

Original Article	Can L-Carnitine Protect Against Amiodarone-Induced Optic Neuropathy in Rabbits? <i>Mohammad Deiaa EL-Shafei¹ and Mohamed E.A. Mostafa²</i> <i>¹Histology and Anatomy² Departments, Faculty of Medicine, Cairo University</i>
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ABSTRACT

Background: Amiodarone (AD), the most effective anti-arrhythmic drug, has been reported to cause optic neuropathy, which may result in permanent visual loss.

Aim of the work: The current study aimed to clarify the effect of amiodarone administration on the structure of optic nerve and to evaluate the role of L-Carnitine (LC) in protection against such effect.

Materials and Methods: This study was carried out on 20 male rabbits divided into five equal groups (four rabbits each). Group I (control group) received no treatment. Group II received saline. Group III received LC. Group IV received AD. Group V received both LC and AD. Animals of treated Groups (II-V) received daily dose for four weeks, then animals of all groups were sacrificed one day after the last dose. Their optic nerves were subjected to light microscopic, electron microscopic and morphometric studies.

Results: After AD administration, there was a significant decrease in the number of total and apparently normal nerve fibers while the apparently degenerated ones were increased. There were areas of thickened perineurium. The myelin sheath showed areas of loss of lamellar pattern, splitting or thickening. Many axons appeared with areas of shrinkage and separation from the myelin sheath. The axoplasm of some fibers looked with inclusion bodies or with disrupted organelles. The astrocytes, oligodendrocytes and microglia cells were increased in number. Some oligodendrocytes appeared degenerated. Some oligodendrocytes and astrocytes looked with swollen mitochondria with disrupted cristae. Concomitant administration of LC with AD has minimized the AD-induced effects on the optic nerve.

Conclusion: It is concluded that AD administration caused several degenerative effects in the optic nerve. These degenerative effects were greatly ameliorated by concomitant administration of LC. Thus, it is recommended to use LC with AD for treatment of cardiac arrhythmias.

Key Words: Amiodarone, optic nerve, l-carnitine.

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INTRODUCTION

Amiodarone (AD) is used in the treatment of ventricular tachycardia and fibrillation and in restoration of sinus rhythm in atrial fibrillation. The widespread use of this drug has increased after approval by the American Heart Association for its use in advanced cardiac life support protocols (Nagra *et al.*, 2003). Amiodarone is considered the most effective anti-arrhythmic drug and is widely prescribed (Zimetbaum, 2007).

Amiodarone (AD) blocks potassium (K) channels that prolong the QT interval and myocardial repolarization. It blocks also sodium (Na) channels and interferes with B-adrenoceptors and Calcium (Ca) currents. The onset of amiodarone effect is after three days to

three weeks (Lacy *et al.*, 2002). It has an extensive uptake and accumulation by tissues and long elimination half-life up to 6 months (Vassallo & Trohman, 2007).

Amiodarone caused vacuolization in many human cells, such as macrophages and smooth muscle cells, where the drug was present in a concentrated form in numerous cytosolic vacuoles (Morissette *et al.*, 2008). The 48–72 hours treatment with AD induced the formation of lipid-filled vacuoles in human smooth muscle cells. The high liposolubility of amiodarone may favor its entry to cells by simple diffusion (Haggie & Verkman, 2009).

Amiodarone produces a constellation of side effects, many of which may be related to drug-induced phospholipidosis and/or vacuolar sequestration as concentric inclusions in peripheral blood leukocytes (Adams *et al.*, 1986). Amiodarone is known to generate oxygen-free radicals when the amiodarone molecule is chemically reduced and iodine is cleaved from it. The generated free radicals cause an increase in cellular lipid peroxidation and drug-lipid inclusions (Vereckei *et al.*, 1993).

Thyroid dysfunction, in the form of hypothyroidism or hyperthyroidism, was induced by AD. This may be related to its iodinated structure which interferes with thyroid gland metabolism (Singh *et al.*, 2007). Serious pulmonary toxicity that includes interstitial pneumonia, fibrosis and foamy macrophages, vacuolar cells containing multilamellar bodies, were encountered with AD treatment (Diaz-Guzman *et al.*, 2008; Ruangchira-Urai *et al.*, 2008). Liver impairment in the form of non-alcoholic steatohepatitis that can lead to cirrhosis was reported (Morissette *et al.*, 2008).

Blue-gray skin discoloration and photosensitivity were reported in 4–9% after AD administration. This might be explained by the actual presence of amiodarone in enlarged vacuoles in neutrophils, macrophages, fibroblasts and endothelial cells of the dermis (Ammoury *et al.*, 2008). The AD-induced pigmentation was maximal in exposed skin areas as UV light exposition increases the density of neutrophils and macrophages in the skin (Halliday *et al.*, 2008).

Amiodarone has also been reported to cause optic neuropathy which may result in permanent visual loss (Arnold, 2001). Other ocular side effects, which may cause visual impairment, include anterior subcapsular lens opacities, multiple chalazia and dry eye syndrome (Domingues *et al.*, 2004). The ocular changes caused by AD were described in the form of lysosome-like intracytoplasmic membranous lamellar bodies in extraocular muscle fibers, corneal epithelial, stromal and endothelial cells, conjunctival epithelium, sclera cells, lens epithelium, iris, ciliary body, choroid, retina, axons of the optic nerve and the endothelium of all ocular blood vessels. These inclusions represent lipofuscin or a drug lipid complex that cannot be metabolized by lysosomal phospholipases

(Turdumambetova *et al.*, 2005). Corneal opacities usually appear in both eyes. They are invisible to the naked eye but can be seen by slit-lamp. Corneal microdeposits and intracytoplasmic inclusions have been observed in the optic nerve axons of patients with AD-induced optic neuropathy (Li *et al.*, 2008). Moreover, AD was found to cause many secretory granules in the cytoplasm of the lacrimal gland cells. Ultrastructure study of these cells showed the presence of inclusions in their cytoplasm with homogeneous and dense structure (Fereshteh, 2008).

L-Carnitine (LC) is a natural compound, primarily located in mitochondria of kidney and liver cells and is proven to exert a protective effect against mitochondrial toxic agents (Arrigoni-Martelli & Caso, 2001). L-carnitine has been used as an antioxidant against oxidative stress produced by toxic agents in many organs such as liver, heart, stomach and kidney (Chang *et al.*, 2005; Derin *et al.*, 2004).

Many studies have reported visual impairment or loss following AD treatment but few literatures have described the effect of AD treatment on the structure of the optic nerve. Thus, the present work aimed to clarify the effect of AD administration on the structure of the optic nerve and to evaluate the role of LC in protection against such effect.

MATERIALS AND METHODS

Drugs And chemicals

- Amiodarone hydrochloride (Sanofi-aventis, France). It was available as 200 mg tablets.
- L-carnitine (Mepaco, Ismailia, Egypt). It was available as 350 mg capsules.
- Other reagents were of analytical grade and of highest quality available and were obtained from commercial sources.

