The Possible Role of Propolis in Ameliorating Paclitaxel-Induced Peripheral Neuropathy in Sciatic Nerve of Adult Male Albino Rats

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Original Article

ABSTRACT

Background: Paclitaxel is a neurotoxic chemotherapeutic agent. The sensorimotor peripheral neuropathy is a serious dose limiting complication of paclitaxel therapy. Propolis is a natural honeybee product collected from plants. It has broad biological and pharmacological activities and a proposed neuroprotective role.

Aim: To assess the possible protective role of propolis on paclitaxel-induced peripheral neuropathy in rat's sciatic nerve.

Material and Methods: Twenty-four adult male albino rats were divided into four equal groups; control group, propolis-treated group (50 mg/kg orally once daily), paclitaxel -treated group (16 mg/kg intraperitoneally once a week), and both paclitaxel & propolis-treated group. Animals were treated for 5 consecutive weeks. Specimens of sciatic nerve were processed for histological study by light and electron microscopy. Immunohistochemical study was carried out using CD68 antibody. Morphometric study and statistical analysis of light microscopic data were performed for all groups.

Results: Compared to the control group, the sciatic nerve specimens of the paclitaxel-treated rats showed splitting of myelin lamellae with infolded myelin loops, together with invagination and evagination of the myelin sheath. Changes in the axons included formation of myelin figures, compression and irregularity. Schwann cells showed cytoplasmic vacuolation and dilated rER. Immunohistochemical study revealed a significant increase in the number of CD68 positive cells. On the other hand, minimal changes were observed in rats treated concomitantly with both paclitaxel and propolis, with a non-significant increase in CD68 positive cells.

Conclusion: Paclitaxel induced structural changes in the myelinated fibers of sciatic nerve of albino rats. The concomitant treatment with propolis ameliorated these changes.

Keywords: CD68, paclitaxel, peripheral neuropathy, propolis

Revised: 26 December 2016 , **Accepted:** 03 March 2017

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ISSN: 1110-0559, 2017, Vol 40. , No. 2

INTRODUCTION

Chemotherapy induced peripheral neuropathy (CIPN) is a common disabling and potentially dose-limiting adverse effect in cancer treatment[1]. Paclitaxel is an effective chemotherapeutic drug obtained from the bark of the western yew tree (Taxus brevifolia). It is widely used in the treatment of breast cancer, ovarian cancer, lung neoplasms and gastrointestinal tumors[2,3]. Moreover, it has an excellent antitumor activity in phase III breast cancer during clinical trials[4]. Paclitaxel is a microtubule stabilizing agent active against cancer cells and also the neurons are affected by paclitaxel[5].

It has been reported that paclitaxel treatment is associated with many serious non-hematological complications as peripheral neuropathy[6]. The incidence of paclitaxel-induced peripheral neuropathy is high ranging from 57%-83% accompanied with severity in 2%-33% of patients[2- 7]. In vivo toxicity of paclitaxel was reported in mature neurons in the hippocampus[8]. In addition, it induced mitochondrial degeneration in rat kidney, heart and brain[9].

Paclitaxel produces significant increase in latency and decrease in amplitude and conduction velocity in peripheral motor nerves. Moreover, it inhibits the usual regenerative response of axons and Schwann cells in crush injuries of nerves in animal models. In vitro study of paclitaxel neurotoxicity showed proliferation and aggregation of neurotubules in cultured sensory neurons[1].

CIPN is manifested by numbness, dysesthesia, paraesthesia and sensory loss in the distal lower extremities. Patients may also suffer from intermitting sharp, shooting leg pain[10]. Most cases may develop myalgias or arthralgias [2]. However, in its severe form, dose reduction

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is required reducing the efficacy of the drug especially in breast cancer treatment[11].

The pathogenesis of CIPN is attributed to the development of axonopathy and myelinopathy[4- 10]. Paclitaxel was found to be concentrated in dorsal root ganglia then in sciatic nerve and spinal cord due to paclitaxel transport along centrifugal and centripetal branches of the dorsal root ganglia neuron axon[12].

The precise mechanism involved in paclitaxel neurotoxicity is believed to be promotion of microtubule polymerization and prevention of depolymerization inhibiting the axonal transport within the neurons. This leads to disruption of peripheral nerve signaling causing sensory, motor, or autonomic peripheral neuropathy influencing the patient routine daily activities or even causes significant disability[8- 11].

Mitotoxicity hypothesis was approved for paclitaxel-induced neurotoxicity. It stated that the mitochondrial damage and dysfunction by paclitaxel causes oxidative damage due to increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) resulting in development of axonopathy and neuropathy[10- 7].

Several neuroprotective agents have been introduced to ameliorate the neurotoxicity associated with paclitaxel as acetyl-L-carnitine[13], amifostine, nerve growth factor [12], glutamine[2- 6], vitamin E[14], lithium[5], and benfotiamine [1] but they have limited successful protective effect.

Propolis or bee glue is a natural resinous balsamic material with promising protective and therapeutic effects [15]. It is collected by honeybees from the exudates of various plants and modified in the beehive by mixing with salivary secretion and wax. More than 150 compounds are found in propolis but it is composed mainly of polyphenols and flavonoids[16]. It has a wide range of biological and pharmacological activities including anticancer, anti-inflammatory, antioxidant, antimicrobial[17,18], anti-apoptotic, antiviral, antifungal, hepatoprotective, cardioprotective, immunomodulatory, anti-diabetic, local anesthetic effect and neuroprotective effects[19- 21].

Propolis acts as a powerful ROS scavenger and a neuroprotective agent. It improves the neuroregeneration process after sciatic nerve injury[20- 22]. Moreover, it has a protective role on cisplatin-induced neurotoxicity in rats [23].

AIM OF THE WORK

So, this work aimed to study the possible ameliorating effect of honeybee propolis on paclitaxel-induced peripheral neuropathy in sciatic nerve of adult male albino rats.

MATERIAL AND METHODS

I-Animals:

Twenty four adult male albino rats (225–250 grams) were recruited in this experiment. Animals were housed in clean properly ventilated cages under the same environmental conditions and fed on a standard laboratory diet. Two-weeks pre-experimentation period, all rats were allowed to be acclimatized to the laboratory conditions. This study protocol was approved by the local ethical Committee of Faculty of Medicine, Tanta University, Egypt.

II-Used drugs:

1- Paclitaxel 100 mg vial (6 mg/ml) with the brand name Taxol was manufactured by Corden Pharma Latina S.P.A. (Sermoneta, Latina, Italy) for Bristol-Myers Squibb Company (Roma, Italy).

2- Propolis was purchased in the form of capsules (each contains 400 mg pure propolis) with a brand name Biopropolis. It is produced by Sigma Pharmaceutical Industries for International Business Establishment Co. (IBE Pharma, Cairo, Egypt).

III-Experimental design (Groups):

Animals were divided into four groups each included six rats:

Group I (control group): was subdivided into two equal subgroups; subgroup IA were left untreated, subgroup IB received intraperitoneal (IP) injection of 1ml normal saline solution once a week for five consecutive weeks.

Group II (propolis-treated group): Animals of this group received propolis at a dose of 50 mg/kg dissolved in 1ml distilled water orally once daily for five consecutive weeks (24).

Group III (paclitaxel-treated group): Animals received IP injection of paclitaxel at a dose of 16 mg/kg diluted in 1ml normal saline once a week for five consecutive weeks (25).

Group IV (paclitaxel & propolis-treated group): Animals received the above mentioned doses of paclitaxel and propolis concomitantly for five consecutive weeks.

