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LIGHT AND TRANSMISSION ELECTRON MICROSCOPICAL OBSERVATIONS ON RAT SCIATIC NERVE INDUCED BY ELECTROCUTION

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ABSTRACT

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The main objective of the present study was to investigate possible alterations of the sciatic nerve of rats exposed to 220 V for 30 seconds by light and transmission electron microscope (TEM). Electric current was applied on the thigh region near the gastrocneamus muscles of rats. The sciatic nerve was taken immediately at 30 and 60 minutes and fixed in 4% cold glutaraldehyde. They were then processed for both light and TEM. Light microscope showed irregularity of the shape of the nerve bundles and fibers compared to the control. Moreover, annulations of the myelin sheath were detected and mast cell infiltration was observed around the myelin sheath, which suggested a response of the nerve tissue to injury. TEM showed that the myelin sheath of non-exposed rats had no remarkable morphological changes: the thickness was in the range (1.41±0.7) µm. On the contrary, the exposed nerve showed a remarkable increase in thickness (2.32±0.8) µm. The nerves from exposed rats were fragmented either in localized areas of the nerve where they appeared "bulby" or onion-like or in the surroundings of the entire nerve. No changes were observed in Schwann cells. Mast cells were detected around the affected nerve fibers showed empty vesicles, suggested degranulation. These results can be a helpful tool in veterinary forensic medicine.

Key words: *Electric current, Forensic toxicology, Microscopy, Morphological changes, Myelin sheath.*

INTRODUCTION

Electroporation damage is the tissue damage induced by the electric field and seems to affect mostly the plasma membrane. It is observed that cell membranes lose their anatomical structure when exposed to trans-membrane potentials above 600-800mV (Anders *et al.,* 2001). Chizuka *et al.* (1983) stated that myelin is a highly specialized multi-lamellar membrane that results from the elaboration of Schwann cells in the peripheral nervous system. Schwann cell elaborates a highly lipid-rich plasma memberane which wrap tightly around the axon many times it is known that the large, myelinated nerve fibres, should be damaged more than the non-myelinated axons. Electrical shock may induce the greatest trans-membrane potential among the largest diameter nerve fibres and those with the thickest membrane. The injury to the peripheral nerve could result in de-myelination and re-myelination, axonal degeneration and regeneration, loss of nerve fibers (focal, multifocal, diffuse), and endoneural edema (Chizuka *et al.,* 1983).

Yunxia *et al.* (2003) reported that inflammatory cells and their mediators are known to contribute to neuropathic pain following nerve injury. Mast cells play a key role in non-neural models of inflammation and we propose that mast cells and their mediators (in particular histamine) are important in the development of neuropathic pain. The study suggests that mast cell mediators such as histamine released within hours of nerve injury contribute to the recruitment of leukocytes.

The objective of the present study is to describe the possible alterations occur in sciatic nerve of rats exposed to 220 V by light and transmission electron microscopy.

MATERIALS and METHODS

Materials
Electrical domestic current:
Alternating current (220 V) using ordinary wire connected to electrical stabilizer was applied at the thigh region of all investigated rats.

Animals
Twelve rats were purchased from Assiut Lab. Animal House and acclimated for two weeks. These rats were divided in two groups exposed and non-exposed. The exposed rats were subjected to AC current 220 volt for 30 seconds through a wire fixed in the thigh region (gastrocenmous muscles). Rats were dissected after 0, 30, and 60 minutes post-exposure.

Sampling
The sciatic nerve was taken immediately after exposure, minced into small pieces and fixed in 4% cold glutraldehyde. Samples were processed and sectioned used ultratome, dehydrated, and stained with toulidine blue and examined by light microscopy. TEM samples were processed, sectioned and stained with lead citrate and uranyl acetate and examined by Joel X100 CXII TEM at Assiut Electron Microscope Unit and photo-graphed using Digital Camera CCD Jacan, Model XR. Measurements of nerve sheath from both exposed and non-exposed rats were made using the CCD camera and expressed as the Mean $\pm$ SEM.

