



T. R.
ONDOKUZ MAYIS UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY

**POTENTIAL EFFECTS OF AQUEOUS *ADANSONIA DIGITATA*,
EXTRA VIRGIN OLIVE OIL AND *OCIMUM BASILICUM*
ESSENTIAL OIL ON SCIATIC NERVE CRUSH INJURY-INDUCED
NEUROPATHY IN A RAT MODEL: A STEREOLOGICAL AND
HISTOLOGICAL STUDY**

PhD Thesis

Nagi Mahmoud Ahmed BAKHIT

Supervisor

Assoc. Prof. Dr. Mehmet Emin ÖNGER

SAMSUN
2021

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ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled “Potential Effects of Aqueous *Adansonia digitata*, Extra Virgin Olive Oil and *Ocimum basilicum* Essential Oil on Sciatic Nerve Crush Injury-Induced Neuropathy in a Rat Model: A Stereological and Histological Study” prepared by Nagi Mahmoud Ahmed BAKHIT and supervised by Assoc. Prof. Dr Mehmet Emin ONGER was found successful and unanimously accepted by committee members as PhD thesis of the department of Histology and Embryology, following the examination on the date

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ÖZET

SULU *ADANSONIA DIGITATA*, SAF SIZMA ZEYTİNYAĞI VE *OCIMUM BASILICUM* ESANSİYEL YAĞININ SİYATİK SİNİR HASARINA BAĞLI NÖROPATİK SIÇAN MODELİ ÜZERİNE POTANSİYEL ETKİLERİ: STEREOLOJİK VE HİSTOLOJİK BİR ÇALIŞMA

Nagi Mahmoud Ahmed BAKHIT

Ondokuz Mayıs Üniversitesi

Lisansüstü Eğitim Enstitüsü

Histoloji ve Embriyoloji Anabilim Dalı

PhD, Aralık/2021

Danışman: Doç. Dr Mehmet Emin ONGER

Bu çalışmada *Adansonia digitata* (AD)'nın, saf sızma zeytinyağının (EVOO) ve *Ocimum basilicum* esansiyel yağının (EOOB) siyatik sinir hasarı üzerine etkilerini araştırmak amaçlanmıştır. 64 adet *Wistar albino* rat sekiz eşit gruba (n=8) bölünmüştür: Kontrol, Hasar, Hasar+AD, Hasar + EVOO, Hasar+ EOOB, AD, EVOO, EOOB grupları. Hasar gruplarında her hayvanın sağ siyatik sinirine cerrahi işlem ile hasar verilmiştir. Hasar+AD grubu ratlara 400 mg/kg/gün AD, Hasar+EVOO grubu ratlara 2ml/kg/gün EVOO, Hasar+EOOB grubu ratlara ise 1500 mg/kg/gün EOOB postoperatif 10. güne kadar oral yoldan verilmiştir. AD, EVOO ve EOOB grubundaki ratlara ise yukarıda belirtilen aynı süre boyunca sırasıyla aynı dozlarda maddeler verilmiştir. Çalışma süresi 21 gündür. Süre sonunda mikroskopik, fonksiyonel ve stereolojik değerlendirmeler yapılmıştır.

Stereolojik sonuçlar, hasar grubuna kıyasla, Hasar+AD (p=0,00), Hasar+EVOO (p=0,00) ve Hasar+EOOB (p=0,01) gruplarında ortalama miyelinli akson sayısı açısından anlamlı düşüş olduğunu göstermiştir. Ayrıca, Hasar+AD grubunda Hasar grubuna kıyasla miyelin kılıf kalınlığındaki artışın anlamlı derecede farklı olduğu bulunmuştur (p=0,01). Fonksiyonel değerlendirmeler açısından; SFI değerlerine göre Hasar grubuna kıyasla, Hasar+AD (p=0,00), Hasar+EVOO (p=0,00) ve Hasar+EOOB (p=0,00) gruplarında anlamlı artış tespit edilmiştir. Amplitüt değerlerine göre Hasar+EVOO grubunda Hasar grubuna kıyasla önemli artış (p=0,00) tespit edilmesine rağmen, latans verileri açısından Hasar+AD ve Hasar+EVOO gruplarında Hasar grubuna (p=0,00) kıyasla önemli azalma tespit edilmiştir.

Sonuç olarak, AD iyileşmeyi artırıcı etkisini miyelinli akson sayısı, miyelin kılıf kalınlığı, SFI ve latans parametreleri üzerinden göstermiştir. Yine EVOO, en iyi rejeneratif kapasitesini miyelinli akson sayısının yanı sıra amplitüt, latans ve SFI parametreleri üzerinden göstermiştir. Ayrıca, miyelinli akson sayısı ve SFI parametreleri açısından EOOB de rejenerasyon potansiyelini göstermiştir. Bununla birlikte, mikroskopik değerlendirmeler de AD, EVOO ve EOOB'nin rejeneratif etkilerini ortaya koymuştur. Bu yüzden AD, EVOO ve EOOB nörorejenerasyon alanında rejeneratif maddeler olarak kullanılabilir.

Anahtar Sözcükler: Sulu *Adansonia digitata*, Saf sızma zeytinyağı, *Ocimum basilicum* esansiyel yağı, Periferal nörorejenerasyon, Stereoloji

ABSTRACT

POTENTIAL EFFECTS OF AQUEOUS *ADANSONIA DIGITATA*, EXTRA VIRGIN OLIVE OIL AND *OCIMUM BASILICUM* ESSENTIAL OIL ON SCIATIC NERVE CRUSH INJURY-INDUCED NEUROPATHY IN A RAT MODEL: A STEREOLOGICAL AND HISTOLOGICAL STUDY

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This study sought to investigate the impact of *Adansonia digitata* (AD), extra virgin olive oil (EVOO) and *Ocimum basilicum* essential oil (EOOB) on sciatic nerve following crush injury. The 64 male *Wistar* rats were divided into eight groups (n=8); Cont, Inj, Inj+AD, Inj+ EVOO, Inj+EOOB, AD, EVOO and EOOB groups. The rats of injured groups were received crush surgery on the right sciatic nerve. The rats treated orally for 10 days postoperative; as the Inj+AD rats were received AD (400mg/kg/day), the Inj+EVOO received EVOO (2ml/kg/day), as well the Inj+EOOB were treated with EOOB (1500mg/kg/day), while the rats of AD, EVOO and EOOB groups were received the same dose that mentioned above for the same duration respectively. The study duration was 21 days. Microscopic, functional and stereological assessments were employed.

The stereological results showed that a significant decreases in the mean of myelinated axons were found when compare the Inj+AD and Inj (p=0.00), Inj+EVOO and Inj (p=0.00), Inj+EOOB and Inj (p=0.01) groups. Also, the increasing of myelin sheath thickness was significantly differed between Inj+AD and Inj (p=0.01) groups. Regarding functional evaluations results; a high significant increases were seen (p=0.00) when compare Inj+AD and Inj, Inj+EVOO and Inj, Inj+EOOB and Inj groups in terms of SFI. Whereas, amplitude results showed a high significant increasing was found among Inj+EVOO and Inj groups (p=0.00). On the another hand, we observed high significant decreases between Inj+AD and Inj, Inj+EVOO and Inj groups (p=0.00) in terms of latency value.

In conclusion, AD improved the recovery in terms of myelinated axons, myelin sheath thickness, SFI and latency. Also, EVOO showed excellent regenerative capacity regarding amplitude, latency and SFI besides myelinated axons number. Additionally, EOOB afforded regrowth potential in terms of axons number and SFI. Moreover, microscopic evaluations were proved the regenerative impacts of AD, EVOO and EOOB. Therefore, AD, EVOO and EOOB could be used as antioxidant substances in the neuroregenerative field of medicine.

Keywords: Aqueous *Adansonia digitata*, Extra virgin olive oil, *Ocimum basilicum* essential oil, Peripheral neuroregeneration, Stereology.

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LIST OF ABBREVIATIONS

ABPP	: polypeptides of <i>Achyranthes bidentata</i> Blume
AD	: <i>Adansonia digitata</i>
CMAP	: Compound Muscle Action Potential
CNS	: Central Nervous System
Cont	: Control
DM	: Diabetes mellitus
EM	: Electron Microscope
EMG	: Electromyography test
EOOB	: <i>Ocimum basilicum</i> essential oil
EPO	: Evening primrose oil
EVOO	: Extra virgin olive oil
hSCs	: Human Schwann Cells
HT	: Hydroxytyrosol
Inj	: Injury
LM	: Light Microscope
MUFA	: Monounsaturated Fatty Acids
OB	: <i>Ocimum basilicum</i>
PBS	: Phosphate Buffered Saline
PN	: Peripheral Neuropathy
PNIs	: Peripheral Nerve Injuries
PNs	: Peripheral Nerves
PNS	: Peripheral Nervous System
SCs	: Schwann Cells
Sec	: Seconds
SFI	: Sciatic Functional Index
SLIs	: Schmidt-Lanterman incisions
TEM	: Transmission Electron Microscope
USA	: United States of America
WD	: Wallerian Degeneration
°C	: Degree centigrade
µm	: Micrometer

2D : Two-Dimensional
3D : Three-Dimensional

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1. INTRODUCTION

The peripheral nerve fibres are quickly damaged as they are the most fragile structure in the body. Therefore, more attention has been paid to peripheral nerve injuries (PNIs) which are considered a worldwide and serious public health problems that result in significant disabilities. The causes of PNIs include traumatic and non-traumatic injuries. These injuries are linked to socioeconomic disabilities, leading to partial or complete sensory and motor impairments (Sasso et al., 2020).