Animals And Treatments

This study was carried out on 20 male Newziland white rabbits, weighing 2.5-3 Kg. They were obtained from the animal house, Faculty of Medicine, Cairo University. Rabbits were housed in stainless steel cages under normal hygienic conditions and allowed free access of water and

food throughout the study in accordance with the international guidelines for the care and use of laboratory animals. They were divided into five groups:

- **Group I (control group):** Four rabbits received no treatment.
- **Group II (saline-treated group):** Four rabbits received daily 3 ml of 0.9% saline orally, by a gastric gavage, for four weeks.
- **Group III (LC-treated group):** Four rabbits, received L-carnitine daily at a dose of 80 mg/kg body weight (bw) dissolved in 3 ml 0.9% saline orally, by a gastric gavage, for four weeks (Diaz *et al.*, 2000).
- **Group IV (AD-treated group):** Four rabbits, received amiodarone hydrochloride daily at a dose of 100 mg/kg bw dissolved in 3 ml saline orally, by a gastric gavage, for four weeks (Iwata *et al.*, 1996).
- **Group V (AD and LC-treated group):** Four rabbits, received amiodarone hydrochloride and LC daily in the same doses as in groups III and IV dissolved in 3 ml saline orally, by a gastric gavage, for four weeks.

One day after the last dose of AD and LC, the animals of all groups were anesthetized, with an intramuscular injection of ketamine (25 mg/kg) and droperidol (1.0 mg/kg). The thorax was opened, a cannula was placed in the left ventricle, the descending thoracic aorta was clamped and the right atrium was opened. Through the cannula, perfusion with 4% glutaraldehyde in 0.9% saline was started. The perfusion was stopped when the venous return from the right atrium became clear (Wang *et al.*, 2005).

The eyes were dissected and the retrobulbar part of the optic nerve, on both sides, was obtained and fixed in fresh 3% glutaraldehyde at 4°C for four hours. Specimens of 1x1 mm were cut from the fixed optic nerve and washed in 0.15 M phosphate buffer, pH 7.4, for two hours (two changes), then postfixed in 1% osmium tetroxide in phosphate buffer for one hour

at 4°C. After that specimens were dehydrated and embedded in epoxy resin. For light microscopy, serial semi-thin sections were cut at 0.5 µm thickness by Seo UMTP-6M ultramicrotome, stained with 1% toluidine blue and examined. For electron microscopy, ultra-thin sections (50-80 nm thick) were prepared using the same ultramicrotome and stained with uranyl acetate and lead citrate (Hayat, 2000). The sections were examined by Seo TEM and photographed under different magnifications.

Fixation was performed by perfusion rather than by immersion as perfusion provides much more constant preservation of cytological details (O'Leary *et al.*, 1968).

Rabbit was selected as an animal model, in the present study, due to the great similarity of the eye in human and rabbit at the anatomical and histological levels (Los, 2008).

Morphometric Study

Using Leica Qwin 500 LTD image analyzer, the number of the myelinated nerve fibers, in the optic nerve of all groups, was studied. The total number, apparently normal and apparently degenerated nerve fibers were counted using oil immersion objective lens (X 100) with a field surface area of 311.8 µm². For each group three slides, of three different specimens, were examined; for each slide 10 randomly chosen fields were studied.

Statistical Analysis

The mean±standard deviation (SD) of each group was performed for each parameter and statistically analyzed. Using analysis of variance (ANOVA), the variables among the different groups were compared. The variables of groups IV and V were compared using the post-hoc Tukey HSD test. Results were considered significant when probability (p) was ≤0.05, highly significant when (p) ≤0.01 and very highly significant when (p) ≤0.001 (Mould, 1989).

RESULTS

Morphometric Study

Statistical analysis of the mean values of total, apparently normal and apparently degenerated nerve fibers revealed no significant change in

Groups II & III versus group I (control group). There was also no significant change of the total number of the nerve fibers in group V versus group I. However, there was a highly significant decrease ($P < 0.01$) in the total and apparently normal nerve fibers in groups IV and V versus Group I. Meanwhile, there was a

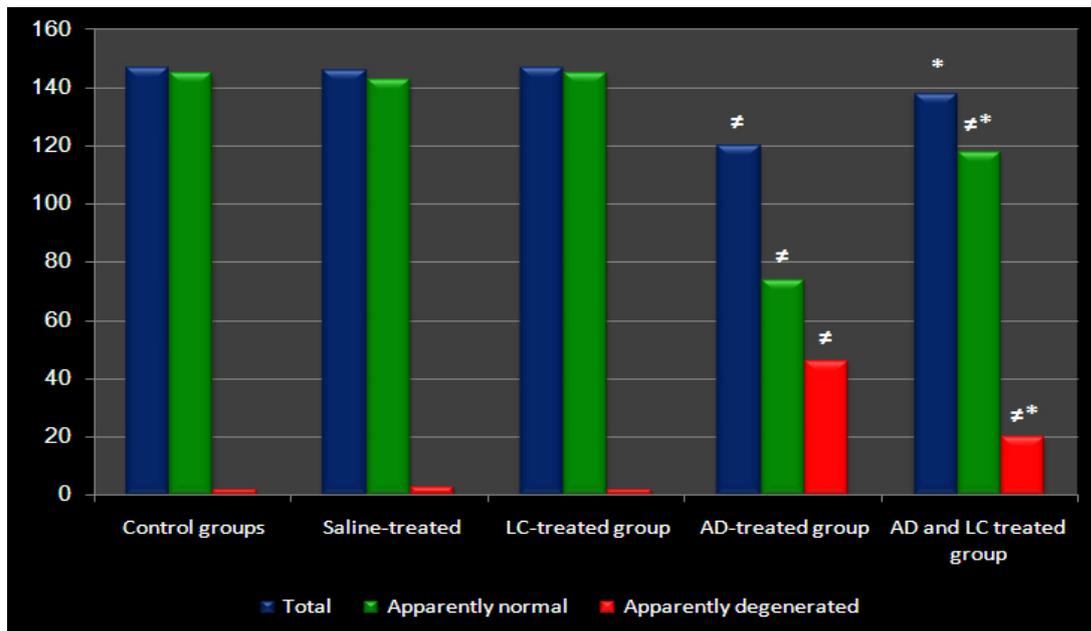
significant increase in the total and apparently normal nerve fibers ($P < 0.05$) in group V versus group IV. Apparently degenerated nerve fibers were highly significantly increased in Groups IV and V versus group I, while there was significant decrease in group V versus group IV (Table 1; Histogram1).

Table 1: Comparison of the mean number of total, apparently normal and degenerated nerve fibers (\pm SD) in 311.8 μm^2 of the optic nerve in the studied groups

	Control group (Group I)	Saline-treated group (Group II)	LC-treated group (Group III)	AD-treated group (Group IV)	AD+LC treated group (Group V)
Mean total number	147 \pm 8	146 \pm 8	147 \pm 8	120 \pm 6 [‡]	138 \pm 7*
Mean number of apparently normal fibers	145 \pm 7 (98.6%)	143 \pm 6 (97.9%)	145 \pm 7 (98.6%)	74 \pm 4 [‡] (61.7%)	118 \pm 5* [‡] (85.6%)
Mean number of apparently degenerated fibers	2 \pm 1 (1.4%)	3 \pm 1 (2.1%)	2 \pm 1 (1.4%)	46 \pm 5 [‡] (38.3%)	20 \pm 3* [‡] (14.4%)

[‡] $P < 0.01$ (highly significant): when compared with Group I

* $P < 0.05$ (significant): when Group V is compared with Group IV



Histogram1: Comparison between the mean number value of the total number, apparently normal and degenerated nerve fibers in the optic nerve of the studied groups

[‡] $P < 0.01$ (highly significant): When compared with Group I

* $P < 0.05$ (significant): When Group V is compared with Group IV

Light Microscopic Study

Sections in the optic nerve of rabbits from group I (control group) revealed the standard architecture of the nerve fascicles surrounded by thin perineurium. Variation in diameter of the nerve fibers was observed. The axons appeared clear with dark ring of myelin around them. Astrocytes, oligodendrocytes and microglia cells could be seen (Fig. 1).