RESULTS

Semithin sections stained with toulidine blue showed the normal appearance of sciatic nerve bundles surrounded by perineurium and epineurium. They have axons surrounded with myelin sheath (Figs.1;2). By thirty minutes of exposure to 220 V, irregularity of the nerve bundles and annulations of the myelin sheath were observed (Fig.3). Moreover, inflammatory cellular reaction was observed in the fascia surrounded the damaged nerve. Mast cells as well as other mononuclear cells were observed in the fascia surrounded the nerve bundles (Fig.4). More irregularity in the shape of the nerve fibers were observed (Fig.5). Transmission electron microscopy of the non-exposed rat sciatic nerve showed axons surrounded by myelin sheath and showann cells. The mean average thickness of the myelin sheath was (1.41±0.7) µm (Figs 6; 7). On the contrary, exposed sciatic nerve by 30 minutes of exposure had marked increase in the thickness of the myelin sheath (2.32±0.8) µm, marked irregularity, fragmentation, and appeared as "bulby" and vacuolated indicating wallerian degeneration Moreover, mast cells with several empty vacuoles were observed (Figs.8;9). By 60 minutes of exposure more fragmentation was observed (Figs.10; 11).

FIGURE LEGENDS

Fig.1: Semithin section of control rat sciatic nerve showed bundles of nerve fibers surrounded with perineurum. Toulidine blue. X5.

Fig.2: Higher magnification of Fig.1. Toulidine blue. X10.

Fig.3: Section nerve drom rats exposed to 220V and collected at 30 minutes showed irregularity of the nerve fibrs (small arrows). Toulidine blue. X10.

Fig.4: Semithin of nerve drom rats exposed to 220V and collected at 30 minutes showed accumulation of inflammatory cells mainly mast cell. (Head arrow). Toulidine blue. X40.

Fig.5: Semithin nerve drom rats exposed to 220V and collected at 60 minutes showed elongation and irregularity in the shape of the nerve fibas (arrows). Toulidine blue. X40.

Fig.6: TEM of from non exposed rats sciatic nerve showed the normal appearance of axons (Ax), myelin sheath (M), and Shwann cell (Sch). Uranyl acetate and Lead nitrate.

Fig.7: TEM of sciatic nerve drom non exposed rots showed the thickness of the myelin sheath as (1.41±0.7) µm. Uranyl acetate and Lead nitrate.

Fig.8: TEM of sciatic nerve fromexposed rats by 30 minutes showed vacualation or bulby appearance of the myelin sheath (Arrow), and elongated mast cells with empty vacuoles (star). Uranyl acetate and Lead nitrate.

Fig.9: TEM of sciatic nerve from exposed rats by 30 minutes showed the thickness of the myelin sheath as (2.32±0.8) µm. Uranyl acetate and Lead nitrate.

Fig.10: TEM of sciatic nerve from exposed rats by 60 minutes showed marked fragmentation of the myelin sheath. Uranyl acetate and Lead nitrate.

Fig.11: TEM of exposed rat sciatic nerve by 60 minutes showed the thickness of the myelin sheath as (2.78±0.8) µm. Uranyl acetate and Lead nitrate.