Globally, more than one million patients are subjected to PNIs annually (Siemionow and Brzezicki, 2009). Furthermore, it is documented that among 20 million American patients, trauma and medical disorders are the risk factors for developing PNIs (Grinsell and Keating, 2014). Furthermore, it is reported that, among 456 cases of nerve damage, axonotmesis represents the most common type of nerve injury (45%), neurotmesis (41%) and neuropraxia (14%), respectively in Brazil (Sasso et al., 2020). Likewise, it has been reported that PNIs incidence is estimated about 13 to 23 per 100,000 individuals every year in the developed nations, consequently, results in temporary or life-long neuronal dysfunction, which eventually leads to economic or social impairments (Hussain et al., 2020; Araújo-Filho et al., 2016; Muheremu and Ao, 2015; Li et al., 2014).

Since the old times, the principles about nerve injuries and nerve repair are recognized. In 1850, Augustus Waller described the concept of “Wallerian degeneration” (WD) as a defect in the distal nerve segment, and this concept marked a milestone in the understanding of nerve injury. The study of nerve healing has dramatically focused on this finding (Waller, 1850). Based on the severity of the injury, scientists have classified the PNIs into various categories. The purpose of classification is to help physicians and scientists to understand the injury mechanism and state the proper treatment precisely. The pioneer scientists who categorized PNIs are Sir Sydney Sunderland and Sir Herbert Seddon (Choi et al., 2016).

One of the milder examples of chronic compression injuries is a crush nerve injury. It is an excellent example of axonotmesis nerve injury (grade two) due to the

damage only in nerve fibres without the supporting structures; thus, the regeneration occurs rapidly in such type of injury (Sasso et al., 2020).

Crush injury is usually caused by severe traumatic pressure on a nerve with a blunt instrument such as a surgical clamp that cannot lead to a complete transaction. Multiple pathophysiological changes, including morphological and metabolic, were developed when the peripheral nerve was injured. These changes take place proximal to the injury site, distal to the injury site, the cell body, and the target organ; however, changes in the injury site start instantly. The WD creates a microenvironment that is attributed to growth and recovery after a traumatic injury. This process takes less than one week after an injury as long as there are common axonal damage signs. These signs are; disruption of the axon's cytoskeleton and loss of integrity of the cell membrane. The proximal stump degeneration is termed axonal degeneration (Geuna et al., 2009).

Furthermore, swelling develops in both ends of the affected nerve (George and Griffin, 1994; Waller, 1851). After damage immediately, the distal part of the injured nerve experiences WD process, this process includes Schwann cells (SCs) proliferation and myelin breakdown. The axon membrane fuses and seals the ends, which take place within the first hours. Then, macrophages target the damaged site and phagocytize cellular and myelin waste over three to six weeks. For establishing a new pathway for axon regeneration, nerve stem collapse must eventually occur due to axial changes at the distal end of the affected nerve. The sudden influx of Ca^{++} and Na^+ ions, triggers a series of apoptotic-like events that aimed to stimulate macrophages using signals generated by SCs, followed by granular degradation of the cytoskeleton as a marker of this phase (Miledi and Slater, 1970).

In contrast, based on the injury site (how far from the soma) and the severity of the injury, changes occurred in the proximal stump. Degradation of the proximal part is minimal and only reaches the Ranvier node; accordingly, apoptosis may occur when the injury site is too close to the soma (Tetzlaff and Bisby, 1989).

When PNI occurs, then the distal part of the axon slowly degenerate. Consequently, the axon and myelin were fragmented due to free radicals and oxidative

reactions, considered critical landmarks of WD (Namikawa et al., 2006). Furthermore, a large body of evidence revealed that in the degeneration and regeneration of PNS, immune cells including macrophages, dendritic cells, neutrophils and lymphocytes achieve a vital role; however, they pooled into damaged sites within hours to days of nerve damage (Chen et al., 2015). Furthermore, many studies stated that the free radicals and increased calcium level mediated oxidative stress and inflammation that could play a key role in degenerative neuropathy like amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and neuropathic pain (Muthuraman and Sood, 2010).

Since ancient times, traditional medicine has been applied to treat human illnesses worldwide through natural products, animals, plants and herbs. Concurrently, the increasing health care costs and the inability of allopathic medicine to treat many diseases have also contributed to the increased utilization of herbal medicines to combat diseases (Karar and Kuhnert, 2017). For example, in Africa (and in Sudan mostly), more than 80% of the population consumes natural medicinal products to treat several diseases (Maroyi, 2013). In contrast, in developing countries, interest in plant-derived medicine has also increased among pharma companies (Tabuti, 2008).

The most widely studied feature regarding the medicinal properties of natural products is its antioxidant effect. The antioxidants serve as the core of human cells' defence functions that scavenge and neutralize all oxidants such as reactive nitrogen species (RNS) and reactive oxygen species (ROS) involved in several human diseases (Moasser et al., 2018; Dunnill et al., 2017). The primary source of natural antioxidants is the herbal plants, as they contain phenolic compounds, including flavonoids and phenolic acids, in addition to vitamins and volatile components. Concurrently, dietary antioxidants have shown to be as promising as possible to treat many conditions such as peripheral neuropathy (PN) including symptoms like neuropathic pain and inflammation. Moreover, they possess other biological activity like antimicrobial properties (Karar and Kuhnert, 2017; Guimarães et al., 2014; Ren and Zuo, 2012; Mishra and Tiwari, 2011). *Adansonia digitata* (AD), extra virgin olive oil (EVOO) and *Ocimum basilicum* essential oil (EOOB) are well known natural potent antioxidants used for medicinal purposes and food sources.

Adansonia digitata L. commonly called baobab, is an indigenous perennial African plant. In many African countries, various parts of plants (fruits, flowers, tubers, twigs, leaves, and nuts) have been used in folk medicine and livelihoods; therefore, it has been known as “the small pharmacy” (Adeoye et al., 2019; Nordeide et al., 1996; Obizoba and Anyika, 1994). The literature revealed the positive effect of baobab fruit in nutritional intake against cancer, coronary heart disease, and oxidation. This is because it contains several vitamins such as vitamins C, E and A in addition to polyphenols that are the most effective components in the treatment of oxidative stress (Carlsen et al., 2010). Simultaneously, other studies indicated that baobab fruits contain a high level of energy, vitamins, ascorbic acid, trace minerals and metals (Glew et al., 2005; WT, 1968).

Extra-virgin olive oil (EVOO) is monounsaturated fatty acids (MUFA) and a dietary role model of the Mediterranean diet. Among all the healthy diets many food experts recommend, the Mediterranean diet is highly recommended as a dietary model with high-fat content, especially with MUFA like olive oil. Olive oil is a significant food due to its high nutritional value and essential dietary fats, preferably EVOO (Bach-Faig et al., 2011; Puertollano et al., 2010). EVOO consists of hundreds of micronutrients, notably antioxidants, as phenol compounds, carotenes and vitamin E (Owen et al., 2000). The interest in their biological and potential therapeutic effects is enormous. However, a large body of reports has indicated that a close relation between minimized morbidity and EVOO intakes; which lowers the incidence of neurodegenerative diseases (Angeloni et al., 2017; Rodríguez-Morató et al., 2015; Khalatbary, 2014), certain types of cancer, cardiovascular illnesses; including stroke, hypertension or coronary conditions and other chronic and metabolic diseases (such as type 2 diabetes mellitus) (D'Alessandro and De Pergola, 2015; Solfrizzi et al., 2011; López-Miranda et al., 2010).

Ocimum basilicum (OB), also known as sweet basil or Rihan (in Arabic), is considered an aromatic annual plant. This kitchen herb belongs to the Lamiaceae family *Ocimum* genus. Aromatic spices are widespread in subtropical and tropical African, Asian and South American areas (Kwee and Niemeyer, 2011; Loughrin and Kasperbauer, 2001). A growing body of reports revealed that OB has curable activities against neurological diseases like sedation, anxiety and depression due to antioxidant

and anti-inflammatory properties (Sestili et al., 2018). *Ocimum basilicum* essential oil (EOOB) ameliorative effect as a neuroprotective agent to combat several central nervous system (CNS) disorders has been reported; it enhances memory after ischemic stroke in mice model (Singh et al., 2018), Multiple sclerosis (Garabadu and Singh, 2020), showed antiepileptic effect (Tsyvunin and Shtrygol, 2015; Oliveira et al., 2009; Ismail, 2006). These protective properties might refer to essential oil constituents, such as methyl chavicol, methyl cinnamate, eugenol, camphor, and linalool. The authors also summarized that EOOB has radical scavenging and antioxidant activities in treating oxidative damage associated with some inflammatory cases (Kavoosi and Amirghofran, 2017; 2/Li et al., 2017; Rodrigues et al., 2016; Tshilanda et al., 2016).

The term stereology is derived from the Greek word stereo, which means something solid. Thus, stereology is defined as a morphometric (multidisciplinary) science that allows actual measurement of objects with three-dimensional (3D) features based on data obtained from their two-dimensional (2D) biological structure images or sections, despite size, shape or orientation, by using several techniques in addition to statistical sampling strategies (Deniz et al., 2018; Geuna and Herrera-Rincon, 2015; Baddeley and Jensen, 2004). Accordingly, the planar sections passing through the biological structures such as organs can represent all components with different orientations and sizes in the organ as sectional profiles (Altunkaynak et al., 2012). In clinical studies, to extract quantitative data, a stereological analysis was performed. In recent years, a quantitative analysis of PNS and CNS degeneration and regeneration has attracted the attention of several authors (Ehrenberg et al., 2017; Ardalan et al., 2016; Kaplan et al., 2010).