Sections of the optic nerve of rabbits from groups II&III showed no obvious changes from group I.

After AD administration (Group IV), some areas showed thickened perineurium. There were an increased number of astrocytes, oligodendrocytes and microglia cells compared to the control group (Fig. 2). Other areas showed swollen nerve fibers with thinning of myelin sheath. Some of these nerve fibers exhibited disrupted myelin forming fermentation chambers (Fig. 3).

Administration of LA with AD (group V) showed nearly the sound organization of the optic nerve similar to that of the control sections. There were areas of thickened perineurium (Fig. 4) and areas of increased number of astrocytes, oligodendrocytes and microglia compared to the control group (Figs. 4, 5).

Transmission Electron Microscopic (TEM) Study:

Ultrathin sections of the optic nerve of rabbits from group I revealed the usual ultrastructural configuration of the nerve fibers. Most of nerve fibers were myelinated with few unmyelinated fibers in between (Fig. 6). The nerve fibers were variable in diameter (Figs. 6, 8). The myelin sheath appeared with compact lamellated pattern (Fig. 7). The axoplasm showed neurotubules, neurofilaments (Figs. 7-9) and mitochondria with preserved cristae (Figs. 8, 9). The astrocytes seemed having euchromatic nuclei with preserved mitochondria in their cytoplasm (Fig. 8). Oligodendrocytes were observed with heterochromatic nuclei and exhibited preserved mitochondria and rough endoplasmic reticulum in their cytoplasm (Fig. 9).

Ultrastructural study of the optic nerve of rabbits from group II&III showed no observable changes from group I.

Amiodarone administration (group IV) resulted in many ultrastructural changes of the optic nerve. In some areas, there were wide spaces separating the nerve fibers (Figs. 10, 15). Some nerve fibers appeared with splitting of the myelin into multilayered whorled masses which appeared to be arising from the inner layers of myelin forming myelin figures (Figs. 10, 16). Some fibers seemed with areas of extensive thinning and disruption of their myelin sheath forming fermentation chambers (Fig. 11). Other fibers exhibited areas of thickening of the myelin sheath (Figs. 11, 13). The myelin sheath of some nerve fibers was compact lamellated in some areas and homogeneous in other areas (Fig. 12). There were areas of shrinkage and separation of the axons (Figs. 10, 11, 13, 16). The axoplasm of some fibers looked with neurotubules and neurofilaments (Fig. 12). The axoplasm of other nerve fibers exhibited inclusion bodies (Fig. 13) or swollen mitochondria with disrupted cristae (Fig. 14). The astrocytes appeared with euchromatic nuclei and their mitochondria showed disrupted cristae (Fig. 14). Oligodendrocytes possessed heterochromatic nuclei (Figs. 15, 16). Their cytoplasm appeared electron dense and devoid of organelles (Fig. 15) or showed vacuoles and swollen mitochondria with disrupted cristae (Fig. 16).

The optic nerve ultrathin sections from rabbits of group V which received LA with AD showed sound structure of most of myelinated nerve fibers which appeared similar to the control group (Figs. 17-19). Some fibers presented areas of thickening of myelin (Figs. 17, 18). Other fibers exhibited myelin figures (Figs. 17, 19). Few nerves seemed with areas of shrinkage and separation of their axons (Figs. 17, 18). The axoplasm looked with preserved neurotubules and neurofilaments (Figs. 17-19). The mitochondria appeared preserved (Figs. 17, 18) or swollen with disrupted cristae (Figs. 18, 19). Astrocytes looked with euchromatic nuclei and preserved mitochondria (Fig. 19). Oligodendrocytes seemed with heterochromatic nuclei. Most of the mitochondria were swollen with disrupted cristae (Fig. 20).

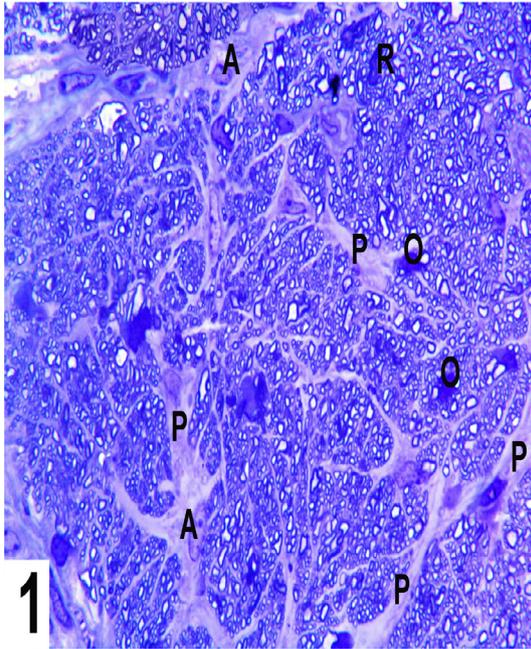


Fig. 1: A photomicrograph of a semi-thin section in the optic nerve of a control rabbit (Group I) displaying the standard architecture of the nerve fascicles surrounded by thin Perineurium (P). The axons appear clear with dark ring of myelin around them. Variation in diameter of the nerve fibers is observed. Astrocytes (A), oligodendrocytes (O) and microglia cells (R) can be seen. Toluidine blue; X 1,000

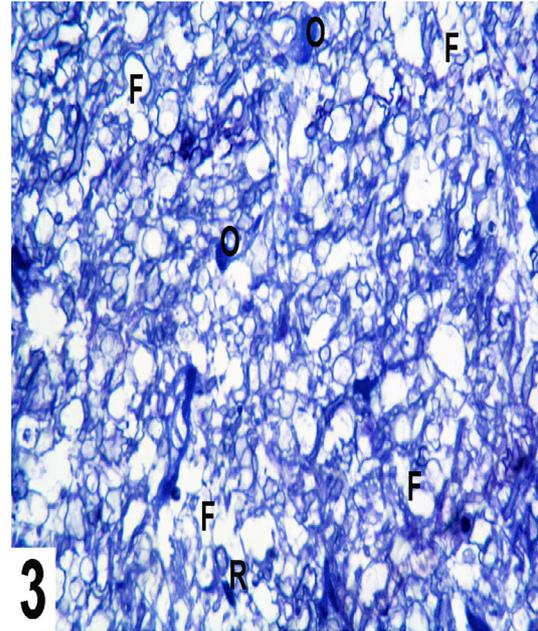


Fig. 3: A photomicrograph of a semi-thin section in the optic nerve of a rabbit from group IV presenting distorted architecture of the nerve fascicles. Most of the nerve fibers appear swollen with thin myelin around them. Disruption of myelin forming fermentation chambers (F) is observed. Oligodendrocytes (O) and microglia cells (R) can be seen. Toluidine blue; X 1,000

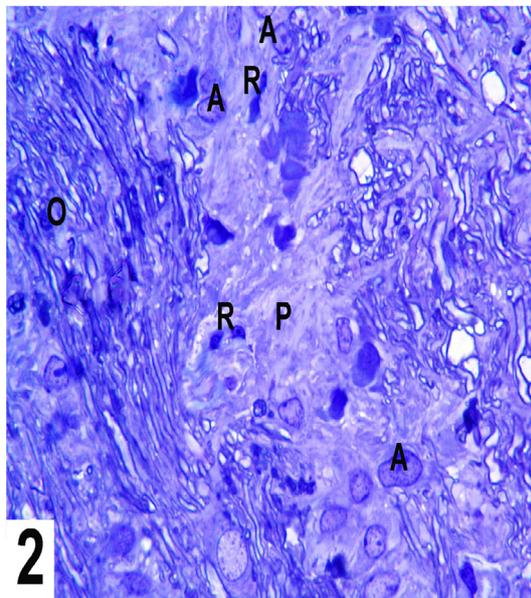


Fig. 2: A photomicrograph of a semi-thin section in the optic nerve of a rabbit from AD-treated group (Group IV) showing nerve fascicles, surrounded by thick perineurium (P). Increased number of astrocytes (A), oligodendrocytes (O) and microglia cells (R), compared to the control group, can be observed. Toluidine blue; X 1,000