IV- Tissue preparation:

One week after the last injection, all animals were anesthetized with IP injection of sodium pentobarbital (50 mg/kg)[26]. The back of both thighs were incised, and the sciatic nerves were exposed near the greater sciatic

foramen. A specimen (5- 7 mm in length) from each sciatic nerve was obtained, put in the fixative, and processed for light and electron microscopic examination.

V-Light & transmission electron microscopic examination:

The sciatic nerve specimens were fixed in 2.5% phosphate buffered glutaraldehyde solution, washed in phosphate buffered saline (PBS), and postfixed in phosphate-buffered 1% osmium tetroxide. Subsequently, the samples were washed in buffer, dehydrated in ascending grades of alcohol, and then cleared in acetone. To obtain cross sections, the samples were oriented longitudinally in flat molds, thereafter, embedded in epoxy resin. Semithin cross-sections (1 µm thick) were stained with 1% toluidine blue and examined by light microscope. Ultrathin sections (80–90 nm) were stained with 8% uranyl acetate followed by lead citrate. Then examined by a JEOL electron microscope at 80 kV in Electron Microscope Unit, Faculty of Medicine, Tanta University, Egypt[27].

Immunohistochemistry:

Samples of the sciatic nerve were immersed in 10% neutral-buffered formalin, washed, dehydrated, and cleared. Then, the samples were embedded in paraffin [28].

CD68 immunohistochemistry:

Cross-sections of the formalin fixed, paraffin embedded sciatic nerve specimens (5µm thick) were deparaffinized, rehydrated and then washed with PBS. The sections were incubated overnight in a humid chamber with primary mouse anti-rat CD68 antibody, a lysosomal protein expressed in the activated macrophage, (1:200 dilution; Monoclonal Antibody (KP1), Thermo scientific, Fremont,USA) and washed three times with PBS. The corresponding biotinylated secondary antibody was added and left for one hour at room temperature, then washed again three times with PBS. Streptavidin peroxidase was added and left for 10 minutes at room temperature and then washed three times in PBS. 3, 3'-diaminobenzidine (DAB)-hydrogen peroxide was used as a chromogen to visualize the immunoreaction. The immunostained sections were counterstained with Mayer's haematoxylin. For negative control sections, the primary antibody was replaced by PBS[29].

Morphometric study:

Image analysis system (Leica Qwin 500 C Image analyzer computer system (Leica Imaging System LTD., Cambridge, England) at Central Research Lab, Faculty of Medicine, Tanta University, Tanta City, Egypt was used for:

1- **Calculation of g-ratio:** The ratio between the axonal diameter divided by the diameter of the axon with myelin, an index of axonal atrophy or myelin thickness changes. Five different non-overlapping fields from each toluidine-blue stained slide were examined at magnification of X1000 in all groups. The axoplasm area and myelin area were measured and then the g-ratio was calculated. The shape of the axons cross sections was not always round so, the whole areas were measured, and then the mean diameter was calculated.

2- **Quantification of the mean number of CD68-immunoreactive (IR) cells (activated macrophages):** In the DAB-stained sections, the number of CD68-IR (positive) cells within the endoneurial connective tissue were counted in four high power (X400) non-overlapping fields. For each group, the results were expressed as mean number of CD68-IR cells.

Statistical analysis:

Data obtained were analyzed by using one-way analysis of variance (ANOVA) followed by Tukey's procedure for comparison between the different groups using Statistical Package for Social Sciences Statistical Analysis Software (version 11.5; SPSS Inc., Chicago, Illinois, USA). The results were represented as mean ± standard deviation. Differences with probability values of $(P) < 0.05$ were regarded as significant[30].

RESULTS

In this study, no deaths were reported during the experiment.

Light microscopic results

In control group (Group I) as well as propolis-treated group (Group II), examination of toluidine blue-stained sections revealed normal architecture of rat's sciatic nerve. The nerve fibers appeared densely packed with scarce endoneurium in between. Regular myelin sheath with minimal enfolding was observed in myelinated nerve fibers. Schwann cell cytoplasm was seen surrounding the myelinated fibers. The unmyelinated nerve fibers appeared as ovoid clusters inbetween the myelinated fibers (Fig. 1).

In paclitaxel-treated group (Group III), the examination of sciatic nerve sections showed marked disruption of most of the myelinated nerve fibers. Noticeable invaginations and evaginations of the myelin sheath as well as irregular myelin thickening were observed in some sections which became marked in some fibers with entrapment of the axon (Figs. 2 -4). In

addition, focal myelin splitting was noticed in some fibers while others showed circumferential myelin splitting (Fig. 5). Severe myelin disruption with multiple different size nerve fibers was also seen in other sections. Another observation was cytoplasmic vacuolation of Schwann cells (Fig. 6). Moreover, migration of the inflammatory cells through the pores of the endoneurial blood vessels and infiltration of the endoneurium was seen (Figs. 6 and 7).

Regarding paclitaxel and propolis-treated group (Group IV), toluidine blue-stained sections showed compact regular myelin sheath nearly similar to control group (group I). Nevertheless, few nerve fibers showed variable degrees of myelin disruption (Fig. 8).

Electron microscopic results

In control group (Group I) as well as propolis-treated group (Group II), examination of ultrathin section showed myelinated nerve fibers with compact regular myelin sheath of uniform thickness. Mitochondria, microtubules and microfilaments were observed in the axoplasm of the myelinated nerve fibers (Fig. 9). Schwann cells with its large nuclei and attenuated cytoplasm were seen surrounding the myelinated nerve fibers completely so the fiber appeared as an actual part of Schwann cells (Fig. 10). Ovoid clusters of unmyelinated nerve fibers were noticed and several unmyelinated axons were seen occupying deep recess in the surface of Schwann cells. Electron-dense bodies and vesicles were observed in the axoplasm of the unmyelinated fibers (Fig. 11). Myelin sheath abnormalities and irregular axons were infrequently noticed in control sections.

In paclitaxel-treated group (Group III), sciatic nerve ultrathin sections showed variable degrees of myelin sheath disruption affecting many myelinated nerve fibers. These were in the form of extensive myelin invaginations and evaginations with irregular outline of several fibers (Fig. 12). Focal splitting of myelin layers were also observed in some fibers (Fig. 13). The myelin of other fibers was extensively split or irregularly folded appearing as one fiber is engulfing another (Figs. 14 and 15). Marked myelin thickening was also noticed (Fig. 16). The axoplasm of the affected fibers was compressed with irregular outline of many myelinated axons (Figs. 15 and 16) in addition to appearance of swollen mitochondria with disrupted cristae, myelin ovoid fragments and myelin figure in some axons (Figs. 17 and 18). Cytoplasmic vacuolation, dilated rER, and dilated perinuclear space were seen in Schwann cells of some myelinated fibers with apparent increase in size (Figs. 19 and 20).

In sciatic nerve sections of paclitaxel & propolis-treated group (Group IV), most fibers were similar to group I, whereas few showed minimal infolding and outfolding as well as minute splitting of the myelin sheath (Figs. 21 and 22). Besides, few cytoplasmic vacuolations of the surrounding Schwann cell were also appeared (Fig. 23).

Immunohistochemical results

CD68-immunostained sciatic nerve sections of the control group (group I) revealed nearly negative immunoreaction for CD68 (Fig. 24). In paclitaxel-treated group (Group III), numerous CD68-IR cells (activated macrophages) were observed in the connective tissue of the endoneurium (Fig. 25) and its small blood vessels (Fig. 26), while in paclitaxel & propolis-treated group (Group IV), few CD68-IR cells were detected in the endoneurium (Fig. 27).