This study sought to explore the efficacy of fruit pulp aqueous extract of *Adansonia digitata*, extra virgin olive oil and *Ocimum basilicum* essential oil on sciatic nerve crush injury-induced neuropathy in *Wistar albino* rat model using different parameters, which include: myelinated axons number, axon diameter, myelin sheath thickness, functional improvement using sciatic-functional index and electromyography from the histology and stereology aspects.

2. GENERAL INFORMATION AND REVIEW OF THE LITERATURE

2.1. Neuron and Peripheral Nerve Structure

The nerve tissue of the body anatomically has organized into two divisions: central nervous system (CNS) that consist of the brain inside the cranial cavity of the skull and spinal cord, which lies inside the vertebral canal, and the peripheral nervous system (PNS) that consist of spinal and cranial nerves, and aggregations of neuronal cell bodies and their supporting cells outside the CNS called ganglia (Pawlina and Ross, 2018). The structural and functional component of the nervous system is the neuron, which is considered the most complex system among the vertebral body systems. The nervous system contains several million neurons, linked together through many connections to form neural circuits that enable the body to respond to continuous external or internal stimuli to integrate and control various body organs and systems. The neuron encompasses a cell body; perikaryon which contains the nucleus, organelles, numerous processes of differing length; such as dendrites and axon. In addition, the neuron has a speciality to receive the impulses from other cells and transmit them to other parts of the system through their processes. The specialized interaction between neurons for information transmission from one neuron to the next cell in the circuit is called synapse or synaptic cleft. Functionally, there are three types of neurons: sensory neurons transmitting sensory information from sensory receptors toward the brain, motor neurons conveying the order from the brain toward the target (muscle or gland), and interneurons, intercalated neurons that communicate between the sensory and motor neurons (Pawlina and Ross, 2018; Mescher, 2013).

Anatomically and based on the number of processes, the neuron has categorized into:

1. Multipolar neurons have a single axon, two or more dendrites, the electrical impulses directed from dendrites and cell body that specialized to receive impulses toward another cell via axon which is represented the conducting

2. portions of the neuron that send the impulses away. Most of the motor and intercalated neurons are multipolar.

3. Bipolar neurons have single axon and single dendrites. Such neurons are associated with special senses receptors (equilibrium, taste, smell, hearing and sight).

4. Unipolar (pseudounipolar) neurons have only a single process that splits close to the perikaryon into two portions, and one lengthens to another cell, whereas another extends to CNS. Most of these neurons are sensory, their cell bodies located in cranial nerve ganglia and dorsal root ganglia.

The neurons are supported by non-conducting cells called neuroglial cells or glia. Those glial cells that found in CNS; central glial cells are oligodendrocytes, astrocytes, microglia, and ependymal cells, whereas in the PNS; peripheral glial cells are SCs, satellite cells and a diversity of other cells linked with particular structures like enteric neuroglial cells which found in ganglia of alimentary tract wall. The role of SCs is to surround the axon of the neuron to form myelin sheath and isolate them from the extracellular matrix and neighbouring cells. The functions of neuroglial cells are 1. Protection of neurons. 2. Repair of injury. 3. Electrical isolation of neurons and their processes that accelerate the transmission of the impulse. 4. metabolic exchange between the vascular system and CNS neuron. 5. The internal fluid milieu regulation of CNS. 6. The neurotransmitters removal from the regions of the synapse. The PNS consists of peripheral nerves (PNs), nerve ending and clusters of neuronal cell bodies, representing outside CNS as peripheral ganglia (Pawlina and Ross, 2018; Mescher, 2013).

2.1.1. Anatomy of Peripheral Nerves

The PNS extends throughout the body using branching formations and blood vessels system. Anatomically and for description purposes, PNS is composed of cranial and spinal nerves with their associated ganglia. The spinal nerves originated from the spinal cord, while the cranial nerves originate from the brain except for the first and second, namely the olfactory and optic nerves. Several nerve trunks are distributed throughout the different body parts due to the functional components and number of fascicles (Snell, 2010; Zacchigna et al., 2007; Sunderland and Smith, 1969; Sunderland

and Bradley, 1949). The cranial nerves (12 paired) and their ganglia leave the skull's cranial cavity through the skull's foramina to distribute to the body's target organs. The arrangement of them from first to 12th nerve as the following:

- I. Olfactory nerve.
- II. Optic nerve.
- III. Oculomotor nerve.
- IV. Trochlear nerve.
- V. Trigeminal nerve.
- VI. Abducent nerve.
- VII. Facial nerve.
- VIII. Vestibulocochlear nerve.
- IX. Glossopharyngeal nerve.
- X. Vagus nerve.
- XI. Accessory nerve.
- XII. Hypoglossal nerve.

On the other hand, the spinal nerves pass through the vertebral column's intervertebral foramina. They are 31 paired in number arranged as follows: eight cervical, 12 thoracic, five lumbar, five sacral and one coccygeal nerve (Snell, 2010).

2.1.2. Embryology of Peripheral Nerve

PNS controls different organs, tissues and systems related to several homeostatic functions. Therefore, The Peripheral Nerves (PNs) development and their axon, SCs, and connective tissue are important to study (Kerns, 2008). A few reports closely described the anatomy and physiological properties of developing mammalian peripheral nerves. For example, literature has proven that axonal conduction began before myelination in neonatal rats' central nervous system (Ziskind-Conhaim, 1988).

In the CNS, early glial cells located within the axons of embryonic nerves before gliogenesis originated from neural crest cells as they navigate their way to meet distal targets throughout body tissues and establish functional links between the CNS the rest of the body. However, axon–glial organization of embryonic nerves is seen early (embryo day E14/15 in rat hind limb and E12/13 in mouse) (Jessen et al., 2015; Harrison, 1924). During the early stages of SCs common development, they produce a protective coat around neuronal processes and ensheath large-calibre fibres in adults and are also significantly related to neurons. The control and bidirectional cell to cell talking among SCs and neurons are significant for later maintenance and development of PNS function and morphology (Lobsiger et al., 2002). A considerable number of SCs are necessary for the normal development of PNS (Atanasoski et al., 2008). In contrast, the proliferation and differentiation of SCs guided by signalling pathways and growth factors have not yet been recognized (K. Jessen and Mirsky, 2002; Lobsiger et al., 2002).

All developmental stages of SCs in rodents, from early gliogenesis to myelination, have been studied in detail (Woodhoo and Sommer, 2008). Fundamentally, several neurulation processes produce the central nervous system's cell precursors (Moore and Persaud, 2004). A number of cells, the neural crest cells detach from the ectodermal surface at the margins of the neural fold during neural tube formation (Moore and Persaud, 2004; Rice and Barone, 2000).

The SCs includes two types: myelinating SCs, which form the myelin sheath around the myelinated axons, and Non-myelinating SCs that envelop unmyelinated axons (Kaplan et al., 2009). Oligodendrocytes cells of CNS have identical structural and functional properties of the myelinating SCs. In comparison, the astrocytes cells of CNS are similar to unmyelinating SCs. The previous reports have proved that SCs have a vital role in repairing damaged nerves and regeneration (Kaplan et al., 2009).

In summary, the primary developmental origin of Peripheral nerves is mainly from neural crest cells, ectodermal origin. The peripheral nerves are derived from processes of the neuroblast cells. The SCs are also developed from neural crest cells, then migrate peripherally to wrap around every nerve axon; neurilemma sheath. Likewise, the cranial

and spinal sensory ganglia that have the same developmental origin. The myelination started on the fourth month prenatally (Sadler, 2006).

2.1.3. Histology of Peripheral Nerve

The neuron is considered the functional unit of the nervous system. It consists of a cell body (Perikaryon or soma) and neuronal processes (axons and dendrites). It has three types sensory, motor and interneuron. The neuron's body contains a large euchromatic nucleus, cytoplasm containing abundant rough endoplasmic reticulum (rER), free ribosomes, rER, and ribosomes small bodies called Nissl bodies. Moreover, the cytoplasm also contains numerous mitochondria, Golgi apparatus, lysosomes, microtubules, neurofilaments, transport vesicles and inclusions (e.g., lipofuscin).

Neurons do not divide, but some brain areas contain neural stem cells; such as the olfactory bulb and the dentate gyrus of the hippocampus that have ability to regenerate. Dendrites: are parts of the cell that receive the information; they have many branches that contain cytoplasm similar to that of the cell body. Most of the neuron has many dendrites their diameter is more significant than that of the axon.

Axon: is the part of the cell body that sends information away from the cell body. Axon originates from the axon hillock; it contains microtubules, neurofilaments, mitochondria, and vesicles. A neuron has only one axon. The area between the axon hillock and myelin sheath is called the initial segment.