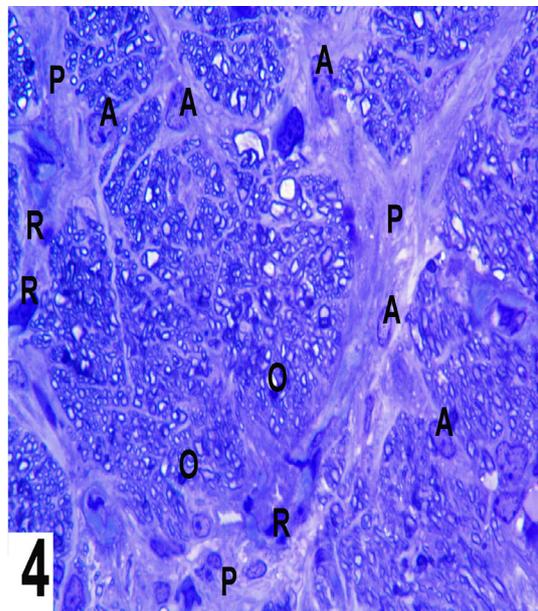


Fig. 4: A photomicrograph of a semi-thin section in the optic nerve of a rabbit from AD and LC-treated group (Group V) exhibiting the sound architecture of the nerve fascicles and surrounded by thickened perineurium (P). Increased number of astrocytes (A), oligodendrocytes (O) and microglia cells (R), compared to the control group, can be seen. Toluidine blue; X 1,000

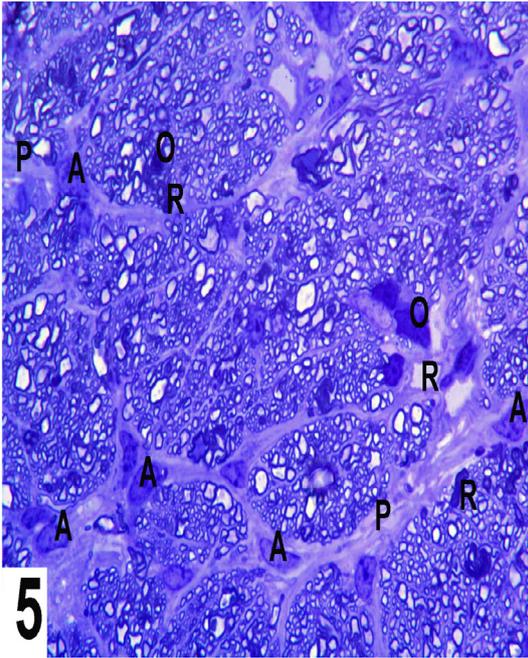


Fig. 5: A photomicrograph of a semi-thin section in the optic nerve of a rabbit from group V displaying the sound architecture of the nerve fascicles and surrounded by Perineurium (P). Many astrocytes (A), oligodendrocytes (O) and microglia cells (R), compared to the control group, can be seen. Toluidine blue; X 1,000

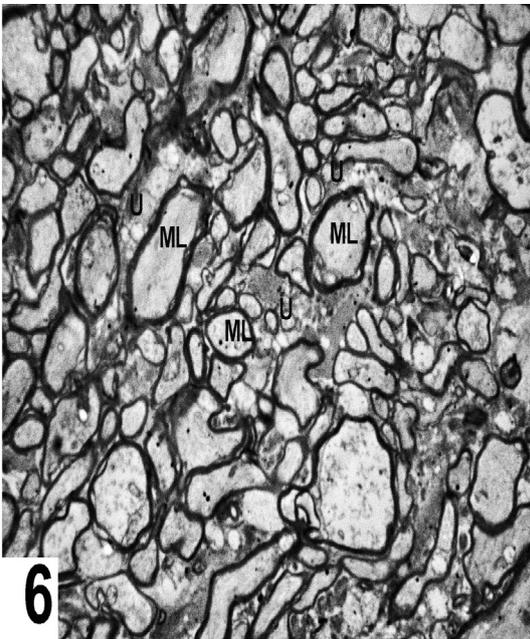


Fig. 6: An electronmicrograph of the optic nerve of a control rabbit (Group I) exhibiting the usual configuration of the nerve fibers which are myelinated (ML) or unmyelinated (U) and of variable diameters. X 3,000

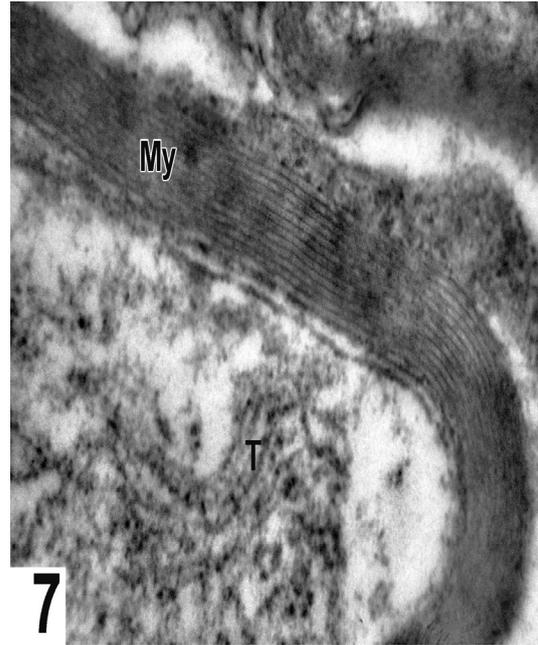


Fig. 7: An electronmicrograph of a part of a myelinated nerve fiber in the optic nerve of a control rabbit (Group I) displaying the usual compact lamellated pattern of myelin sheath (My). Neurotubules and neurofilaments (T) are observed in the axoplasm. X 80,000

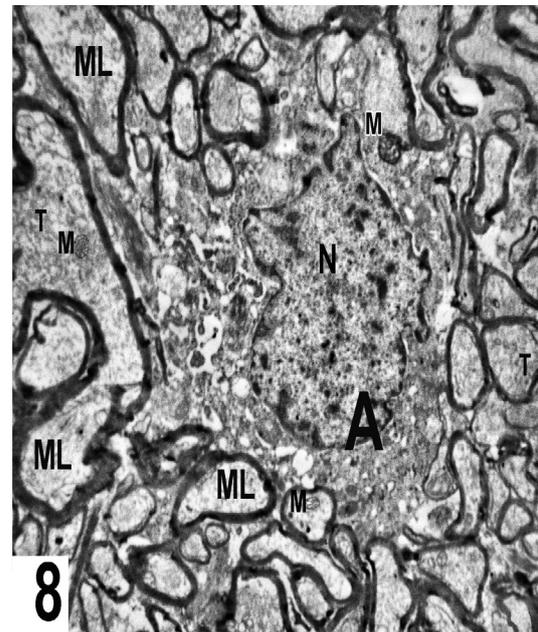


Fig. 8: An electronmicrograph of the optic nerve of a control rabbit (Group I) showing the usual architecture of the Myelinated nerve fibers (ML) of variable diameter. Their axoplasm exhibits mitochondria with preserved cristae (M), neurotubules and neurofilaments (T). An astrocyte (A) appears with euchromatic nucleus (N) and preserved mitochondria (M) in its cytoplasm. X 4,000