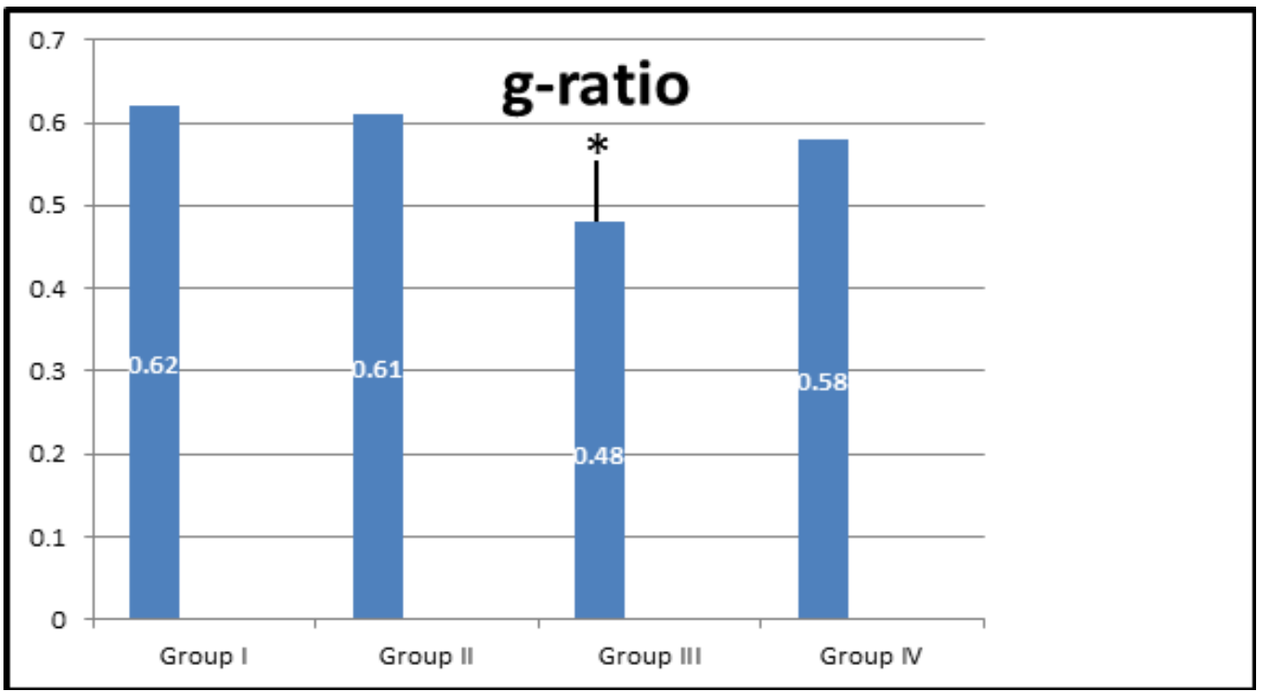
Morphometric and statistical results:

In the paclitaxel-treated group (Group III), the g-ratio was significantly reduced (0.48 ±0.042) compared with the control group (0.62± 0.054), while there were no significant differences (0.58 ±0.053) between the paclitaxel and propolis-treated group (Group IV) and control group (Table 1 and Histogram 1).

The mean number of CD68-IR cells (Activated macrophage) in the paclitaxel-treated group (group III) showed a significant increase (10.5±1.87) compared to the control group (2.17±1.17), while paclitaxel and propolis-treated group (group IV) showed a non-significant increase (3.33± 0.8) compared to the control (Table 1 and Histogram 2).

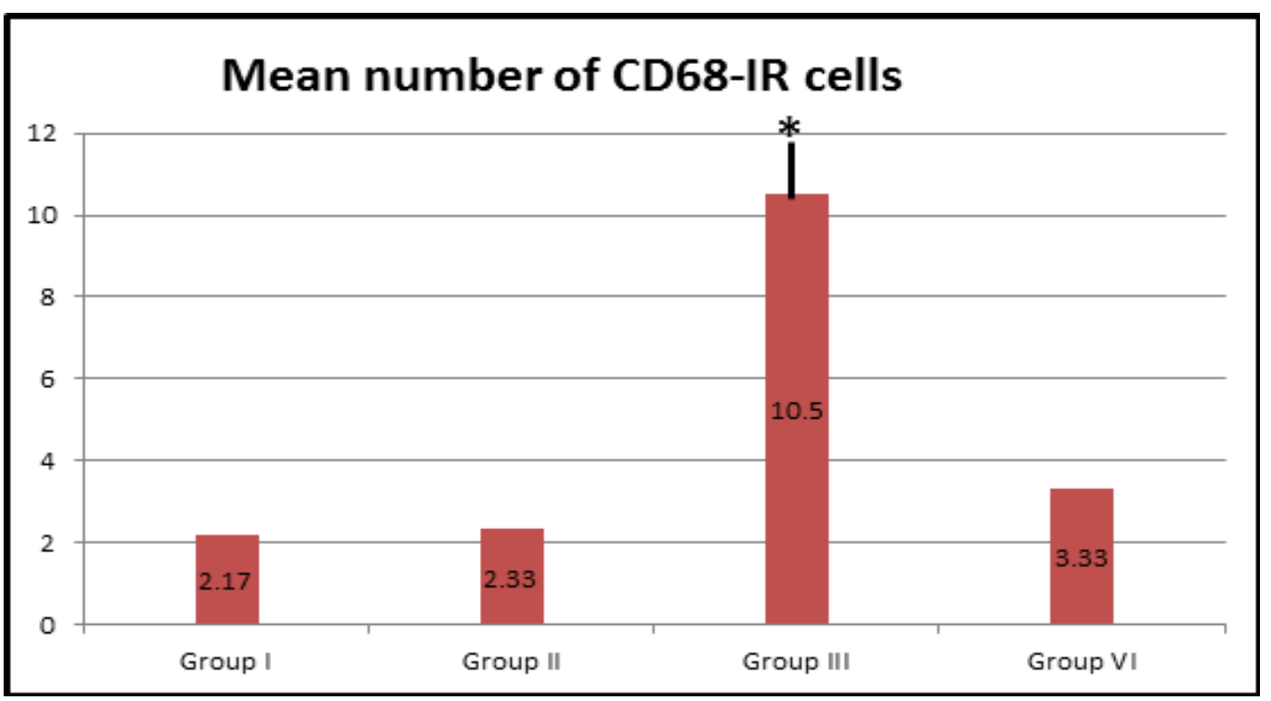
Table 1: Showing morphometric analysis of g-ratio and the mean number of CD68-IR cells in different groups. Data are expressed as mean ± standard deviation, *$P < 0.05$ is significant versus control

Parameters \ Groups	Group I	Group II	Group III	Group IV
Mean number of CD68-IR cells	2.17±1.17	2.33±1.21	*10.5±1.87	3.33±0.8
g-ratio	0.62±0.054	0.61±0.049	*0.48±0.042	0.58±0.053



Histogram 1: Showing g-ratio in different animal groups.

**P < 0.05* is significant versus control.



Histogram 2: Showing the mean number of CD68-IR cells in different animal groups.

**P < 0.05* is significant versus control.

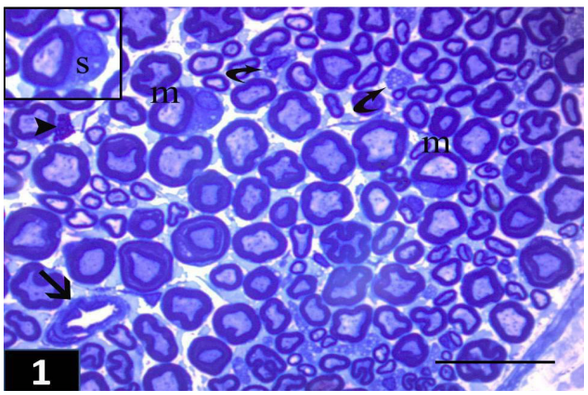


Fig. 1: A photomicrograph of a semithin section in rat sciatic nerve from control group (group I) showing densely packed nerve fibers and scarce endoneurium. The myelinated nerve fibers show regular myelin with minimal infoldings (m). The endoneurial connective tissue shows a mast cell with its metachromatic granules (arrowhead) and a blood capillary (arrow). Notice ovoid clusters of unmyelinated nerve fibers (curved arrows). The inset shows myelinated nerve fiber surrounded by Schwann cell (S) . (Toluidine blue X 1000; Scale bar = 10µm).