Synapses are specific connections between neurons that assist the impulses conductivity from the presynaptic neuron to the postsynaptic neuron. Synapses also appear between axons and target organs cells, such as gland and muscle cells. Synapses between neurons are classified into four types:

- Axodendritic; between axons and dendrites.
- Axosomatic; between axons and the cell body.
- Axoaxonic; between axons and axons.
- Dendro-dendritic is occurring between two dendrites. According to the length of the axon, neurons are classified into two types:

- Golgi type I neurons with a long axon may be one meter long. Examples of these neurons are pyramidal cells of the cerebral cortex, Purkinje cells of the cerebellar cortex, and the spinal cord's anterior horn neurons.

- Golgi type II neurons have a short axon or have no axon; an example of these neurons is stellate cells found in the cerebral and cerebellar cortex.

Neuroglia: are supporting cells generally smaller than the neurons, but their number is higher ten times than the neurons. Neuroglia is distributed throughout the central and peripheral nervous systems.

There are two types in the peripheral nervous system; there are:

- SCs that form the myelin sheath in the peripheral nerve, and each SCs myelinate only one nerve fiber. As well, SCs that associated with unmyelinated axons.

- Satellite cells are found in the ganglia and surround the neuronal cell bodies (Pawlina and Ross, 2018; Mescher, 2013).

The peripheral nerves consist of neural and connective tissue components (Kaplan et al., 2009). The peripheral nerve bulk contains nerve fibres and their supporting SCs. Each fiber and its supporting SCs are held together through three specific functional layers of connective tissue: The endoneurium is a loose connective tissue surrounding each single nerve fiber, while a group of fibres forms fascicle, the perineurium is a specialized connective tissue surrounding each nerve fascicle which contributes to blood-nerve barrier employing tight junction between perineurial cells, the presence of extended basal lamina and existence of transporters all these factors aid to maintain ionic milieu. The epineurium consists of dense irregular connective that surrounds a peripheral nerve and fills the spaces between nerve fascicles (Ross and Pawlina, 2006). The nerve fibres might be myelinated or unmyelinated (Geuna et al., 2009; Flores et al., 2000) depend on the way of myelination; the unmyelinated fibres are held together as a group and covered by single SCs. In contrast, the myelinated fibres are covered SCs for each axon individually (Figure 2.1) (Pawlina and Ross, 2018; Kaplan et al., 2009).

Furthermore, the individual axon-SCs units are housed by tubular spaces that consistently penetrate the surrounding connective tissue matrix. This arrangement of tissue is held at the distal portion of the transactive location in the nerve parts. It gives numerous tubular channels that contain the SCs orphaned by the degeneration of the axons.

These SCs help develop regenerating axons by holding in a linear sequence; each axon develops within these tubular spaces between the innermost part of the surrounding connective tissue and the cell surface of Schwann regenerating axons join the peripheral nerve trunk (Scherer and Easter, 1984).

The axons of neurons are surrounded by a multi-layered myelin sheath formed by SCs in PNS. In contrast, the myelination by oligodendrocytes in the tracts of CNS, both of them have a highly specialized plasma membrane that is important for myelination formation. The biochemical components of the myelin sheath are about 70-80% of lipid (mainly cholesterol), 15-30% of protein (especially myelin-specific protein) (A. V. Waller, 1851). The types of fibres, conduction velocity and function, are illustrated in (Table 2.1).

Table 2. 1. Shows the types of nerve fibres and properties of each type Modified table from (Miller et al., 2009)

Nerve Fiber Types and Properties						
Fiber Class	Myelin	Diameter (Mm)	Conduction Velocity (m/s)	Spinal Cord Tract	Location	Function
A α	+	6-22	30-120	Ipsilateral dorsal column	Efferent to muscles	Motor
A β	+	6-22	30-120	Contralateral spinothalamic tract	Afferent from skin and joints	Tactile, proprioception
A γ	+	3-8	15-35	Ipsilateral dorsal column	Efferent to muscle spindles	Muscle tone
A δ	+	1-4	5-30	Contralateral spinothalamic tract	Afferent sensory nerves	Pain, cold, temperature, touch
B	+	1-3	3-15	Preganglionic	Preganglionic sympathetic	Various autonomic functions
sC	-	0.3-1.3	0.7-1.3	-	Postganglionic sympathetic	Various autonomic functions
dC	-	0.4-1.2	0.1-2.0	Contralateral spinothalamic tract	Afferent sensory nerves	Various autonomic functions Pain, warm, temperature, touch

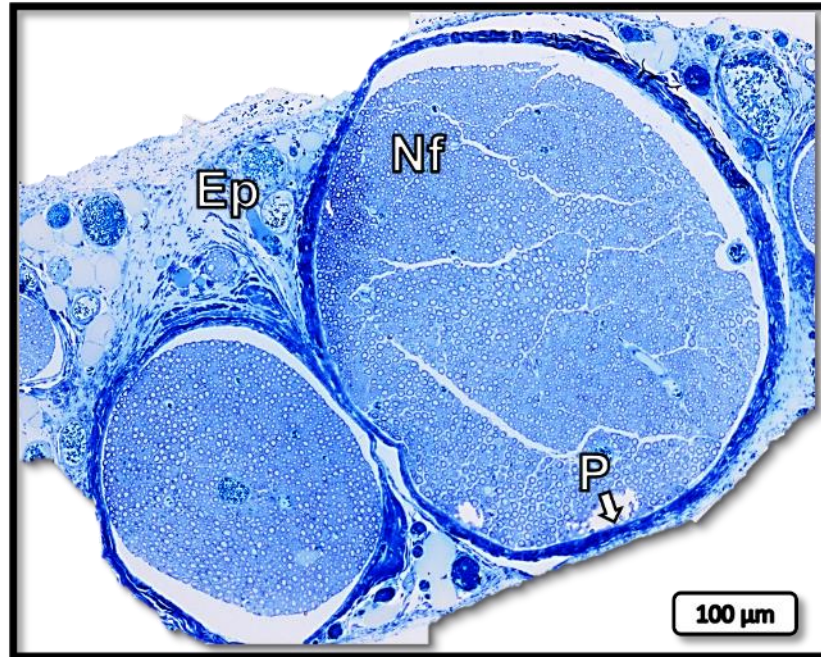


Figure 2.1. Shows the histological features and appearance of peripheral nerves, includes connective tissue epineurium (Ep) and perineurium (P) and nerve fascicle (Nf)

2.2. Peripheral Nerves Injuries (PNIs)

The peripheral nerve fibres can usually be quickly damaged as they are the most fragile structure in the body. The causes of PNIs include traumatic and non-traumatic injuries like partial or complete crushing. These injuries are linked to socioeconomic disabilities, in addition to the functional disability of innervated structures with sensation and motor impairment (Sasso et al., 2020). The crush, the Gunshot wounds, chronic compression, lacerations, major surgery, radiotherapy, trauma, or the full transaction are the risk factors that might cause PNIs (Burt et al., 2017; Secer et al., 2009). When nerve injury has developed, the connection abnormalities between the brain and another body's organs will be detectable (Prakash and Bidkar, 2016).

PNIs draw pronounced attention of the scientific community that is considered a global and severe public health problem resulting in significant disability. PNIs spent more than 150 billion dollars annually on healthcare expenses in the United States of America (USA) (Taylor et al., 2008).

2.2.1. History of Peripheral Nerve Injuries

The PNS has attracted researchers' interest since ancient times (Terzis et al., 1997; Cajal, 1928). Herophilus and Hippocrates were the first researchers who started studying PNIs since the third and fourth centuries, respectively (Terzis et al., 1997). The history of the recovery of injured nerves has unchanged until Galen of Pergamon (Majno, 1991), who was the first to discover the peripheral nerve transaction results. He showed remarkable findings when nerves were damaged. Notwithstanding the fact that Persians document the first specific reference about nerves repair by suture, Rhazes (850-932 AD) and Avicenna (980-1037AD) (Sunderland and Williams, 1992; Seddon, 1942).

Since the old times, the principles about nerve injuries and nerve repair are recognized. In the seventh century, Paul of Aegina was the first researcher to describe wound closure and fixation of nerve endings (Mafi et al., 2012). Throughout the middle years, many of the most recognized experts of surgery described sutures of injured nerves briefly such as the French surgery founder "Lanfranchi" (1296), William of Saliceto (1210-1277) and Guy de Chauliac (1300-1368) in the 14th century. However, the mentioned technique has rarely been implemented due to a lack of knowledge about the peripheral nerves system's anatomy and physiology (Terzis et al., 1997). Theodor Schwann (1810-1887) subsequently issued statements on the composition of the cell named in his honour (Terzis et al., 1997). After Johannes von Purkinje (1787-1869) is addressed the connection between neurons and axons in the early 19th century, his contemporary Robert Remak (1815-1855) was also revealed the difference between myelinated and unmyelinated nerve fibres (McHenry and Garrison, 1969). In 1850, Augustus Waller described the concept of "Wallerian" degeneration", and the loss of the distal nerve portion achieved a landmark in understanding nerve injury. The study of nerve healing has dramatically focused on these findings (Waller, 1850). The use of the functioning microscope was independently endorsed by Kurze and Smith in 1964 (Kurze, 1965; Smith, 1964). The benefits of tension-free recovery have identified by Terzis during the 1970s. It seems that the scientific development in nerve restoration is still in its early stages. At any time, the nerve repair approach may change forever with an important knowledge-based discovery (Terzis et al., 1997).

2.2.2. Classification of Peripheral Nerve Injuries

Based on the severity of the injury, scientists have classified the PNIs into various categories. The purpose of classification is to help physicians and scientists to understand the damage and state mechanism and decide proper treatment precisely. The pioneer scientists of PNIs grading are Sir Sydney Sunderland and Sir Herbert Seddon.

