in their neurotransmitters resulting in their degeneration. Halliwell (2009) owed the central effect of antimony trioxide to dendritic degeneration of Purkinje neurons of the cerebellar region. Lund and Narahashi (2003) recorded that antimony peripheral nerve neurotoxicity might be a result of sodium channel blockage of axonal and mitochondrial membranes. Narahashi (2012) thought that mammalian neurotoxicity of antimony trioxide could be attributed to a general disturbance of cell membrane function in neural tissues. Talts et al. (2008) reported an increase in the density of muscarinic cholinergic receptors in the cerebral cortex and putamen. Calore et al. (2010) referred antimony trioxide neurotoxicity to marked disturbance in Box genes which enhanced apoptosis of brain cells. Sheets (2010) noticed that monoamine oxidase activity increased and sodium potassium ATPase activity decreased in various regions of the central nervous system in rats intoxicated with antimony trioxide. Hossain et al. (2005) believed that antimony trioxide reduced the hippocampal high affinity choline uptake and acetyl choline synthesis was involved in the modulatory effects of antimony trioxide on acetyl choline release and other cholinergic activities. Mubarak et al. (2006) demonstrated that antimony trioxide increased the extracellular level of cerebral dopamine and striatal dopamine as well as cerebral dopamine uptake are differentially affected by antimony trioxide indicating that dopaminergic circuits might be antimony target. On the other hand, Ray et al. (2006) suggested the antimony trioxide affection of peripheral and central nervous systems were secondary to mammalian immunotoxicity.

Concomitant administration of vitamin “E” with antimony trioxide for 12 consecutive weeks in this research had minimized the neurotoxic effect of antimony trioxide on the sciatic nerves of adult male albino rats. Following the intake of vitamin “E”, this study revealed that most of the myelinated nerve fibers were either normal in structure or regenerating and only few fibers showed mild to moderate degree of degeneration. These observation are in accordance with that reported by Gutteridge and Halliwell (2003) who concluded that vitamin “E” administration might accelerate the regeneration process and at the same time it markedly decreased the severity of degeneration of the injured peripheral myelinated nerve fibers. The appearance of many blood vessels in the endoneurium and the increase in the blood supply observed in the current work with vitamin “E” administration may promote the process of regeneration. This confirms the view of Gautan et al. (2006) who mentioned that proper blood flow was important factor for nerve regeneration.

Halliwell (2009) suggested that vitamin “E” neutralized the oxidants generated from myeloperoxidase halide system, released from neutrophils in any tissue damage, thus suppressed the inactivation of antiproteases mediated by these oxidants. Gwino et al. (2008) believed that the protective role of vitamin “E” in antimony trioxide peripheral neurotoxicity might be due to its strong antioxidant effect. Kale et al. (2009) demonstrated that vitamin “E” inhibited the free-radical inducing damage to cell membrane and cytoplasm showed that vitamin “E” was a potential scavenger of reactive oxygen radicals. Kavirai (2003) thought that vitamin “E” prevented the formation and opposed the actions of reactive oxygen and nitrogen species.
generated in vivo and causing damage to the DNA, lipids and proteins. VanRossum et al. (2004) reported that vitamin E was the most effective lipid–soluble antioxidant protecting the polysaturated fatty acids in the cell membranes. The Muller and Hahan (2005) added that these fatty acids were important for cell membrane structure, regeneration and function. El Demerdash et al. (2007) pointed that vitamin "E" was capable of complete protection of lipids against peroxidative damage and spared tocopherol which is a great cell membrane stabilizer.

The present electron microscopic investigation revealed prominent changes in the endoneurium surrounding the myelinated nerve fibers of sciatic nerve following antimony trioxide administration. These changes appeared in the form of distortion and irregularity of the endoneurium which also contained tissue debris. On the other hand, the endoneurium was preserved, appeared intact and more dense around the non-myelinated nerve fibers, following concomitant administration of vitamin "E" with antimony trioxide. These findings are in consistency with the results of Muller and Hahn (2005) who mentioned that the changes in the endoneurium were secondary to the degenerative changes in the myelinated nerve fibers. Ross (2013) added that this dense arrangement of the endoneurium was a normal supporting factor to protect the loose unsupported non-myelinated nerve fibers.