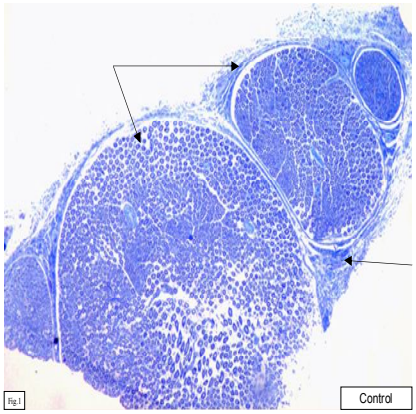


Fig.1

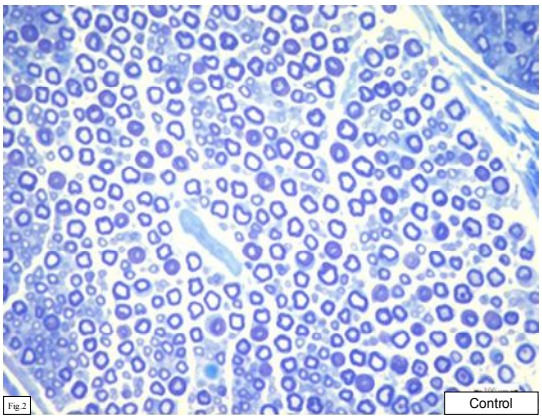
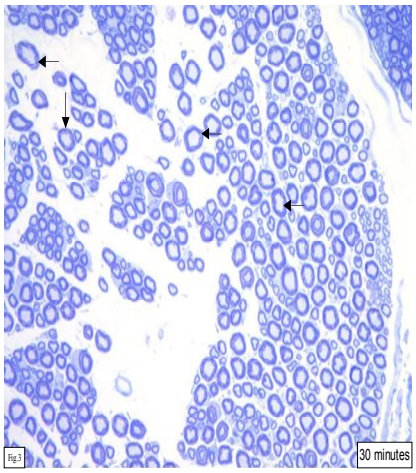


Fig.2



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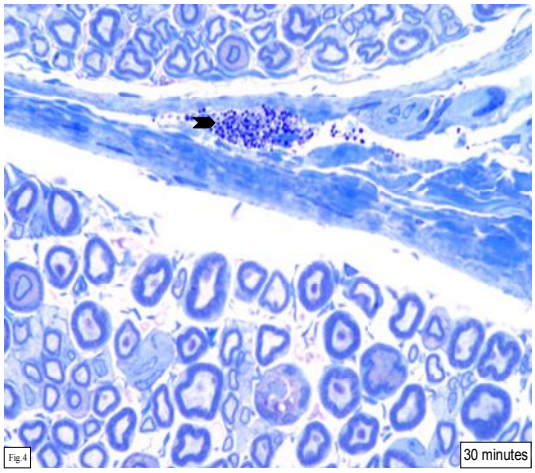