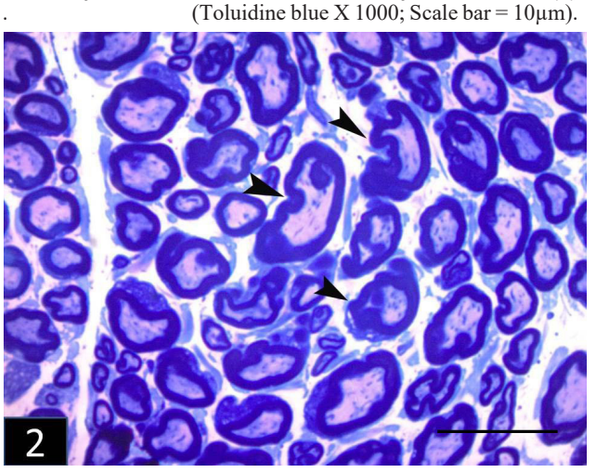


Fig. 2: A photomicrograph of a semithin section in rat sciatic nerve from paclitaxel-treated group (group III) showing invagination and evagination of the myelin of some nerve fibers (arrowheads) (Toluidine blue X 1000; Scale bar = 10µm).

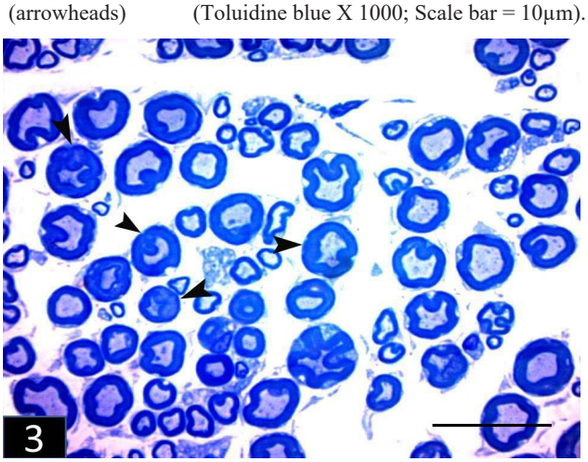


Fig. 3: A photomicrograph of a semithin section in rat sciatic nerve from paclitaxel-treated group (group III) showing irregular thickening of myelin sheath of some nerve fibers (arrowheads)

(Toluidine blue X 1000; Scale bar = 10µm).

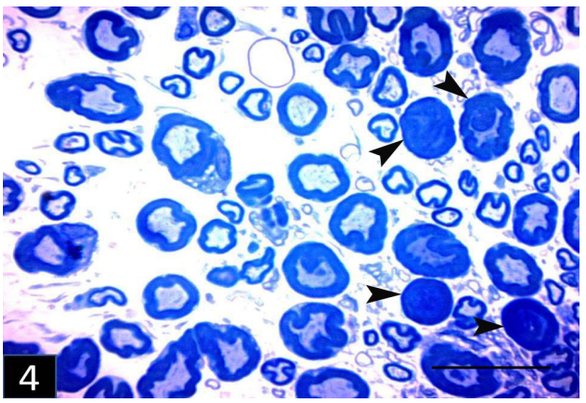


Fig. 4: A photomicrograph of a semithin section in rat sciatic nerve from paclitaxel-treated group (group III) showing marked thickening, invagination and evagination with entrapment of the axons (arrowheads)

(Toluidine blue X 1000; Scale bar = 10µm).

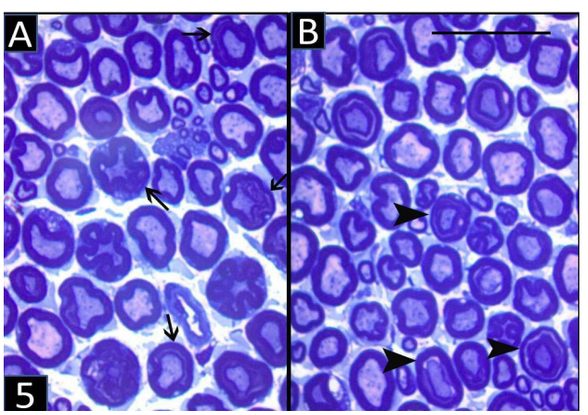


Fig. 5: A photomicrograph of a semithin section in rat sciatic nerve from paclitaxel-treated group (group III), (A) showing focal myelin splitting of some nerve fibers (arrows) and (B) showing circumferential splitting of the myelin of other nerve fibers (arrowheads)

(Toluidine blue X 1000; Scale bar = 10µm).

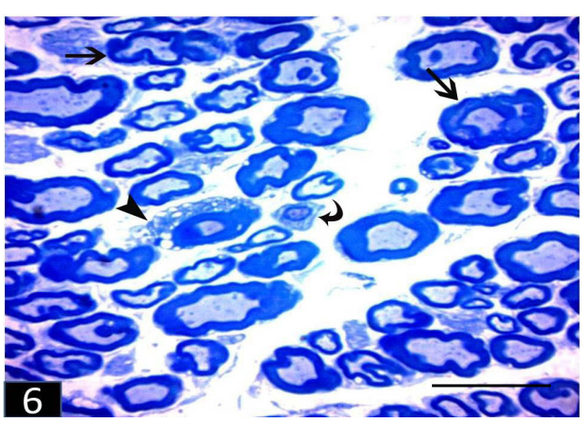


Fig. 6: A photomicrograph of a semithin section in rat sciatic nerve from paclitaxel-treated group (group III) showing severe disruption of the myelinated fibers (arrows) with multiple different size nerve fibers. Notice cellular infiltration of the endoneurium (curved arrow) and cytoplasmic vacuolation of Schwann cell (arrowhead).

(Toluidine blue X 1000; Scale bar = 10µm).

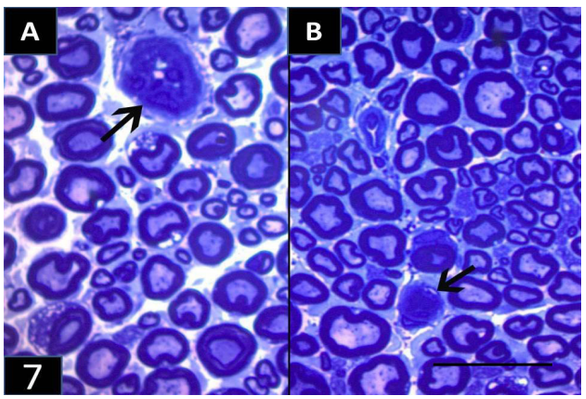


Fig. 7: A photomicrograph of a semithin section in rat sciatic nerve from paclitaxel-treated group (group III), (A) showing inflammatory cells sweeping out from the pores of the endoneurial blood vessels and (B) showing cellular infiltration of the endoneurium (arrows)
(Toluidine blue X 1000; Scale bar = 10µm).