2.2.2.1. Seddon's Classification

In April 1941, during the Physicians Association Conference, depending on the presence of demyelination and the severity of the damage to the axons and surrounding connective tissue of the nerve, Seddon proposed and categorized the PNIs clinico-pathologically into three distinctive types as the following:

- A. Neurotmesis (cutting) is a complete detachment of a nerve into two parts.
- B. Axonotmesis "continuity lesion" is severe axon damage. However, this type's noticeable feature is the segmentation of the axon with its myelin sheath and the survival of nerve supporting connective tissue.
- C. Neuropraxia, "no- action " is defined as impulses blockage due to compression with fast recovery (Seddon, 1942).

2.2.2.2. Sunderland's Classification of Nerve Injuries

In 1951, Sunderland agreed to Seddon's classification and divided PNIs into five subsections based on the exact damage and discontinuity of several connective layers of peripheral nerve (Chhabra et al., 2014). He stated that neuropraxia is grade one that is a condition of compression or crush in nerve fibres that leads to blockage of signal conductivity due to damage in the myelin sheath. Furthermore, Sunderland reclassified the axonotmesis, "which explained by Seddon" into three grades:

- A. Grade two "of Axonotmesis" is a condition in which the damage will be in the nerve fiber and its myelin sheath while the connective tissue is survived (Hussain et al., 2020; Zuniga and Radwan, 2013). Accordingly, it contributes to denervation in target areas and causes sensory / motor control disturbance.

However, the complete functional recovery may take several weeks without surgery, so axon regeneration is essential.

B. Grade three "of axonotmesis" is the axon's damage, myelin sheath and endoneurial layer, whereas the other layers remain intact with more rehabilitation challenges.

C. Grade four "of axonotmesis" is the most severe grade in which the epineurium is only survived, whereas the axonal sheath and all other layers are damaged (Hussain et al., 2020; Flores et al., 2000).

The crush nerve injury is an excellent example of axonotmesis due to the damage only in nerve fibres without the supporting structures, which accelerate regeneration. (Sasso et al., 2020). The differentiation between nerve injury types has shown in Figure 2.2.

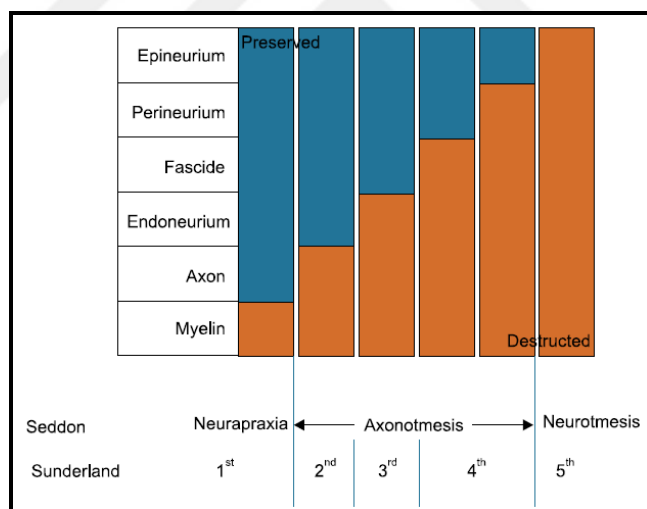


Figure 2.2. Shows the comparison between Seddon's and Sunderland's types with how the severity of each type

2.2.3. Epidemiology and Etiology of Peripheral Nerve Injuries

Globally, there are more than one million patients with PNIs annually (Siemionow and Brzezicki, 2009). Likewise, it has been reported that PNIs incidence is estimated between 13 to 23 per 100,000 individuals every year in the developed nations and consequences in temporary or life-long neuronal dysfunction that can eventually lead to social-economic impairments (Hussain et al., 2020; Araújo-Filho et al., 2016;

Muheremu and Ao, 2015; Li et al., 2014). PNIs represents about 2.8% of multiple-trauma victims. Moreover, most peripheral nerve injuries occur in the upper limb due to traumatic causes. However, PNIs causes suffering to healthy civilians and military officers. They are most at risk of traumatic injuries, although it severely decreases patient's quality of life by affecting sensory and motor functions (Grinsell and Keating, 2014; Ashley et al., 2007; Belkas et al., 2004; Noble et al., 1998). A previous study showed that in 20 million Americans, trauma and medical disorders are risk factors for developing PNIs (Grinsell and Keating, 2014). PNIs spent more than \$150 billion of annual health dollars in the USA. Furthermore, it is reported that in Brazil, among 456 cases of nerve damage, axonotmesis represents the most common type of nerve injury (45%), followed by neurotmesis (41%) and neuropraxia (14%), respectively (Sasso et al., 2020).

Many factors determine the etiology and epidemiology of PNIs and peripheral neuropathy (PN), such as the level of population development and periods of peace or conflict. However, in wartime, the awareness of PNIs was mainly established (Eser et al., 2009). As a consequence of a disease process or trauma, PN may be hereditary or acquired (Richard, 2002).

Traumatic causes include crushing, complete division of nerves, constriction, and stretching. In contrast, there are many acquired PN sources such as diabetes, cancerous, alcohol, autoimmune, chemotherapy, or toxic (Caillaud et al., 2019; Katona and Weis, 2017; Ramchandren, 2017). The clinicians sometimes have not discovered some PN types' specific behavioural science; conditions referred to as idiopathic neuropathies. (Caillaud et al., 2020). Previous studies reported that traumatic PNIs are most common in young individuals as the male to female ratio was 2.4 : 1, and the ulnar, median, radial nerves, brachial plexus and facial nerve, besides the sciatic nerve the lower extremity, were the most common nerve injuries. Moreover, road traffic accidents were the most common cause of injuries for most nerves, except for the facial nerve injuries with iatrogenic causes (Miranda and Torres, 2016; Eser et al., 2009).

2.3.1. Pathophysiology of Nerve Injury

Schmidt-Lanterman incisures (SLIs) are cytoplasmic components of SCs, and they regulate the metabolism of the myelin sheath. Thus, the increase of SLIs reveals that SCs are active in remyelination (Ludwin and Maitland, 1984; Menorca et al., 2013). Compression injuries have various mechanisms (Pham and Gupta, 2009; Tapadia et al., 2010). One of the milder examples of WD is chronic compression injury. It has been suggested that the macrophages and their mitogenic factors, like interleukins 1,6,10, and 12, play a vital role in enhancing SCs activity (Gray et al., 2007). Besides, micro-progressive damage in a chronic compression does not trigger the inflammatory process, macrophages arrive slowly after the proliferation SCs (Gupta and Channal, 2006). Several in vitro studies performed in well-controlled rooms applied shear stress injury, the results revealed that demyelination, proliferation, and remodelling of SCs might cause alteration of the expression of the SCs protein with significant changes, which attributed to B4 integrin expression (Frieboes and Gupta, 2009).

Crush injuries are usually caused by severe traumatic pressure on the nerve with a blunt instrument such as a surgical clamp that does not lead to a complete transaction. Multiple pathophysiological changes, including morphological and metabolic changes, develop at the site of injury when the peripheral nerve is injured. These changes take place in proximal and distal segments of the injury site, the cell body, and the target organ. However, changes in the injury site start instantly (Geuna et al., 2009). Each part will be discussed as the following:

2.3.1.1. Wallerian Degeneration (WD)

In 1850, Augustus Waller pronounced his significant observations of the experiment after axotomy of frog glossopharyngeal and hypoglossal nerves. He defined WD as a defect in the distal nerve segment, and this concept marked a milestone in understanding nerve injury (Waller, 1850). This process takes less than one week after injury associated with common axonal damage signs; these signs are disruption of the axon's cytoskeleton and loss of cell membrane integrity. Thus, the proximal stump

degeneration is termed axonal degeneration. Furthermore, swelling develops to both ends of the affected nerve (George and Griffin, 1994; Waller, 1851).

2.3.1.2. Changes in the Distal Part

After damage immediately, the distal part of the injured nerve experiences WD process. This process includes SCs proliferation and myelin breakdown. The axon membrane fuses and seals the ends and this takes place within the first hours. Then, macrophages target the damaged site and phagocytize cellular and myelin waste over three to six weeks. During the early days of axonal collapse, this process's main features are the granular disintegration of microtubules and neurofilaments, these changes are attributed to proteolysis (Lubińska, 1982; Schlaepfer, 1977; Vial, 1958). SCs proliferation induced by loss of SCs- axon integration. One of the critical SCs functions is to control and upregulate neurotrophic factors such as NGF (Geuna et al., 2007; Funakoshi et al., 1993; Thoenen et al., 1988; Heumann, 1987).

Laminin and fibronectin are considered as neurite outgrowth-promoting factors that regulate the progress of regenerating fibers of the distal segment subsequent to the organization of proliferating SCs into columns (Büngner bands) which allow regenerating axons to grow between their basal membranes (Hall, 1997; Liu, 1996; Baron-Van Evercooren et al., 1982).

For establishing a new pathway for recent axon regeneration, nerve stem collapse must eventually occur due to axial changes at the distal end of the affected nerve. The sudden influx of ions, mainly Ca^{++} and Na^{+} , triggers a series of apoptotic-like events aimed to stimulate macrophages using signals from SCs, followed by granular cytoskeleton degradation as a sign of this phase (Miledi and Slater, 1970). It has also reported that, in the distal stump of the affected tibial nerve, neurotrophin-3 and ciliary neurotrophic factor are down-regulated. In contrast, messenger c of brain-derived neurotrophic factor and glial cell line-derived neurotrophic element are found to be upregulated. If axon does not regenerated, the distal stump would retain these modifications for six months after the damage (Onger et al., 2016; Dieu et al., 2005).