Fig.4

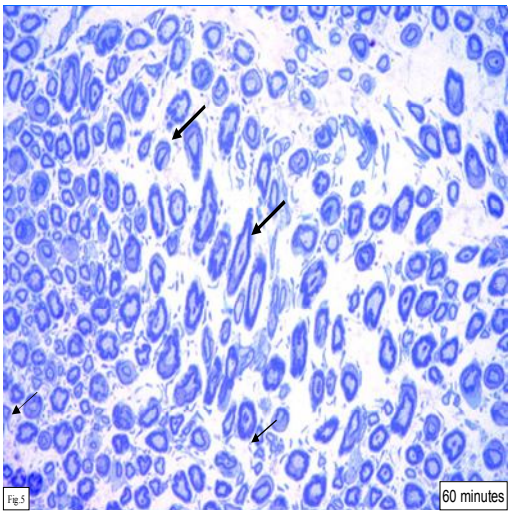

Fig.5

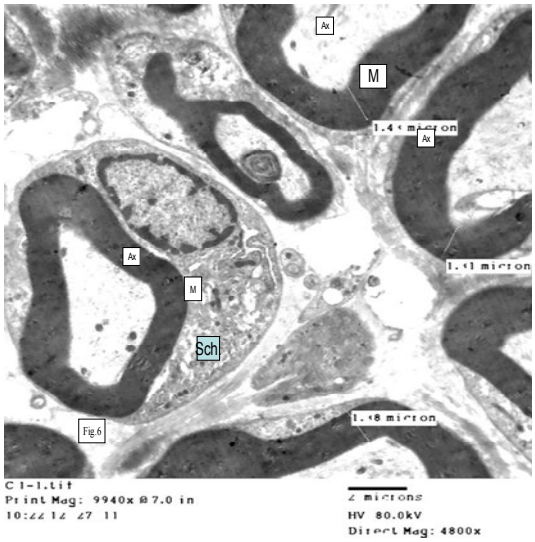

Fig.6

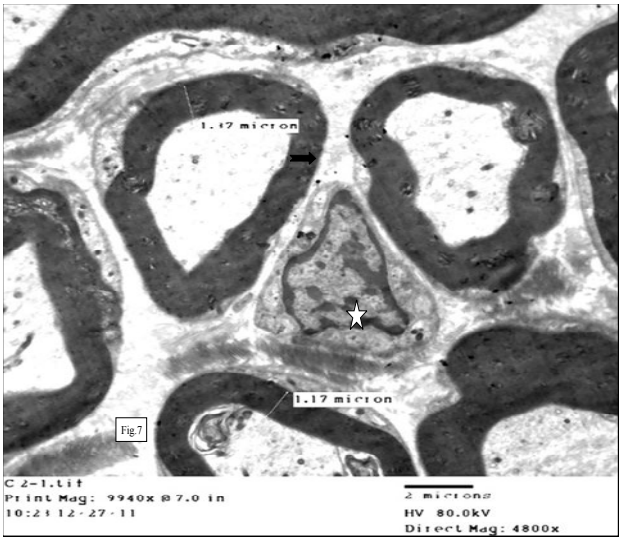

Fig.7

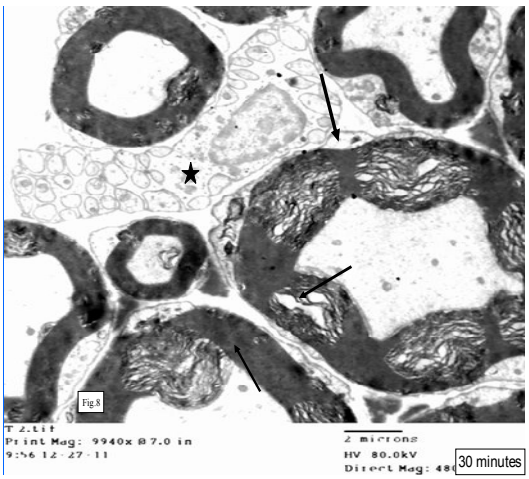

Fig.8

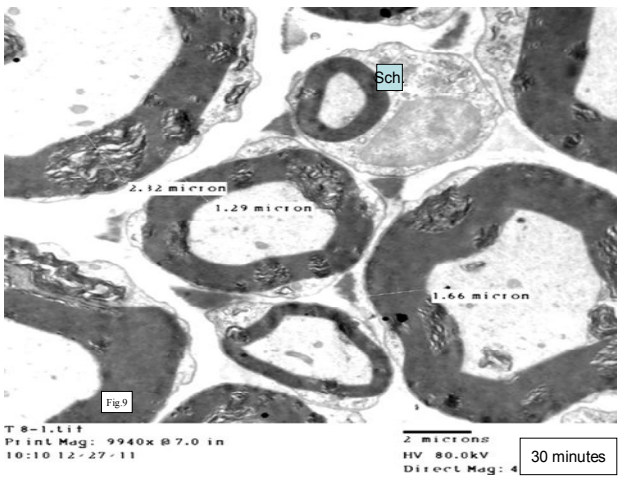

Fig.9

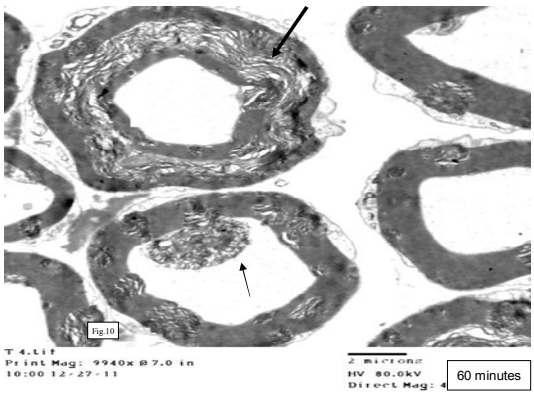

Fig.10

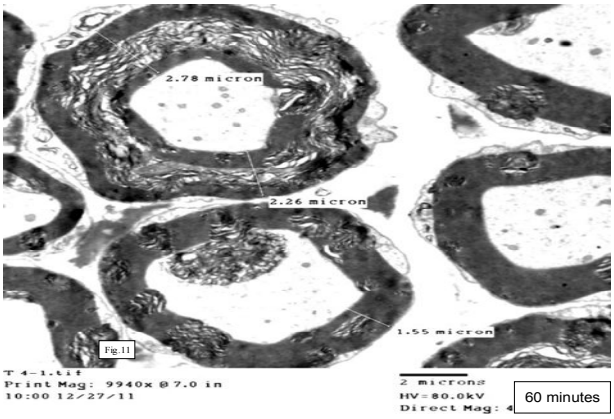

Fig.11

DISCUSSION

Kun-Wu *et al.* (2005) described the histolopathological changes in peripheral nerves exposed to electricity in rats. The blood vessel had embolism within the injured nerve. A large number of nerve fibers experienced Waller degeneration while the myelin sheath was vacuolated. Some nerve fibers experienced Waller degeneration and disintegration. Regenerative myelin appeared in some rats at about the fourth week after injury. The point of entry of the electric currents showed obvious Waller degeneration and disintegration of the myelin sheath, while some nerves showed a regenerated myelin sheath by the second week after injury. The morphology (such as quantity and diameter) of the injured myelin was basically normal by the fourth week after injury. In the present study, vacuolation, disintegration of the nerve fibers were observed at 30 and 60 minutes post-exposure compared to the non-exposed. Moreover, the severity of alteration observed in the nerve fibers was time-dependant.

Mast cells were also observed outside the damaged nerve fibers at 30 minutes post-exposure. Several reports indicated the recruitments of inflammatory cells in the injured peripheral nerves in response to trauma and legation. Degranulation of mast cells was also evident by TEM in the present study. Toews *et al.