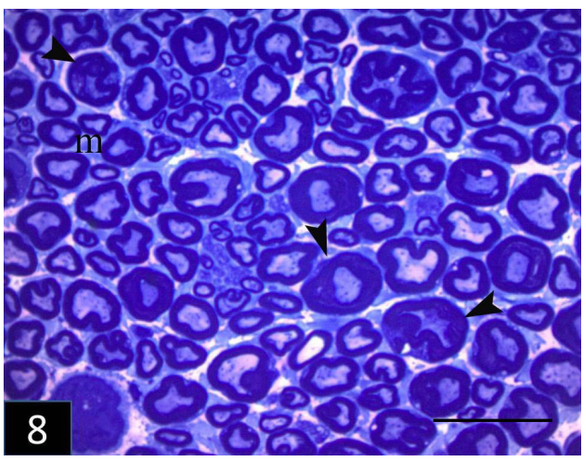


Fig. 8: A photomicrograph of a semithin section in rat sciatic nerve from paclitaxel & propolis-treated group (group IV) showing regular myelin sheath nearly similar to control group (m) with occasional areas of myelin disruption of few nerve fibers (arrowheads)
(Toluidine blue X 1000; Scale bar = 10µm).

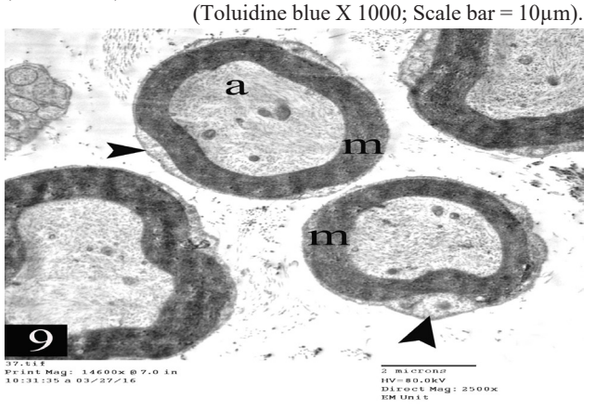


Fig. 9: A photomicrograph of an ultrathin section of sciatic nerve from a control rat (Group I) showing compact regular myelin sheath of the myelinated nerve fibers (m). The myelinated axons are surrounded by Schwann cells (arrowheads). The axoplasm (a) contains mitochondria, microtubules and microfilament
(X 14600).

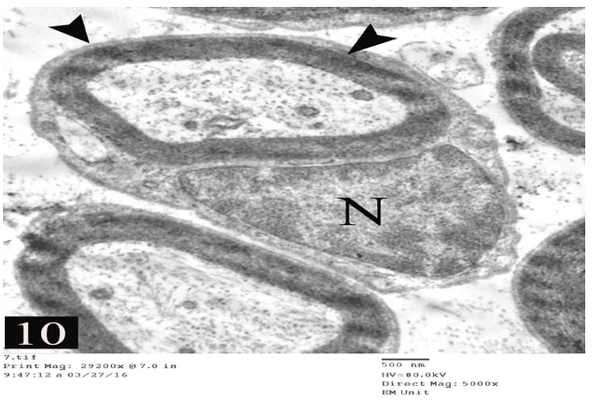


Fig 10: A photomicrograph of an ultrathin section of sciatic nerve from a control rat (Group I) showing myelinated nerve fiber completely enveloped by Schwann cell to be an actual part of the cell (arrowheads). Notice, the large nucleus (N) of Schwann cell surrounded by attenuated cytoplasm
(X 29200).

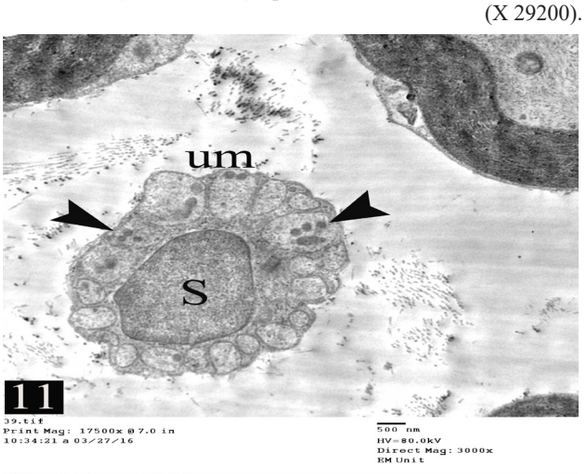


Fig. 11: A photomicrograph of an ultrathin section of sciatic nerve from a control rat (Group I) showing a group of unmyelinated nerve fibers (um) occupying deep recess in the surface of Schwann cell (S). Notice presence of electron dense bodies (arrowheads) in their axoplasm (X 17500).

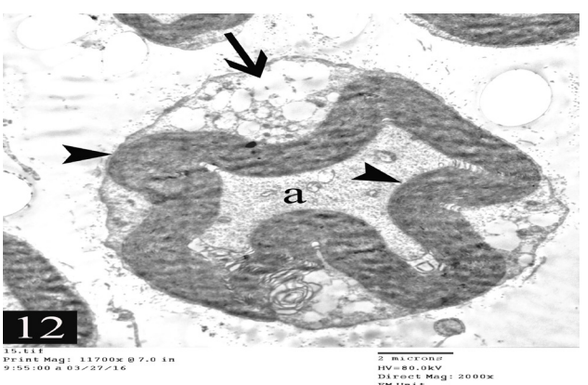


Fig. 12: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing invaginations and evaginations of the myelin sheath (arrowheads) with irregular outline of the axons (a) of some myelinated nerve fibers. Notice cytoplasmic vacuolation of the surrounding Schwann cell (arrow)
(X 11700).

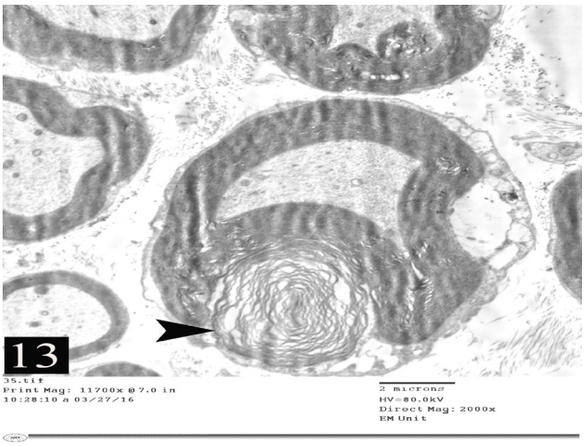


Fig. 13: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing focal splitting of the myelin sheath layers of some fibers (arrowhead) (X 11700).

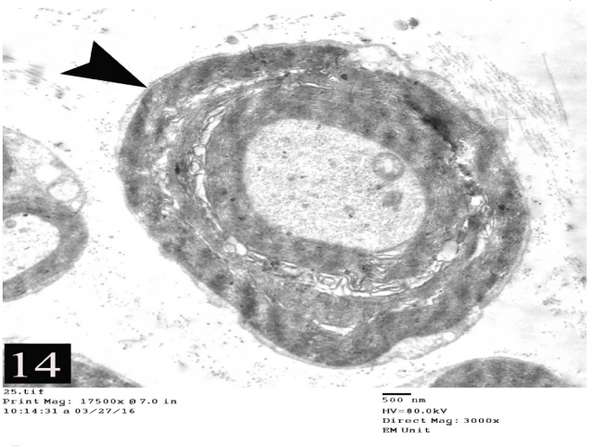


Fig. 14: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing extensive splitting of myelin sheath appearing as one fiber engulfing another (arrowhead) (X 17500).