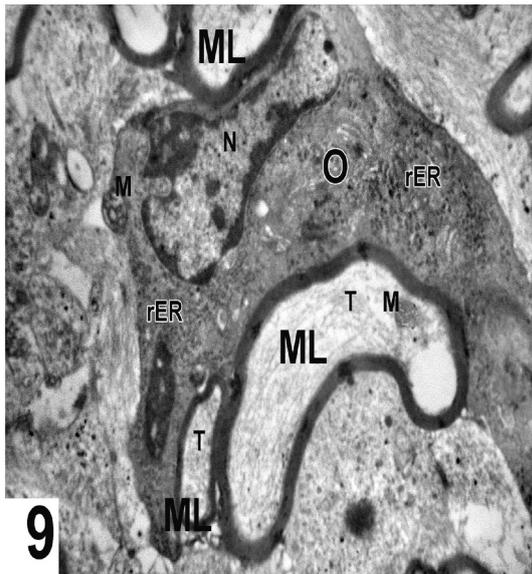


Fig. 9: An electronmicrograph of the optic nerve of a control rabbit (group I) displaying the usual presentation of the myelinated nerve fibers (ML). The nerve fibers show neurotubules and neurofilaments (T) in their axoplasm. One of them shows a mitochondrion (M) with preserved cristae. An oligodendrocyte (O) has heterochromatic nucleus (N) and cytoplasm which exhibits preserved mitochondria (M) and rough endoplasmic reticulum (rER). X 8,000

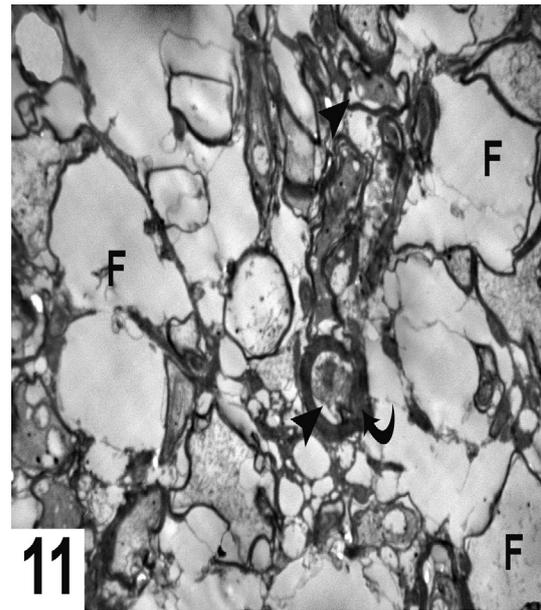


Fig. 11: An electronmicrograph of the optic nerve of a rabbit from group IV displaying myelinated nerve fibers. Some fibers show areas of extensive thinning and disruption of their myelin sheath forming fermentation chambers (F). A nerve fiber shows area of thickening of its myelin (curved arrow). Areas of shrinkage and separation of axons (arrowheads) are observed. X 3,000

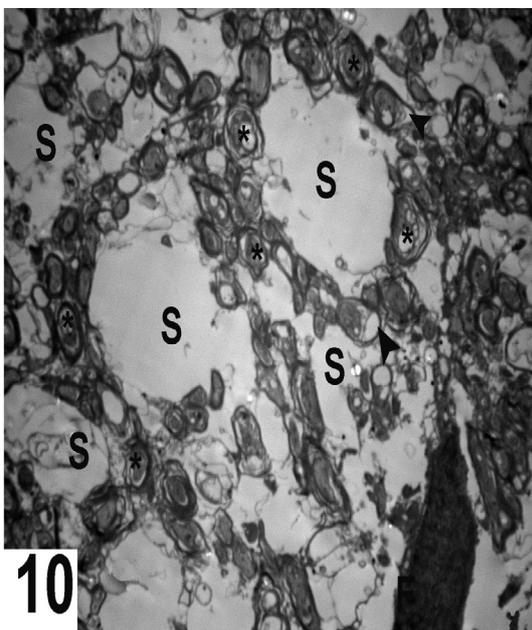


Fig. 10: An electronmicrograph of the optic nerve of a rabbit from AD-treated group (group IV) exhibiting myelinated nerve fibers. Few of them show splitting of the myelin into multilayered whorled masses forming myelin figures (asterisks). The nerve fibers are separated by wide spaces (S). Some fibers display areas of shrinkage and separation of their axons (arrowheads). X 3,000

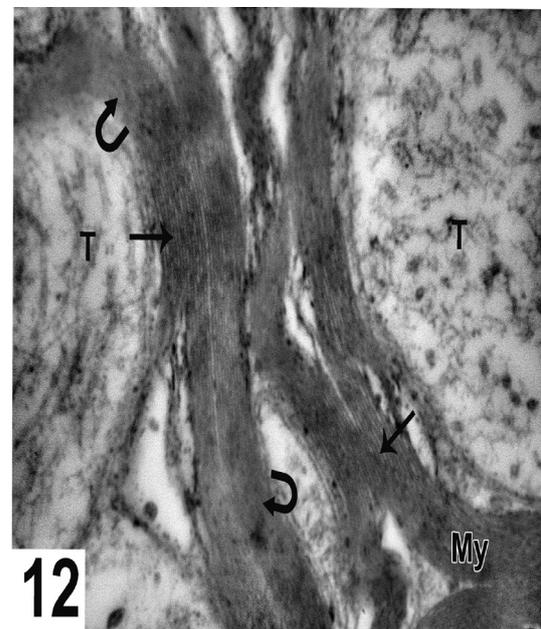


Fig. 12: An electronmicrograph of parts of three myelinated nerve fibers in the optic nerve of a rabbit from group IV displaying the myelin sheath (My) with compact lamellated areas (arrow) and homogenous areas (curved arrows). The axoplasm shows neurotubules and neurofilaments (T). X 60,000

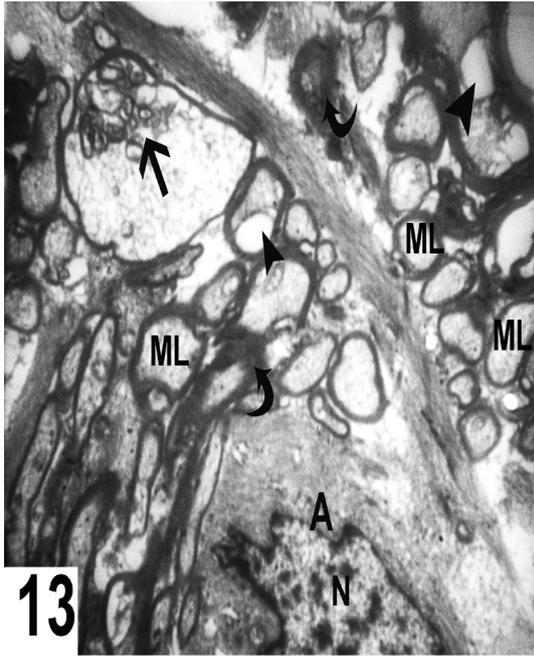


Fig. 13: An electronmicrograph of the optic nerve of a rabbit from group IV showing myelinated nerve fibers (ML). The myelin exhibits areas of thickening (curved arrows). Others show areas of shrinkage and separation of their axons (arrow heads). A nerve fiber shows inclusion bodies (arrow). Part of an astrocyte (A) appears with its nucleus (N). X 4,000