Throughout the current experiment, the non-myelinated nerve fibers were not affected and appeared normal. This is supported by Kale et al. (2009) who pointed that antimony trioxide was concentrated more in body fat and accordingly it was directed to the myelin sheath which is lipid in nature, thus the non-myelinated nerve fibers escaped from the neurotoxic effect of antimony trioxide.

Throughout the present work, the Schwann cells appeared normal and their number increased during regeneration with vitamin "E" administration. This was explained by Tseng et al. (2012), who found that remyelination of regenerating axons after antimony trioxide neurotoxicity took place in a manner similar to that for developing nerve fibers. Tipton and Singer (2013) described that, in peripheral nerves, myelination begins with invagination of a single nerve fiber into the cytoplasm of a Schwann cell so that the fiber is embedded in a groove in the Schwann cell plasma membrane, thus forming a mesaxon and as myelination proceeds, the mesoxons wraps around the axon thereby enveloping the axon in a spiral layers of Schwann cell cytoplasm. As this process continues, the cytoplasm is excluded; the inner layers of plasma membrane fuse with each other so that the axon becomes surrounded by several layers of modified membranes which together constitute the laminated myelin sheath (Sheets, 2010).

Considering the results of the current study and correlating them to those of other investigators, it can be concluded the antimony trioxide administration results in marked peripheral neurotoxicity. Moreover, vitamin "E" administration greatly minimizes the peripheral neurotoxic effect of antimony trioxide and enhances the process of nerve regeneration.

Based on the results of the present study, it is recommended to avoid the use of antimony and its compounds in different industries as much as possible. It is also advisable for workers and persons exposed to antimony to wear protective clothes and masks and to avoid daily exposure to this environmental toxin to prevent their accumulation in body tissues and internal organs. Workers exposed to antimony compounds should be supplemented with high doses of vitamin "E". Using antimony, especially in industry, should be under the governmental control. Lists for permitted insecticides in Egypt have to be prepared in view of the recent findings concerning their safety aspects. National education programs have to be carried out to limit the use of harmful environmental toxins. Educational messages have to be published in television, newspapers, magazines and internet concerning the health hazards associated with the uncontrollable use of antimony compounds.

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Figure 1. A photomicrograph of a transverse section in the sciatic nerve of a control rat showing parts of two nerve fascicles surrounded by epineurium (E) and perineurium (P). Each fascicle contains many nerve fibers (arrows) which are surrounded by endoneurium (arrow heads). Blood vessels (V) of various sizes can be seen in the epineurium, perineurium and endoneurium.

(Osmic acid; X 100)

Figure 2. A photomicrograph of a transverse section in the sciatic nerve of a control rat showing non-myelinated (N) and myelinated (arrows) nerve fibers surrounded by endoneurium (E).

(Osmic acid; X 400)

Figure 3. A photomicrograph of a transverse section in the sciatic nerve of a control rat showing small to large non-myelinated (N) and myelinated (arrows) nerve fibers surrounded by endoneurium (E). Note the presence of many blood vessels (V) in the endoneurium.

(Toluidine blue; X 1000)
Figure 4. An electronmicrograph of a transverse section in the sciatic nerve of a control rat showing non-myelinated axons (A) of various sizes are embedded in Schwann cells (S) with mesaxons (arrows). A nucleus (N) of one Schwann cell is seen. Note the variable number of fibers enclosed by each Schwann cell. The axoplasm of non-myelinated nerve fibers contains mitochondria (M) and numerous neurofibrils (arrow heads). Schwann cells are surrounded by dense endoneurium (E).