* (1998) stated that following injury to the peripheral nervous system, circulating monocytes/macrophages are recruited to the damaged tissue, where they play vital roles during both nerve degeneration and subsequent regeneration. Deborah and helme (1985) mentioned that substance P is a putative mediator of neurogenic inflammation, where it is postulated to be released from nerve terminals in the skin in response to noxious and electrical stimulation.

In conclusion, this study showed alterations of the sciatic nerve are time dependant and mast cell degranulation was profound and could contributed to the neuropathic pain.

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ملاحظات بالميكروسكوب الضوئي والالكتروني النافذ على العصب الوركى للفئران بعد تعرضه للكهرباء

دعاء صفوت ، عادل شحاتة اسماعيل ، محمود على عبد الناصر ، عمر هاشم بيومى ، صلاح حسن عفيفى

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تهدف هذه الدراسة لملاحظة التغيرات الناشئة للعصب الوركى للفئران عند تعرضه لتيار كهربائى ٢٢٠ فولت لمدة ٣٠ ثانية باستخدام كل من الميكروسكوب الضوئي والالكتروني النافذ. تم تطبيق التيار الكهربائي على عضلة gastrocneamus للفئران. تم اخذ العصب الوركى مباشرة بعد التعرض وذلك بعد ٣٠ ، ٦٠ دقيقة. وكذلك عينات من فئران غير معرضة للتيار الكهربائي وتثبيته في ٤% جلوترالدهايد وتمريره وتقطيعه وصباغته بالتوليديين الأزرق لفحصه بالميكروسكوب الضوئي. وأيضا تم اخذ قطاعات وصباغتها لفحصها بالميكروسكوب الالكتروني النافذ. اظهر الفحص بالميكروسكوب الضوئي تغيرات ممثلة في عدم انتظام ألياف العصب وكذلك تكسير في غلاف الميالين المحيط بالعصب وظهوره على هيئة حلقات عند ٣٠ دقيقة. كان هناك تجمع لخلايا التهابية Mast cells حول العصب. وعند قياس سمك العصب عند ٣٠ دقيقة µm (1.41±0.7) مقارنة بضوابط التجربة. أما بعد ٦٠ دقيقة كان هناك تكسير شديد لغلاف الميالين وزيادة في سمك الغشاء بمقدار µm (2.32±0.8) مقارنة بضوابط التجربة. لم يستدل على اى تغيرات في خلايا شوانن Schwann cells