2.3.1.3. Changes in the Proximal Part

Based on the injury site (how far from the soma) and the severity of the injury, changes occur in the proximal stump. Degradation of the proximal part is very minimal and only reaches the closest node of the Ranvier. Accordingly, apoptosis may occur when the injury site is too close to the soma. In severe damage, chromatolysis in the proximal portion begins to shift towards the regenerative phenotype (Tetzlaff and Bisby, 1989). Previous reports revealed that the neurofilaments involved in maintaining the axon diameter are downregulated. At the same time, other factors such as endogenous neuroprotectants such as heat-shock protein-27 and some proteins like GAP-43, tubulin and actin are upregulated (Hall, 2005; Lewis et al., 1999; Costigan et al., 1998; Fu and Gordon, 1997; Tetzlaff et al., 1991). The neurons change from recovery to maintenance when neuron maturity is required, and the axon reaches the target organ. The key to axon regeneration is the SCs which are considered as the main mediator of WD. In addition to recruiting macrophages that cleans SCs debris by phagocytosis. This process occurs under the control of protein MAC-2, which supports SCs phagocytosis (Menorca et al., 2013).

2.3.2. Degeneration and Regeneration

Since ancient times, great attention was given to the damage and repair of nerve cells. Paul Aegina in the 7th century was the first to describe wound closure and nerve ends apposition (Mafi et al., 2012). If PNI occurs, then the distal part of the axon would slowly degenerate. Consequently, the axon and myelin will be dismantled due to free radicals and oxidative reactions which considered a critical landmark of WD (Namikawa et al., 2006). It is known that the PNS is not entirely isolated; accordingly, a complex multicellular response induced by affected axons includes multiple components such as non-differentiation of SCs and activation of the immune response (DeFrancesco-Lisowitz et al., 2015; Gaudet et al., 2011). Moreover, based on the active regenerative reaction of damaged axons and the simultaneous participation of immune cells, significant regeneration of axons achieved. A large body of evidence revealed that in the degeneration and regeneration of PNS, immune cells, including macrophages, dendritic cells, neutrophils and lymphocytes perform an essential role however they are pooled

into damaged sites within hours to days from nerve damage time. (Chen et al., 2015), (figure 2.3). The degeneration and regeneration cycle also involves inflammatory mediators, such as prostaglandins, histamine, cytokines, chemokines and growth factors (Tamaddonfard et al., 2014). Many studies stated that the free radicals and increased calcium level mediated oxidative stress and inflammation which play a vital role in degenerative neuropathy like amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and neuropathic pain (Muthuraman and Sood, 2010).

A deep understanding of cellular and tissue-level events following nerve injury is necessary to improve healing and regeneration. The WD exclusive features include: (a) disunion of resident SCs from associated fibres, (b) transition of these SCs into a "repair SCs" phenotype, (c) breakdown of the barrier between blood and nerve, and (d) inflow of macrophages into the tissue that following "repair SCs," phagocytize axonal and myelin-derived debris (Jessen et al., 2015; Chen et al., 2007). Macrophages promote the repair of SCs during the regeneration process and mediate axonal regrowth and re-innervate the target tissue. (Cattin et al., 2015; Mietto et al., 2015; Mokarram et al., 2012). To ensure proper peripheral nerve healing, activities of many distinct cell types such as neurons, SCs, and immune cells are important. Peripheral axons can regenerate following nerve damage and reconnect with their destinations, giving a proper route. Functional healing can be improved with clinical therapies that accelerate axonal regeneration. However, the proximal end of the damaged nerve experience neuroma and scar tissue without a clear pathway, which inhibits the growth of regenerating nerves, through autocrine circuits and block apoptosis, SCs of the injured part could support their survival and improve axonal growth toward the distal stump (Mokarram et al., 2012).

The practical outcome of peripheral nerve regeneration is always poor despite the regeneration ability, mainly because of severe damaged peripheral nerves might be very distant from their targets to recombine. However, the regeneration-time ratio is ~1 mm/day or ~1 inch/month; hence the length of the axon determines how long the reinnervation and remyelination takes (Morisaki et al., 2011; Yan et al., 2011; Lehmann et al., 2010). Thus, healing in older individuals is slower than in children (Seddon et al.,

1943). In addition, clinically, inappropriate functional recovery is a significant challenge, especially following serious damages. It has been documented that there are a number of innovations in regenerative medicine (Li et al., 2014).

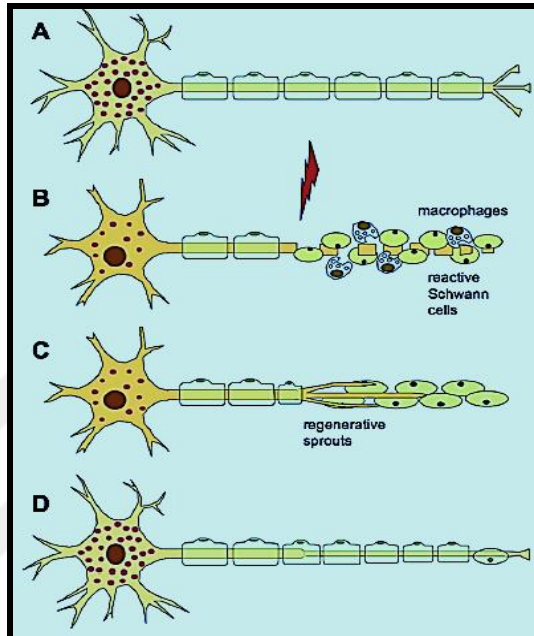


Figure 2.3. Shows stages of peripheral nerve regeneration after injury. As A. normal axon. B. Wallerian degeneration. C. alignment of SCs as bands of Büngner, D. Fully regenerated axon. Modified image from (Allodi et al., 2012)

2.4. Natural Antioxidant Substances

Dire consequences such as total or partial loss of motor and sensory functions leading to lifelong impairment may occur when treatment for PN is not sufficient (Noorafshan et al., 2011; Sun et al., 2009; Burnett and Zager, 2004; Noble et al., 1998). With current therapeutics, the challenges in diagnosing and fully understanding PN pathological processes result in an inadequate cure. Several clinical approaches, including physical rehabilitation, microsurgical reconstruction and pharmacotherapy (Roglio et al., 2008; Sabatier et al., 2008; Marqueste et al., 2004) have been applied for the treatment of PN. Nevertheless, pharmacotherapy (with adjuvant therapy) has promised to be the most useful for neurorehabilitation (Zhang et al., 2014). Therefore much focus has been paid to studying successful drugs that could improve axonal regeneration (Ma et al., 2013).

Since ancient times, traditional medicine is used to treat human illnesses worldwide by compounds derived from natural products, animals, plants and herbs. Concurrently, the increasing health care costs and the inability of allopathic medicine to treat many diseases have also contributed to the increase in herbal medicines to combat diseases (Karar and Kuhnert, 2017). In Africa nations such as Sudan more than 80% of the population consumes natural medicinal products to treat several diseases (Maroyi, 2013). In contrast, in developing countries, interest in plant-derived medicines has also increased among pharma companies. The pharmaceutical outcome is a potential drug for medicinal plants with a long history of healthy and prosperous consumption (Tabuti, 2008). However, nineteen new naturally-derived drugs were released and approved by the European medicines agency and the food and drug administration between 2005-2010 (Gebauer et al., 2002).

The most widely studied feature regarding the medicinal properties of natural products is its antioxidant effect. The antioxidants serve as the core of defence functions of the human cells that scavenge and neutralize all oxidants such as reactive nitrogen species and reactive oxygen species involved in several human diseases (Moasser et al., 2018; Dunnill et al., 2017). By reducing oxidative stress, antioxidants play a significant role in preventing infectious diseases, coronary artery disease, cancer, diabetes, high blood pressure, stroke, and Alzheimer's disease (Lako et al., 2007). Additionally, dietary antioxidants have shown to be as promising as possible to treat many conditions such as PN and its symptoms like neuropathic pain and inflammation. Moreover, they had been possessed other biological activity like antimicrobial (Karar and Kuhnert, 2017; Guimarães et al., 2014; Quintans et al., 2014; Ren and Zuo, 2012; Mishra and Tiwari, 2011). *Adansonia digitata*, extra virgin olive oil and *Ocimum basilicum* essential oil are well known natural potent antioxidants used for medicinal purposes and food sources.

2.4.1. Aqueous *Adansonia digitata* (AD)

Adansonia digitata L. (commonly called baobab), tree of life, monkey bread or “Ose” in Yoruba language, is an indigenous perennial African plant. In Sudan, the baobab tree's indigenous names are 'Humar,' 'Homeira,' or 'Tebaldi, whereas 'gunguleiz' referred to the fruits. It belongs to the family Bombacacea that is the most widespread of

the *Adansonia* species in several arid regions in Sudan and Africa (Figure 2.4) (Gebauer et al., 2002). It extends from Namibia to Ethiopia and northern Transvaal, Sudan, and the Sahara's southern fringes (El Amin, 1990).