(Uranyl acetate and lead citrate; X 5000)

Figure 5. An electronmicrograph of a transverse section in the sciatic nerve of a control rat showing a myelinated nerve fiber and a part of Schwann cell (S) with its nucleus (N). The myelinated nerve fiber consists of central axoplasm (A), which contains mitochondria (M) and numerous neurofibrils (arrow heads), and is surrounded by thick compact laminated myelin sheath (arrows) with onion-peel appearance. Loose endoneurium (E) is also seen.

(Uranyl acetate and lead citrate; X 5000)

Figure 6. A photomicrograph of a transverse section in the sciatic nerve of a rat from group II showing Wallerian degeneration (arrow heads) affecting most of the myelinated fibers. Marked decrease in number of intact myelinated nerve fibers (arrows). Non-myelinated fibers (N) appear healthy. The endoneurium (E) is markedly augmented.

(Osmic acid; X 400)
Figure 7. A photomicrograph of a transverse section in the sciatic nerve of a rat from group II showing Wallerian degeneration (arrow heads) affecting many myelinated fibers. The non-myelinated fibers (N) appear healthy. There is marked increase of endoneurium (E).

(Toluidine blue; X 1000)

Figure 8. An electronmicrograph of a transverse section in the sciatic nerve of a rat from group II showing a group of severely degenerated myelinated nerve fibers. The axoplasms (arrow heads) are degenerated with loss of their neurofibers and contain myelin fragments. The myelin sheaths reveal pleomorphic destruction (short arrows), fragmentation and rupture (long arrows). The non-myelinated nerve fibers (N) appear intact. The endoneurium (E) is distorted, irregular and contains tissue debris.

(Uranyl acetate and lead citrate; X 5000)

Figure 9. An electronmicrograph of a transverse section in the sciatic nerve of a rat from group II showing two severely degenerated myelinated nerve fibers. The myelin sheaths are absent around the nerve fibers and its fragments (arrow heads) replace the degenerated axoplasms. Complete destruction of myelinated nerve fibers with appearance of pleomorphic vacuoles (arrows) in their axoplasm and myelin sheath. The endoneurium (E) is irregular and contains tissue debris.

(Uranyl acetate and lead citrate; X 5000)
Figure 10. A photomicrograph of a transverse section in the sciatic nerve of a rat from group III showing that most of the myelinated nerve fibers are either intact control-like (arrows) or regenerating with beaded myelin sheaths (R). Few nerve fibers reveal Walarian degeneration (arrow heads). Non-myelinated nerve fibers appear healthy (N). Many blood vessels (V) are seen in the endoneurium (E).

(Osmic acid; X 400)

Figure 11. A photomicrograph of a transverse section in the sciatic nerve of a rat from group III showing that most of the myelinated nerve fibers are either control-like (arrows) or regenerating with beaded myelin sheaths (R). Few nerve fibers reveal Walarian degeneration (arrow heads). Non myelinated nerve fibers are intact (N).

(Toluidine blue; X 1000)

Figure 12. An electronmicrograph of a transverse section in the sciatic nerve of a rat from group III showing normal large Schwann cell (S) with prominent large nucleus (N) and abundant cytoplasm surrounding mildly degenerated myelinated nerve fiber which has pleomorphic defects in myelin sheath (arrow heads) and healthy axoplasm (A). Completely regenerated nerve fibers (R) are also seen. Healthy loose endoneurium (E) is observed surrounding the myelinated nerve fibers and Schwann cell.

(Uranyl acetate and lead citrate X 5000)
Figure 13. An electronmicrograph of a transverse section in the sciatic nerve of a rat from group III showing a group of myelinated nerve fibers. Two of these fibers are regenerating (R) and one of them reveals moderate degeneration (arrow) affecting the myelin sheath only but not the axoplasm (arrow head). Another group of non-myelinated nerve fibers (N) appears intact. The nerve fibers are surrounded by healthy endoneurium (E) that appears more dense around the non-myelinated nerve fibers. (Uranyl acetate and lead citrate X 5000)

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