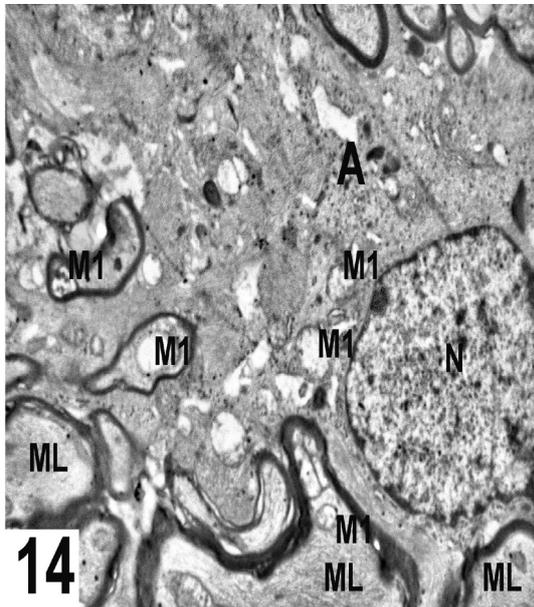


Fig. 14: An electronmicrograph of the optic nerve of a rabbit from group IV showing myelinated nerve fibers (ML). Their axoplasm of some fibers exhibits swollen mitochondria (M1) with disrupted cristae. An astrocyte (A) seems with euchromatic nucleus (N) and mitochondria (M1) with disrupted cristae. TEM X 6,000

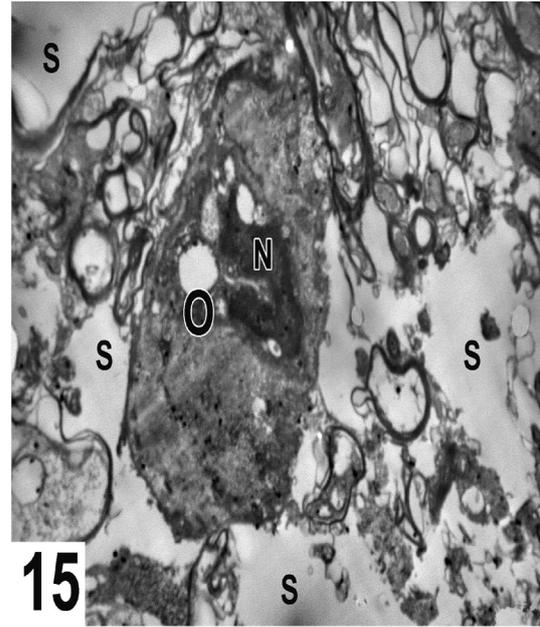


Fig. 15: An electronmicrograph of the optic nerve of a rabbit from group IV presenting myelinated nerve fibers separated by wide spaces (S). An oligodendrocyte (O) appears with heterochromatic nucleus (N) and electron dense cytoplasm devoid of organelles. X 6,000

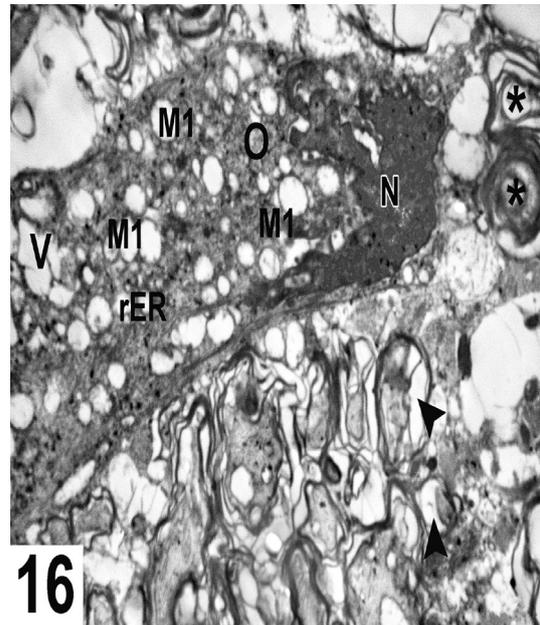


Fig. 16: An electronmicrograph of the optic nerve of a rabbit from group IV displaying degenerated myelinated nerve fibers. Some fibers show myelin figure (asterisks) with splitting of the myelin. Axons show areas of shrinkage and separation (arrowheads). An oligodendrocyte (O) appears with heterochromatic nucleus (N). Its cytoplasm contains vacuoles (V) and swollen mitochondria (M1) with disrupted cristae. X 6,000

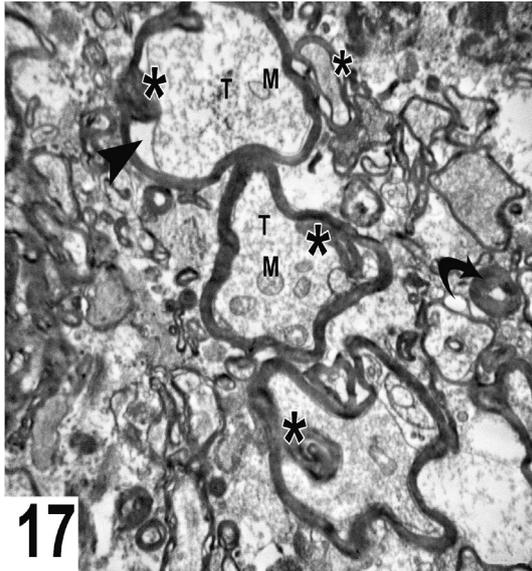


Fig. 17: An electronmicrograph of the optic nerve of a rabbit from LC and AD-treated group (group V) exhibiting myelinated nerve fibers. Many fibers appear with sound structure similar to the control group. Some fibers look with myelin figure (asterisks) or present with areas of thickening of myelin (curved arrow). One nerve shows areas of shrinkage and separation of axon (arrowhead). The axoplasm shows preserved mitochondria (M), neurotubules and neurofilaments (T). X 6,000

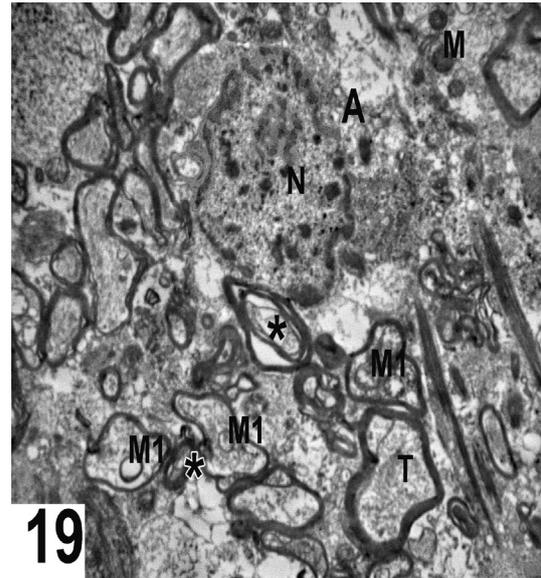


Fig. 19: An electronmicrograph of the optic nerve of a rabbit from group V exhibiting myelinated nerve fibers. Many fibers appear with sound structure similar to the control group. Few of them look with myelin figure (asterisks). The axoplasm displays preserved neurotubules and neurofilaments (T). The Mitochondria are swollen with disrupted cristae (M1). An astrocyte (A) appears with euchromatic nucleus (N) and preserved mitochondria (M). X 6,000