AD is essential to the people's livelihood in waterless zones since it is a multipurpose deciduous tree, considered the largest succulent plant globally. In many African countries, various parts of plants; fruits, flowers, tubers, twigs, leaves, and nuts have been used in folk medicine and livelihoods; therefore, it has been known as “the small pharmacy” (Adeoye et al., 2019; Nordeide et al., 1996; Obizoba and Anyika, 1994), where several products were known in Khartoum markets in the oldest century. The European commission and the food and drug administration in USA have identified baobab pulp as a new food (Khamis et al., 2020).

Generally, the baobab fruit consists of pulp and large seeds embedded in the dried acidic pulp and shell (Figure 2.5). It used as refreshing drinks, ice cream, desserts, and sauces. It is highly recommended for use as a daily energy intake for children and pregnant women due to its carbohydrate and protein composition. Baobab is a convenient source of amino acids as it is considered a low protein diet. Therefore, the pulp of dried baobab fruit would also be beneficial as an ingredient for adding fibre to the meat and improving its overall nutritional profile. Because it is high in soluble fibre, it may be a saturated ingredient with a low glycaemic index food compared to its profile with other food (Chadare et al., 2008; Osman, 2004). The literature revealed the positive effect of baobab fruit in nutritional intake against cancer, coronary heart disease, and oxidation. It contains several vitamins such as vitamins C, E and A in addition to polyphenols that were the most effective component in the treatment of oxidative stress. Simultaneously, other studies indicate that baobab fruits contain a high level of energy, vitamins, ascorbic acid, trace minerals and metals (Glew et al., 2005; WT, 1968).



Figure 2.4. Baobab tree in Sudan. Modified image from (Gebauer et al., 2002)



Figure 2.5. Shows the baobab fruit and the baobab fruit seeds with and without fruit pulp, Modified images (Gebauer et al., 2002)

2.4.1.1. Chemical Composition of Aqueous *Adansonia digitata*

The literature about the chemical characterization of phenol compounds of baobab fruit pulp is insufficient. Based on geographic regions and plants, the results reported high inconsistency. For instance, phenylethanoid glycosides, hydroxycinnamic acid glycosides, and iridoid glycosides are the main components in a Nigerian baobab's

phytochemical analysis for fruit pulp using UHPLC-DAD-HR-ESI-MS technique (Li et al., 2017). The study of fruit sample from Cameroon using LC-ESI-MS/MS technique revealed that it is rich in tiliroside, procyanidins and flavanol glycosides (Sokeng et al., 2019). The same study was conducted for a Malawi baobab fruit pulp; the results showed the epicatechin, gallic acid, and procyanidin B2 are dominants (Tembo et al., 2017). It is worth noting that all samples of fruit extracts show high antioxidant efficacy due to flavonoids, glycosides, saponins, tannins, terpenoids and steroids components (Gwarzo, 2013).

Generally, baobab dried fruit pulp is very high in fibres, vitamin C (more than ten times than orange, over 100 mg/100 g) (Sabina et al., 2020) and low fat in terms of macronutrients. Compared to other fruits, the pulp contains low sugar. Moreover, the magnesium, iron and calcium are significant components. Although it is worth mentioning that baobab fruit extract might play a protective role as an antioxidant because it contains an extraordinary amount of polyphenolic compounds and phytochemicals contents (Sokeng et al., 2019; Braca et al., 2018; Fernandes de Oliveira et al., 2012; Lamien-Meda et al., 2008; McDougall et al., 2005). The high content of nutrients and polyphenols in baobab fruit has been widely recognized. However, based on a comparative study conducted to evaluate the total phenolic and flavonoid contents for fourteen species of wild fruits from Burkina Faso, the *Adansonia digitata* L. was the second-highest species (Lamien-Meda et al., 2008). The percentages of each component shown in Table 2.2.

Table 2. 2. Chemical composition of baobab fruit, Modified image from (Nour et al., 1980)

Constituents (dry weight basis)	
Total soluble solids (%)	79.3
Alcohol insoluble solids (%)	57.3
Total sugars (%)	23.2
Reducing sugars (%)	19.9
Total pectin (% galacturonic acid)	56.2
Starch (%)	–
Protein (% N)	2.6
Fat (%)	0.2
Crude fibre (%)	5.7
Ash (%)	5.3
Ascorbic acid (mg/100 g)	300.0
Iron (mg/100 g)	8.6
Calcium (mg/100 g)	655.0
Phosphorus (mg/100 g)	50.8
Moisture	6.7
pH	3.3

2.4.1.2. Biological Activities of Aqueous *Adansonia digitata*

AD parts have attracted the interest of researchers to measure the potential efficacy and beneficial effects. The aqueous extract of *A. digitata* fruit was tested to assess the biological activities. The result showed an anti-metabolic syndrome expressed through anti-inflammatory, weight loss, hypoglycemic, hypolipidemic, cardio, hepatic, renal-protective activities (Suliman et al., 2020). However, Another study indicates that baobab fruit pulp extract could be considered a cardioprotective agent against isoproterenol, which induces oxidative stress in rats (Ghoneim et al., 2016). It also reduces the glycaemic reaction and enhances many other metabolic parameters due to its polyphenolic components (Coe et al., 2013). The antidiabetic effect and hypoglycemic activity of fruit pulp extract have been extensively studied to evaluate the efficacy, and researchers agreed that it showed proven antidiabetic activity (Ahmed et al., 2019; Muhammad et al., 2016; Bako et al., 2014). Several reports conclude that baobab fruit

extract has a significant protective effect on a rat liver (Khamis et al., 2020; Hanafy et al., 2016). Moreover, baobab fruit aqueous extract has many uses, as a cosmetic agent to treat skin pigmentation (Zeitoun et al., 2020). The authors also summarized that *A. digitata* fruit-pulp had shown antiviral activity against herpes simplex virus, respiratory syncytial virus and influenza virus (Selvarani, 2009) and polio (Anani et al., 2000).

The aqueous bark extract of AD has also been investigated to obtain biological activities; it has an antidepressant role in enhancing the mice model's behaviour (Shehu et al., 2019). Moreover, a recent study stated that it could cure the pain as an analgesic agent in Wistar rats (Owoyele and Bakare, 2018). It also showed antihypertensive and antioxidant effects on a rat model (Ntchapda et al., 2020) and antimalarial activity (Adeoye and Bewaji, 2018; Musila et al., 2013).

On the other hand, an aqueous leaf extract of AD showed ameliorative effects in many studies. It can reduce oxidative stress in lead acetate-induced cortical neurodegeneration (Atuadu et al., 2020). However, it is a good antioxidant with the inhibitory properties of alpha-glucosidase, alpha-amylase and aldose reductase and may also be useful in treating type 2 diabetes (Ironi et al., 2017). Moreover, it has strong antidiabetic and excellent hypolipidemic properties (Ebaid et al., 2019; Adegoke et al., 2017), an antinociceptive effect (Mumtaz et al., 2017). Concurrently, it has been reported that it can cure infectious diseases caused by Gram-negative bacteria (Djeussi et al., 2013). In like manner, the seed extract of AD has also been evaluated; the reports declared that it has also been scientifically proved for its hepato-protective activity (Cao et al., 1993), antioxidant activity (Al-Juhaimi et al., 2020; Khamis et al., 2020).

Traditionally, various parts of AD tree have been consumed in nutrition and folk medicine in Africa and other countries like India to manage several clinical conditions, including dysentery, diarrhoea and cardiovascular disorders (Rahul et al., 2015). The previous studies brought out information about consumption of the baobab fruit pulp as a panacea to cure almost any disease, such as fever, diarrhoea, malaria, tuberculosis, microbial infections, toothache, anaemia, and dysentery, etc. (N'guessan et al., 2010; Nguta et al., 2010; Van Wyk and Gericke, 2000; Breyer-Brandwijk, 1962). Likewise, baobab fruit pulp used as an immunostimulant (Bako et al., 2014). In Mali, it was well

known since a hundred years back the baobab fruit pulp flour is also used to stimulate milk production in breastfeeding women and treat children's diarrhoea (Braca et al., 2018). It is documented in India that it has external use to treat some illnesses as the baobab dried baobab leaves crushes and use to relieve the pain. In contrast, the pulp is used by mixing it with buttermilk for dysentery and diarrhoea (Sidibe, 2002).

2.4.2. Extra-Virgin Olive Oil (EVOO)

Among all the healthy diets recommended by many food experts globally, the Mediterranean diet is highly recommended as a dietary role model with high-fat content, especially with monounsaturated fatty acids (MUFA) like olive oil. Olive oil is the center of the Mediterranean food pyramid. It is a significant vital food due to its high nutritional value and an essential source of dietary fats, preferably extra virgin olive oil (EVOO) (Bach-Faig et al., 2011; Puertollano et al., 2010). Virgin olive oil consists of hundreds of micronutrients, notably antioxidants, as phenol compounds, carotenes and vitamin E (Owen et al., 2000).

The primary source of olive oil is the olive tree's fruit (*Olea Europaea L.*) and produced by an industrial process using some solvents. The olive oil production process begins with the olive fruit powder to extract the oil, then mixing the olive paste until it is combined in the liquid phase, then the separation phase using centrifugation or filtration and pressure. The temperature can be increased during the process stages that can determine the class of olive oil. The detailed olive oils taxonomies and sub-classes are lampante olive oil, olive-pomace oils, olive oil, refined olive oil, virgin olive oil (VOO) and EVOO, as shown in Figure 2.6. EVOO extracted from olive fruits based on a particular preparation protocol that does not change the properties and composition. According to the standards of European Union regulations, the oleic acid component determines the oil's acidity, and EVOO must have free acidity (no more than 0.8 grams of oleic acid per 100 grams of olive oil) (Aparicio-Soto et al., 2016).