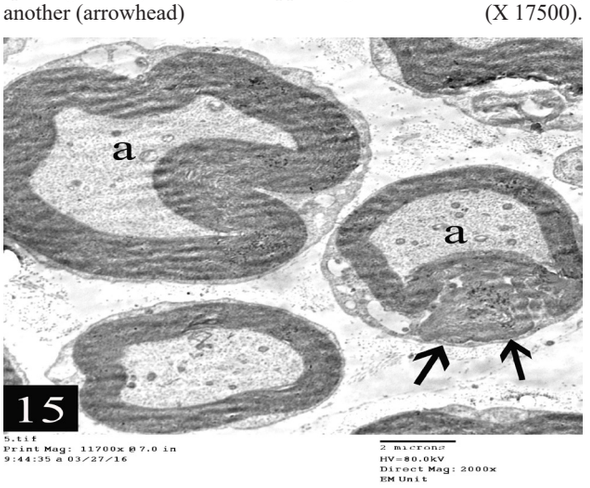


Fig. 15: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing irregularly folded myelin sheath (arrows) appearing as a fiber engulfing another. Notice compression of the axoplasm of the myelinated nerve fibers (a) (X11700).

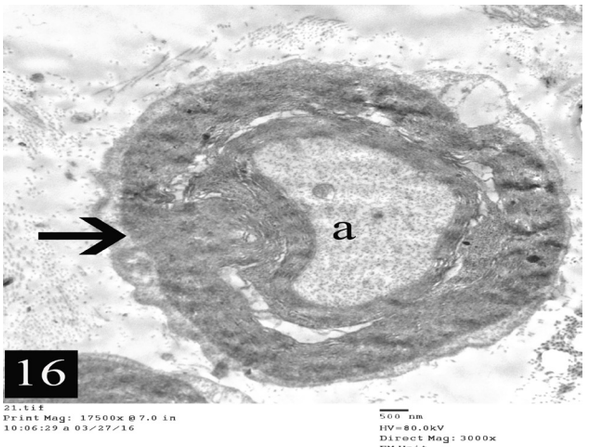


Fig. 16: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing markedly thickened myelin sheath (arrow) with compression of the axon (a) (X 17500).

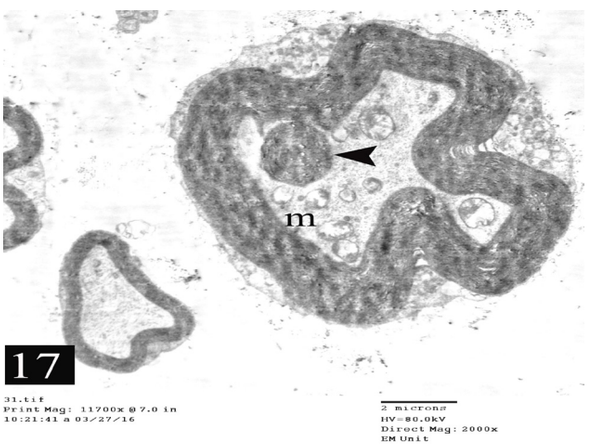


Fig. 17: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing myelinated axon with swollen disrupted mitochondria (m) and myelin ovoid fragment (arrowhead) in the axoplasm. Notice irregular outline of the axon (X11700)

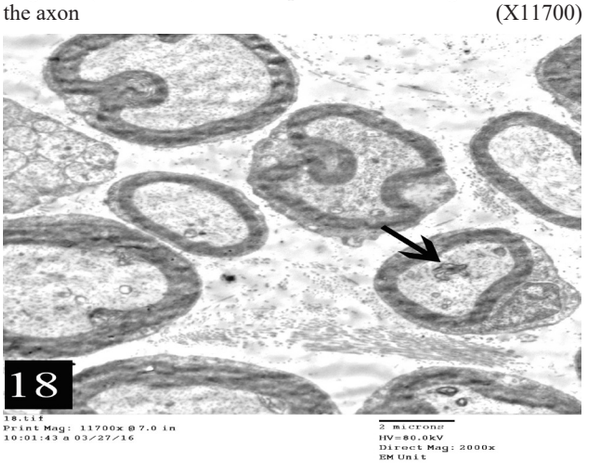


Fig. 18: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing myelin figure (arrow) of some axons (X11700).

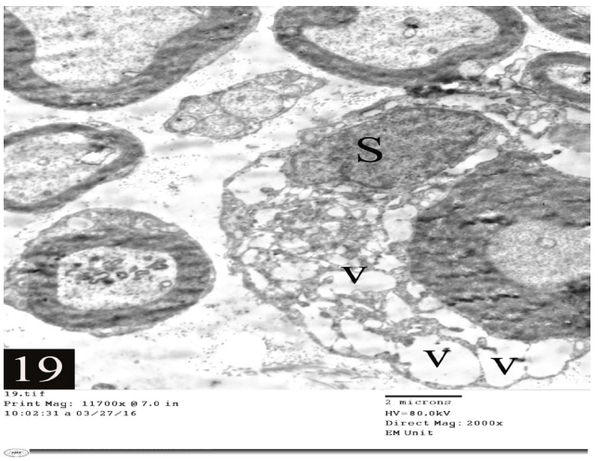


Fig. 19: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing severe cytoplasmic vacuolation (V) of Schwann cell (S) with apparent increase in its size (X 11700).

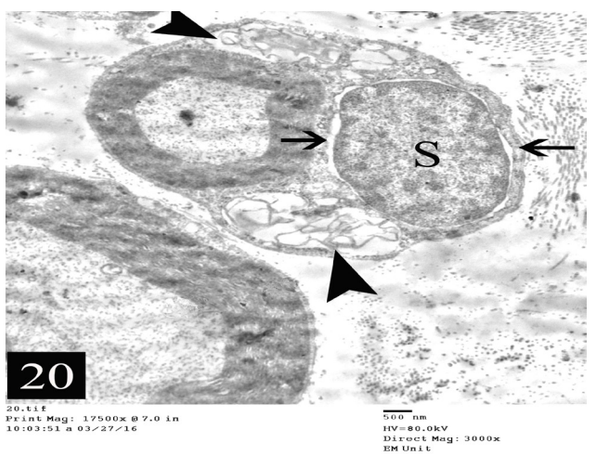


Fig. 20: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel-treated rats (Group III) showing dilated rER (arrow heads) and dilated perinuclear space (arrows) of Schwann cell (S) (X 17500).

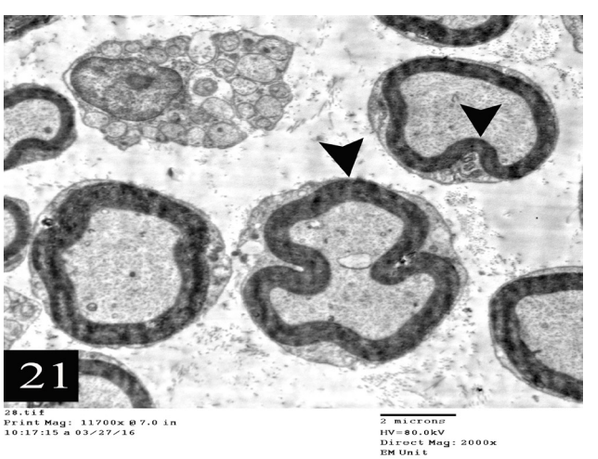


Fig. 21: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel & propolis-treated rats (Group IV) showing compact myelin sheath with minimal infolding and outfolding (arrowheads) (X 11700).

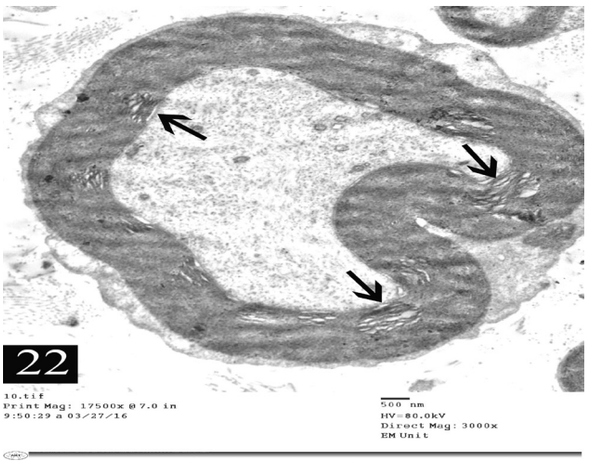


Fig. 22: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel & propolis-treated rats (Group IV) showing minute splitting of the myelin sheath of few fibers (arrows) (X 17500).