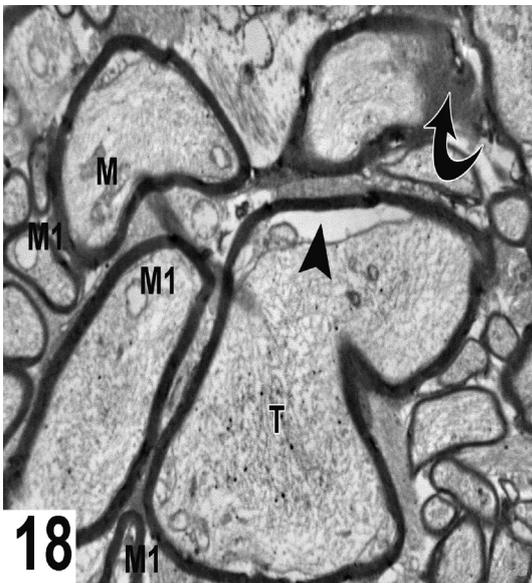


Fig. 18: An electronmicrograph of the optic nerve of a rabbit from group V presenting few myelinated nerve fibers. Many fibers appear with sound structure similar to the control group. One fiber shows area of thickening of myelin (curved arrow). Other fibers show area of shrinkage and separation of axons (arrowhead). The axoplasm displays preserved neurotubules and neurofilaments (T). The mitochondria are preserved (M) or swollen with disrupted cristae (M1). X 8,000

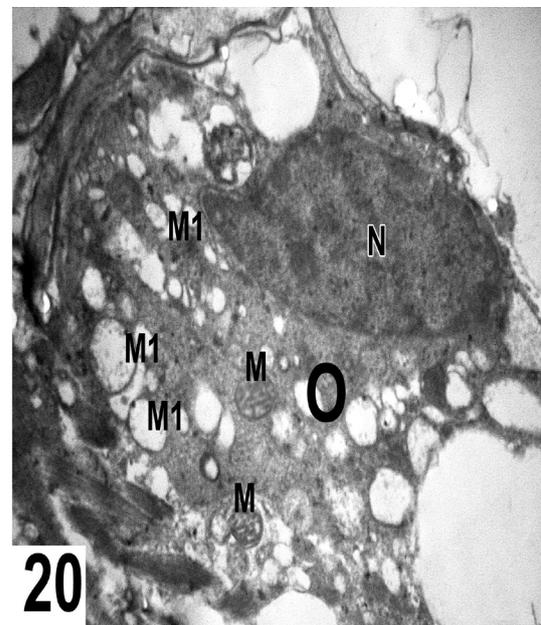


Fig. 20: An electronmicrograph of the optic nerve of a rabbit from group V showing an oligodendrocyte (O) with dark heterochromatic nucleus (N). Most of the mitochondria in their cytoplasm were swollen with disrupted cristae (M1) and few were preserved (M). X 3,000

DISCUSSION

Amiodarone (AD), the most effective anti-arrhythmic drug, has been reported to cause optic neuropathy, which may result in permanent visual loss (Arnold, 2001).

In the present work, statistical analysis of the apparently normal nerve fibers revealed a high significant decrease ($P < 0.01$) in groups treated with AD alone or with AD and LC versus control group. Light microscopic examination of the optic nerve of AD-treated group revealed areas of thickened perineurium compared to the control group. This result is in agreement with that reported by Khan (2004) who studied the effect of experimental enucleation of the eye on the optic nerve in rabbits. It could be suggested that the increased perineurium thickness may be due to shrinkage of nerve fascicles as a result of decreased nerve fibers content.

In the present study, variation in diameter of the nerve fibers was observed in control and experimental groups. Most of the nerve fibers were myelinated with few unmyelinated fibers in between. This variation in diameter in the mammalian optic tract was suggested by Guibal and Baker (2009) to reflect the addition of nerve fibers of different classes during development forming a deep to- superficial and first born-to-last born organization in the optic tract.

In the current study, AD administration resulted in many ultrastructural changes in the optic nerve. There were apparently degenerated nerve fibers distributed among the apparently normal ones. This could explain the findings of Nagra *et al.* (2003) who described visual field defects as a result of AD-induced optic neuropathy.

In the present work, the myelin sheath of some nerve fibers was compact lamellated in some areas and homogeneous in other areas. These areas of homogeneity were also observed by Hokoc *et al.*, (2002) who studied the effect of malnourishment on the ultrastructure of the optic nerve in rats. They proposed this finding as a sign of degeneration. Some nerve fibers, in the present study, appeared with splitting of the myelin sheath. The splitted myelin sheath seemed with multilayered whorled masses, which appeared to be arising from the inner layers of myelin, forming myelin figures. Such

splitting was reported by Hinman *et al.*, (2006) who studied the age-related changes in the optic nerve of monkeys. They added that this splitting might lead to concomitant disruption at sites of axoglial contact. Moreover, Van der Lugt and Venter (2007) reported that Wallerian degeneration and eventual fibrosis and atrophy of the optic nerves followed the myelin splitting. The myelin figures were also described by Saggu *et al.* (2010), 24 hours after experimental injury of the optic nerve. They claimed this splitting of the myelin sheath, which form myelin figures, as a sign of Wallerian degeneration. In other nerve fibers, in the present study, the myelin sheath showed areas of thickening. The same results were obtained by Hinman *et al.* (2006) who added that the thickening of the myelin sheath may interfere with nerve impulse propagation. The myelin sheath in the current study exhibited areas of extensive thinning and disruption forming fermentation chambers. These results are in agreement with those reported by Hokoc *et al.* (2002) and Khan (2004). Changes in the myelin sheath, found in the current study, may interpret the visual loss occurred as a side effect of AD treatment in man as they hinder the nerve conduction.

The current work revealed many axons with areas of shrinkage and separation from the myelin sheath. The same results were obtained by Hinman *et al.*, (2006) who added that this shrinkage might lead to concomitant delay in nerve conduction. Moreover, Saggu *et al.* (2010) described the axonal separation as a sign of Wallerian degeneration which occurred seven days after experimental injury of the optic nerve.

The axoplasm of some fibers, in the present work, looked with inclusion bodies. The same inclusion bodies were observed by Dake *et al.* (1985). They reported that inclusion bodies affected many tissues and suggested that AD induces a systemic metabolic abnormality in lysosomal functions. Moreover, Mansour *et al.* (1988) found the same inclusion bodies in the optic nerve of a post mortem human case treated by AD. They claimed that the optic nerve damage, in AD-related optic neuropathy, was primarily lipidosi. Those inclusion bodies were found also in the cornea, by high incidence (70–100%), in patients receiving AD (Mantyljarvi *et al.*, 1998). Moreover, Bicer *et al.*, (2002) reported that AD caused corneal deposits in 16% of AD-treated dogs. They

explained that this lower prevalence compared to humans might be due to species variations particularly in volume of lacrimal secretion or the need for longer administration. In addition, sunlight was believed to exacerbate the corneal deposits in humans and all dogs in their study were housed indoors. *Mehraein (2008)* found that the tissue lysosomal phospholipid content increased in animals receiving AD. They added that AD strongly bind with these phospholipids forming intra lysosomal inclusion bodies and render them indigestible by phospholipases. On the other hand, *Domingues et al. (2004)* reported that the optic neuropathy and the intracytoplasmic inclusions may be unrelated. They added that AD-induced structural or functional changes in the optic nerve did not mean that it was the cause of the visual loss in optic neuropathy.

In the present work, the affected axons appeared with disrupted organelles. Similar results were obtained by *Saggu et al., (2010)*, 24 hours after experimental injury of the optic nerve using N-methyl-D-aspartate. They described the disruption of organelles as a sign of Wallerian degeneration. On the other hand, *Bicer et al., (2002)* reported that AD caused no changes in the structure of the optic nerve.