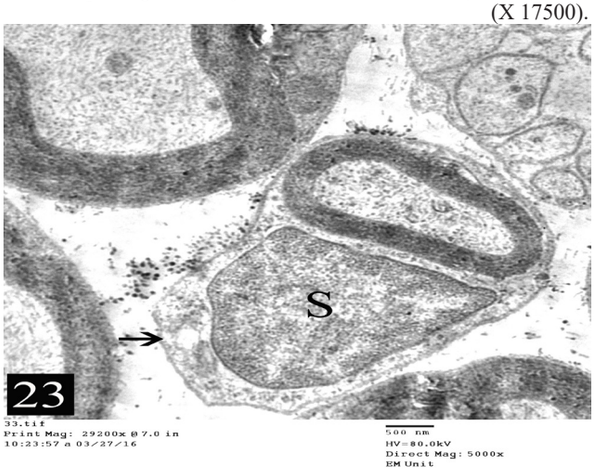


Fig. 23: A photomicrograph of an ultrathin section of sciatic nerve of paclitaxel & propolis-treated rats (Group IV) showing few cytoplasmic vacuolation (arrow) of Schwann cell (S) (X 29200).

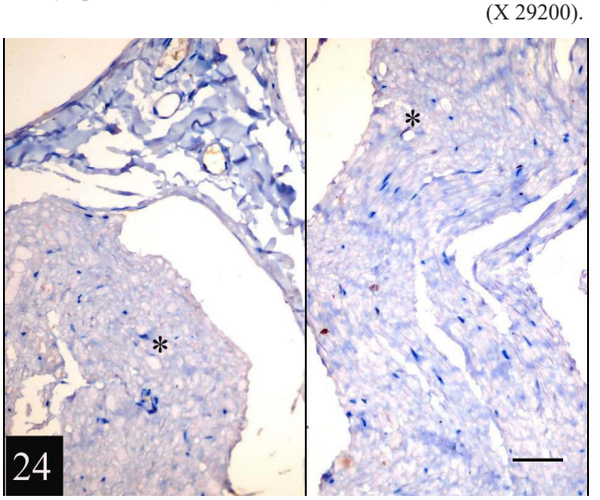


Fig 24: A photomicrograph of a section in rat sciatic nerve from the control group (Group I) showing a negative immunoreaction for CD68 in the endoneurium (*). (CD68 immunostaining X 400; Scale bar = 10µm)

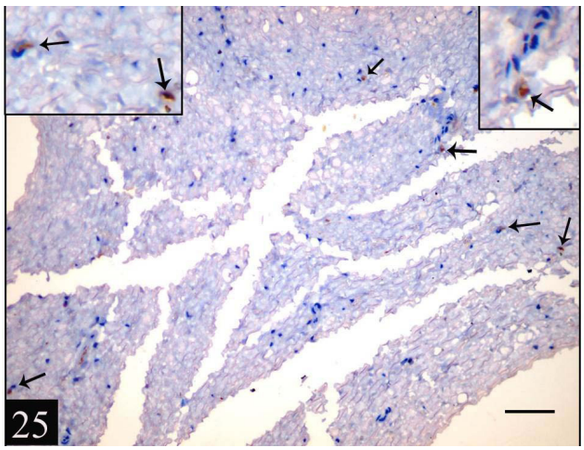


Fig. 25: A photomicrograph of a section in rat sciatic nerve from the paclitaxel-treated group (Group III) showing numerous CD68-IR (positive) cells in the endoneurium (arrows). Insets show a higher magnification of CD68-IR cells. (CD68 immunostaining X 400, insets X1000; Scale bar = 10µm).

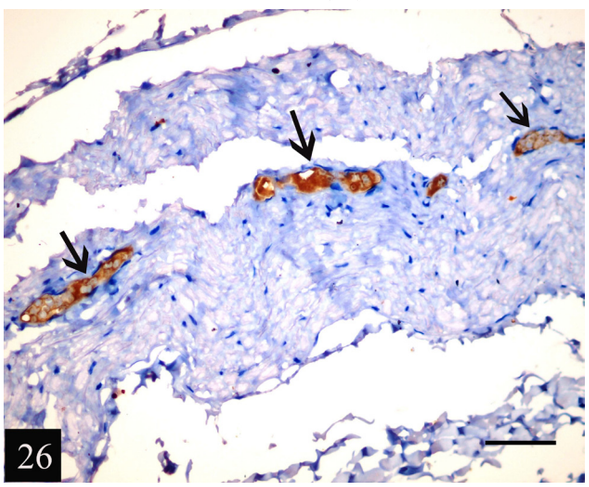


Fig. 26: A photomicrograph of a section in rat sciatic nerve from the paclitaxel-treated group (Group III) showing numerous CD68-IR (positive) cells in the blood vessels (arrows) of the endoneurium. (CD68 immunostaining X 400; Scale bar = 50µm).

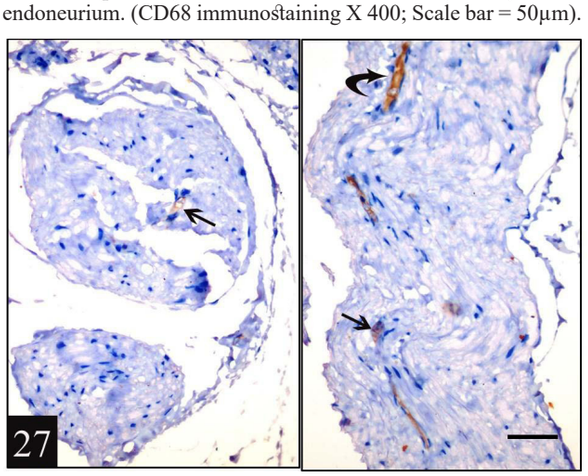


Fig. 27: A photomicrograph of a section in rat sciatic nerve from the paclitaxel & propolis-treated group (Group IV) showing few CD68-IR cells in the endoneurium (arrows) and its blood vessels (curved arrow). (CD68 immunostaining X 400; Scale bar = 10µm).

DISCUSSION

In the present study, paclitaxel induced neurodegenerative changes in sciatic nerve of albino rats. These neurodegenerative changes were in agreement with previous reports indicating that paclitaxel specifically affect the peripheral nervous system as it does not cross the blood brain barrier[31]. Our results showed that sciatic nerve had degenerative changes in the form of axonopathy (irregular compressed axoplasm, swollen mitochondria and myelin figures) and myleinopathy (irregular thickening, disruption and splitting). These findings coincide with previous reports whereas sciatic nerve of paclitaxel-treated animals showed axonopathy including collapse and fragmentation of the myelin sheath of some axons[32].

In our study, the axoplasm of some myleinated fibers was compressed with irregular outline. A possible mechanism for these findings is apoptosis as cell volume shrinkage and cell membrane changes (blebbing) are features of apoptosis[33]. Previous investigators suggested that apoptosis plays an important role in paclitaxel toxicity. Paclitaxel stabilizes microtubules leading to cellular mitosis arrest and hence cell death (apoptosis)[34]. Microtubule stability affects axonal transport of growth factors leading to abnormal nerve physiology and loss of axonal integrity[7]. In addition, paclitaxel can evoke apoptotic cascade by stimulation of cytochrome C release which activates caspase signaling pathway and also by binding to mitochondrial anti-apoptotic Bcl2 resulting in its inactivation[10, 35].