In the present work, there were increased numbers of astrocytes and microglia in groups IV and V. The astrocytes' mitochondria looked swollen with disrupted cristae. The same results were reported by *Khan (2004)*. Astrocytes have been considered previously as passive structural elements involved in blood– brain barrier and repair process, by scarring, following central nervous system (CNS) injury. However, astrocytes were proved to be dynamic cells with multiple functions in potassium homeostasis, metabolism, axon–glial signaling and participate in the physiology of nodes of Ranvier (*Butt et al., 2004*). Moreover, astrocytes regulate blood flow in CNS, release neurotransmitters and energy substrates and provide structural rigidity. This rigidity maintains the extracellular environment and modulates synaptic function and plasticity (*Rossi et al., 2007*). It could be suggested that the increase number of astrocytes, through their forementioned functions, was to share in the regeneration of the affected nerve fibers. However, the increase of the microglia, through

its phagocytic function, was to remove the debris of the degenerative processes. This suggestion is supported by the findings reported by *Saggu et al. (2010)*.

In the existing work, there were increased number of oligodendrocytes in groups IV and V. Some of these oligodendrocytes appeared degenerated (especially in group IV) and others exhibited swollen mitochondria with disrupted cristae. This finding is in accordance with that reported by *Saggu et al., (2010)*. It has been confirmed that oligodendrocytes form the myelin sheath in CNS that facilitate rapid conduction of axons. The oligodendrocytes are also essential for axon integrity and function in the segregation and clustering of sodium and potassium channels at nodes of Ranvier (*Butt et al., 2004*). It was added that demyelination or dysmyelination disrupts the nerve conduction, leading to axonal degeneration and neurological disabilities (*Chen et al., 2009*). It could be suggested that the degenerative changes in the oligodendrocytes found in the present work participated in the degeneration observed in the myelin sheath. Moreover, the increased number of oligodendrocytes was to compensate for the degenerated oligodendrocytes, to repair the myelin sheath of the affected nerve fibers and to preserve the non-affected ones.

The exact mechanism of cellular toxicity of AD and its major metabolite, desethylamiodarone, is uncertain (*Purvin et al., 2006*). Selective accumulation of intracytoplasmic lamellar inclusions in large axons of the optic nerve was documented in a patient who had taken amiodarone (*Mansour et al., 1988*). These inclusions might represent lipofuscin or a drug lipid complex that could not be metabolized by lysosomal phospholipases (*Turdumambetova et al., 2005*). It was of debate, whether the inclusion bodies in AD-treated animals reflected the ongoing cytotoxic process or whether these bodies were directly toxic to the cells (*Mehraein et al., 2008*). It was postulated that lamellar inclusions accumulated in glial cells produced swelling of these cells. This caused secondary axonal compression, blockage of axoplasmic flow and optic neuropathy. Although this mechanical model for optic neuropathy was possible, a biochemical dysfunction inducing optic neuropathy appeared more plausible

(Garrett *et al.*, 1988). However, no inclusion bodies were encountered in the glial cells in the existing study. On the other hand, biochemical pathogenesis of the optic nerve was described as AD generates oxygen-free radicals when the AD molecule is chemically reduced and iodine is liberated. The generated free radicals cause an increase in cellular lipid peroxidation and drug-lipid inclusions (Vereckei *et al.*, 1993).

Concomitant administration of LC with AD (group V) has minimized the degenerative effects of AD alone (group IV). The protective role LC may be explained by many theories. Hagen *et al.* (2002) attributed the protective role of LC in inhibition of lipid peroxidation by scavenging of reactive oxygen species and increasing antioxidant enzyme activities. Arrigoni-Martelli and Caso (2001) also postulated that LC effectively inhibited mitochondrial injury and mitochondrial-dependent apoptosis induced by oxidative stress of various types of cells. Meanwhile, Yano *et al.* (2008) assigned the protective role of LC to prevent mitochondrial membrane depolarization and abrogation of cellular ATP depletion. They added that these events cause apoptosis and necrosis after exposure of pulmonary epithelial cells to AD. Moreover, Ishii *et al.*, (2000) ascribed the protective role of LC to its anti-apoptotic effect. They studied the anti-apoptotic effect of LC in primary cultured neurons. Furthermore, Athanassakis *et al.*, (2001) stated that LC is essential cofactor of several enzymes necessary for the transformation of long chain fatty acids in regulation of carbohydrate metabolism and maintenance of cell membrane structure. These postulations interpret the results of the present work which revealed that LC administration improved the number and integrity of the optic nerve fibers and glial cells of the animals exposed to AD.

In conclusion, AD caused many degenerative effects in the optic nerve which were greatly ameliorated concomitant administration of LC. Thus, it is advisable to limit the use of AD to life-saving interventions. Moreover, the use of LC with AD has to be considered in such cases. Meanwhile, routine ocular fundus examination with monitoring of the visual acuity and field testing are indicated.

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هل يستطيع إل-كارنيتين أن يقي من اعتلال العصب البصري المستحدث بالأميودارون في الأرانب؟

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ملخص البحث

استهدفت هذه الدراسة توضيح تأثير إعطاء الأميودارون على تركيب العصب البصري و تقييم دور إل-كارنيتين في الحماية من هذه الآثار. وقد أجريت هذه الدراسة على عشرين أرنباً بالغاً تم تقسيمهم إلى خمس مجموعاتٍ متساوية (أربعة أرانب لكل مجموعة). المجموعة الأولى (مجموعة ضابطة) لم تعطى أى شئ. المجموعة الثانية تم إعطاؤها محلولاً ملحياً. المجموعة الثالثة تم إعطاؤها إل-كارنيتين. المجموعة الرابعة تم إعطاؤها أميودارون. المجموعة الخامسة تم إعطاؤها كل من إل-كارنيتين و أميودارون. و قد تم إعطاء حيوانات المجموعات المعالجة (٥-٢) جرعة يومية لمدة أربعة أسابيع ثم تم التضحية بحيوانات كل المجموعات بعد يوم واحد من آخر جرعة. وقد تم تعريض أعصابها البصرية للدراسة بالمجهرين الضوئي والالكتروني والدراسة القياسية.

وقد أظهرت نتائج هذه الدراسة أنه بعد إعطاء الأميودارون كان هناك نقص في العدد الكلي للألياف العصبية و عدد الألياف التي تبدو طبيعية بينما زادت تلك التي تبدو غير طبيعية. وكانت هناك مناطق من زيادة سمك الغلاف العصبي. كما ظهر بالغلاف النخاعي مناطق إما فقدت النمط اللفانفي أو بها انشطار أو زيادة في السمك. وظهر بكثير من المحاور العصبية مناطق من الانكماش و الانفصال عن الغلاف النخاعي. وكان جبلة المحورة لبعض الألياف محتوية إما على أجسام ضمنية أو عضيات ممزقة. وقد كان هناك زيادة في أعداد الخلايا النجمية و خلايا الدبق العصبي الناقصة و الدقيقة. و ظهرت ببعض خلايا الدبق العصبي الناقصة و الخلايا النجمية حبيبات خيطية منتفخة و ممزقة الأعراف. و قد أدى إعطاء إل-كارنيتين مع الأميودارون إلى تقليل كبير في الآثار المحدثة بالأميودارون على العصب البصري. يستنتج أن إعطاء الأميودارون قد أدى إلى تأثيرات تدهورية عديدة في العصب البصري وقد تناقصت كثيرا تلك التأثيرات التدهورية مع إضافة إل-كارنيتين. لذلك يوصى باستخدام إل-كارنيتين مع الأميودارون لعلاج اضطرابات نبض القلب.