Paclitaxel neurotoxicity may be due to oxidative stress as previous research revealed that reactive oxygen species (ROS) affects blood vessel function reducing perfusion of several organs which include peripheral nerves leading to ischemia-reperfusion effects and subsequent neuropathy [36].

Our study revealed swollen disrupted mitochondria in the affected myleinated axons, which coincides with many investigators who suggested that mitochondrial swelling is a characteristic sign in paclitaxel axonopathy[37, 38]. This could be explained by affection of Mitochondrial permeability transition pore (mPTP) which is a high conductance channel in mitochondrial inner membrane that contains different proteins including adenine nucleotide translocator (ANT), mitochondrial phosphate carrier (PiC), voltage-dependent anion channel (VDAC), and cyclophilin-D (Cyp-D). Stimulation of mPTP opening by paclitaxel leads to mitochondrial depolarization and Ca++ release. Increase Ca++ mediated neuronal excitability results in mitochondrial structural changes[10]. Another explanation for mitochondrial swelling in paclitaxel-treated animals is that paclitaxel binds to β-tubulin moiety which is associated with VDAC. Opening of VDAC by paclitaxel causes mitochondrial swelling and vacuolation[9, 39].

Some affected myleinated axons showed myelin figures which are stacks of phospholipid bilayer. This finding may

be attributed to cellular damage, during which, proteolysis cut the links between cell membrane and cytoskeleton. As a result, phospholipids become free leading to myelin figures formation[40].

In our study, the myelin sheath of affected axons showed degenerative changes in the form of irregular thickening, disruption and splitting consistent with Abd-El-Hafez, 2014[41]. Noteworthy, myleinopathy or demyelination commonly followed axonal affections[42],whereas, the myelin membrane is susceptible to toxic substances that can result in structural changes like edematous splitting and disruption[43].

The presence of myelin ovoid fragments was in accordance with many investigators[44] that can be explained by toxic injury caused by paclitaxel. Nerve injury is usually followed by wallerian degeneration characterized by axonal degeneration, degradation of the myelin sheath and its fragmentation into small ovoid-like structures[45, 46].

The present study revealed structural changes in Schwann cells in the form of hypertrophy, dilated rER, dilated perinuclear space and vacuolation. It was suggested that Schwann cells structural changes are features combined with axonal injury[44]. Also, it was proposed that Schwann cell hypertrophy and vacuolation are signs of cell activation which may be a reaction to toxic injury[25].

Our study showed endoneurium infiltration with macrophage in paclitaxel-treated animals. CD68 is a glycoprotein found in cytoplasmic granules in many cells of macrophages lineage. Sections of sciatic nerves from paclitaxel-treated animals showed a significant increase in density of CD68-immunoreactive macrophages within the endoneurium compared to non-treated rats consistent with Peters et al, 2007[42]. The presence of many macrophages could be explained by proliferation of resident macrophages or migration of hematogenous macrophages[47]. The function of macrophages in injury site is to get rid of degenerating neuronal debris and to help in subsequent regeneration[48].

Cavaletti *et al.* 1995 considered the decrease in g-ratio an indicator for axonal atrophy rather than myelin thickening[25]. Our research revealed a significant decrease in g-ratio in paclitaxel-treated group compared to the controls. Such significant decrease in g-ratio can cause dysfunction in nerve impulse conduction[49].

The concomitant administration of propolis with paclitaxel reduced the neurodegenerative changes induced by paclitaxel supported by previous reports where propolis stimulates the differentiation of neural stem cells into neurons and enhances the transcription of pro-neural genes thus it has an anti-apoptotic effect[50]. Besides, propolis attenuates caspase-3 activities enhancing the anti-apoptotic effect[51]. In addition, it acts as an antioxidant drug by decreasing the extent of lipid peroxidation (LPO) and increasing the activities of enzymatic antioxidants[52]. Moreover, it is a powerful ROS scavenger[53] as it enhances the levels of reduced glutathione (GSH) suggesting GSH-dependent detoxification of free radicals[54]. Propolis also inhibits the migration of neutrophil thus reduces the inflammatory response[55].

CONCLUSION

Propolis had a neuroprotective effect against paclitaxel-induced peripheral neuropathy in rats. It may, therefore, be a useful common supplement for the patient undergoing neurotoxic chemotherapeutic medications. However, further studies are recommended to evaluate different doses of propolis for the optimal protection.

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربى

الدور المحتمل للبروبوليس فى تحسين اعتلال الأعصاب الطرفية المحدث بعقار باكليتاكسيل فى العصب الوركى لذكور الفئران البيضاء البالغة

أميرة عدلى كساب و هبه حسن القلينى

قسم الهستولوجيا - كلية الطب - جامعة طنطا- مصر

المقدمة: عقار باكليتاكسيل هو علاج كيميائي سام للأعصاب. اعتلال الأعصاب الطرفية الحسيه والحركيه مشكلة خطيرة تؤثر على جرعة العلاج بالباكليتاكسيل. البروبوليس هو منتج طبيعى لنحل العسل يجمعه من النباتات ويتمتع بأنشطة حيوية و دوائية واسعة ودور مقترح في حماية الأعصاب.

الهدف من العمل:تقييم الدور الوقائي المحتمل للبروبوليس على اعتلال الأعصاب الطرفية المحدث بعقار باكليتاكسيل فى العصب الوركى للفئران.

مواد و طرق البحث: تم تقسيم أربعة وعشرون من ذكور الفئران البيضاء إلى أربع مجموعات متساوية. المجموعة الضابطة، المجموعة التي عولجت بالبروبوليس (50 ملجم / كجم بالفم مرة واحدة يوميا)، مجموعة معالجة بعقار باكليتاكسيل (16 ملجم / كجم داخل الغشاء البريتونى مرة واحدة أسبوعيا)، والمجموعة التي عولجت بكلاهما. تم اعطاء الحيوانات هذه الجرعات لمدة 5 أسابيع متتالية. تم تجهيز عينات من العصب الوركى للدراسة النسيجية بالمجهر الضوئى والمجهر الإلكتروني. كما تم عمل صبغات هستوكيميائية مناعية بإستخدام أجسام ضد سى-دى68 وقد تم عمل دراسة قياسية شكلية وتحليل البيانات احصائيا لكل المجموعات.

النتائج: مقارنة بالمجموعة الضابطة, أظهرت عينات العصب الوركى للفئران المعالجة بعقار باكليتاكسيل انفصال فى طبقات الميلين, التفاف حلقات الميلين, تكيسات (اقلابات) للداخل وللخارج. وشملت التغيرات المحاور العصبية فقد احتوت على أشكال من الميلين والانضغاط وعدم الانتظام. كما لوحظ ظهور فجوات سيتوبلازمية واتساع الشبكة الاندوبلازمية الخشنة لخلايا شوان. وقد أظهرت الدراسة الهستوكيميائية المناعية زيادة ذات دلالة إحصائية في عدد الخلايا الايجابية ل سى-دى68. في المقابل، فقد لوحظ تغيرات طفيفة فى الفئران التى عولجت بالتزامن بكل من عقار باكليتاكسيل و بروبوليس مع وجود زيادة ليست ذات دلالة إحصائية فى عدد الخلايا الايجابية ل سى-دى68..

الاستنتاج: من النتائج السابقة, يمكن استنتاج أن عقار باكليتاكسيل تسبب فى حدوث تغيرات تركيبية للألياف العصبية ذات الميلين فى العصب الوركى للفئران البيضاء التي يمكن أن تتحسن بالعلاج المصاحب بالبروبوليس.