For human consumption purposes, The EVOO components give a unique taste and flavour, differentiating it from other oils that need the refining process, leading to the loss of the vast majority of these minor components. On the other hand, EVOO is unprocessed and unrefined, which considered a real juice and one of the most highly

appreciated edible oils both for important gastronomic qualities and its nutritional values (Yubero-Serrano et al., 2019).

The interest in their biological and potential therapeutic effects is enormous. However, a large body of reports has indicated a close relation between minimized morbidity or mortality and EVOO intake, a lower incidence of neurodegenerative diseases, certain types of cancer, cardiovascular illnesses (stroke, hypertension or coronary cases), and other chronic and metabolic diseases (such as metabolic syndrome such or type 2 diabetes mellitus) (D'Alessandro and De Pergola, 2015; Solfrizzi et al., 2011; López-Miranda et al., 2010). It is well-known that the EVOO comprehend a high amount of MUFA, especially oleic acid, up to 80% of its total lipid composition (Tripoli et al., 2005). Compared to Polyunsaturated Fatty Acids-enriched oils, The MUFA (EVOO) molecule has only one double bond, which reveals its strong antioxidant property, long shelf life and its high stability (Owen et al., 2000). Secondary metabolites of plants available in our diet are primary phenols and polyphenols, including vegetables, fruits, olive oil, tea and wine (Rodríguez-Morató et al., 2015). The polyphenols' effect against neurodegenerative diseases was also mostly documented; they have been shown to have significant neuroprotective effects (Angeloni et al., 2017; Rodríguez-Morató et al., 2015; Khalatbary, 2014).

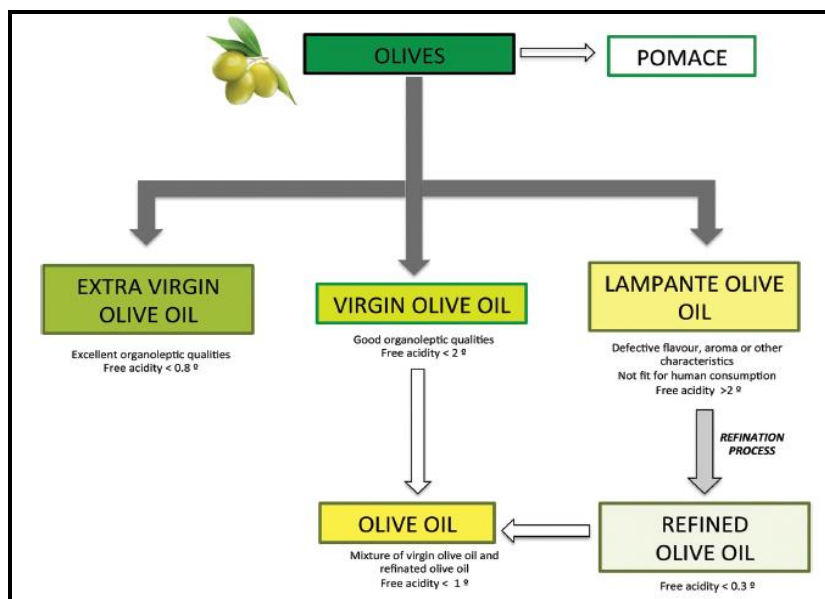


Figure 2.6. Olive oils classification and categories. Modified images from (Aparicio-Soto et al., 2016)

2.4.2.1. Chemical Composition of EVOO

Based on the mechanical preparation process, olive oils are different from other vegetable oils. Notably, EVOO components also differ due to several factors: processing method, geographical area, and growing conditions. EVOO consists of two main components: 90-99% of the oil is glycerol (saponifiable) as the main fraction, and two minor sub-fractions comprised of a phenolic fraction in addition to a non-saponifiable fraction such as; pigments, sitosterols, squalene, and triterpenes that known as a fraction which extracted with solvent (Fitó et al., 2005; Lercker and Rodriguez-Estrada, 2000). Most of the components of the glycerol portion (saponifiable) are fatty acids such as MUFA and oleic acid, which is up to 80% of the lipid content. In comparison, polyunsaturated omega-6 and omega-3 fatty acids (mainly linoleic acid) make up to 3-22% of total content, and about 8-26% of saturated fatty acids; primarily palmitic acid (Quintero-Flórez et al., 2015; Del Coco et al., 2013; Sacchi et al., 1998).

The authors also summarized that more than 30 phenolic constituents are found in olive oil with many variations in concentration and composition with either one or two hydroxyl groups. They are categorized into three types: tyrosol (4-hydroxyphenylethanol) and oleocanthal, simple phenols like hydroxytyrosol (HT) (3,4-dihydroxyphenolethanol) and secoiridoids such as oleuropein (3,4-dihydroxyphenylelenolic acid), and lignans (Cicerale et al., 2012; Waterman and Lockwood, 2007; Vissers et al., 2004). Another study reported that the polyphenols are classified based on the molecular structure into non-flavonoids such as saponin, flavonoids such as flavonols, and isoflavonoids (Simonyi et al., 2005). In the best condition, the content of the phenolic compounds in EVOO can reach up to 60 mg / 100 g (Rigacci and Stefani, 2016). However, all of them have shown strong antioxidant properties (Scoditti et al., 2014). These compounds revealed the beneficial properties of EVOO exclusively at cardiovascular illnesses (Scoditti et al., 2014; Storniolo et al., 2014). There are substantial pieces of evidence that indicate the potential positive properties of EVOO and its phenolic components especially HT in prevention of damage by oxidative stress as cardio- and neuro-protective agent due to it possesses antioxidant,

anti-inflammatory, anti-cancer properties (Robles-Almazan et al., 2018; Cicerale et al., 2012; Tasset et al., 2011; Carrasco-Pancorbo et al., 2005) (Table 2.3).

Table 2. 3. Shows the basic composition of EVOO. It is modified from (Yubero-Serrano et al., 2019)

<i>Triacylglycerols</i>
Oleic acid (C18:1) (55–83% of EVOO)
Linoleic acid (C18:2) (3.5–21% of EVOO)
Palmitic acid (C16:0) (7.5–20% of EVOO)
Stearic acid (C18:0) (0.5–5% of EVOO)
Linolenic acid (C18:3) (specifically α -linolenic acid, 0.1–1.5% of EVOO)
Non-glyceric esters
Tocopherols
Sterols
Terpenic acids
Pigments
Chlorophylls
Carotenoids
<i>Polyphenols</i>
Hydroxytyrosol
Oleuropeine
Tyrosol
Caffeic acid
Ligstroside
Vanillic acid
Hydroxytyrosol esters

2.4.2.2. Biological Activities of EVOO

EVOO has attracted the interest of several researchers; however, epidemiological data indicate that certain antioxidants nutrients like vitamin B, E and Polyunsaturated fatty acids (Roberts et al., 2010) in addition to some foods such as vegetables (such EVOO), fruits, wine and fish (Polidori et al., 2009) could grant a therapeutical effect in a cognitive disease like Alzheimer's Disease (Batarseh and Kaddoumi, 2018; Grossi et al.; Valls-Pedret et al., 2012; Williams and Spencer, 2012). Likewise, a broad range of studies has shown the neuroprotective effects of EVOO and its phenolic components; such as HT and oleuropein on brain damage (Amel et al., 2016), cerebral hypoxia-reoxygenation (González-Correa et al., 2008), against Huntington's disease (Tasset et al., 2011), damage of the brain after hypoxia-reoxygenation in diabetic rats model (De La Cruz et al., 2010), cerebral ischemia (Mohagheghi et al., 2010; Bu et al., 2007), ageing

(Casamenti and Stefani, 2017; Khalatbary, 2013; Farr et al., 2012; Pitozzi et al., 2012; Pitozzi et al., 2010), multiple sclerosis (Weinstock-Guttman et al., 2005), peripheral neuropathy (Ristagno et al., 2012) and spinal cord injury (Impellizzeri et al., 2012; Khalatbary and Zarrinjoei, 2012; Khalatbary and Ahmadvand, 2011). D, Angelo et al. summarized those polyphenols of EVOO could have the ability to cross the blood-brain barrier. Bearing the mentioned considerations in mind, the scientific knowledge in addition to in vitro and in vivo studies regarding the efficiency of EVOO and its phenolic components on PNS indicate that it can prevent the diabetic neuropathy (Ristagno et al., 2012), prevent the neurotoxicity (Qusa et al., 2020), induce the human SCs proliferation that could enhance the nerve regeneration in vitro study (Kamil et al., 2020). Moreover, the interest in their biological and potential therapeutic impacts that counteract body disorders is enormous; especially cardiovascular illness (D'Agostino et al., 2020; George et al., 2019; Vazquez et al., 2019; Covas et al., 2015; Villarejo et al., 2015; Williams and Spencer, 2012; Perona et al., 2006) and other disorders including type 2 diabetes, obesity, liver steatosis and/or further liver tissue damage, cancer, atherosclerosis, metabolic syndrome, amyloid and neurological diseases (Rodríguez-Morató et al., 2015; Frankel, 2011). There is substantial evidence that indicates the ameliorative impacts of EVOO on lung injury (Ghorbel et al., 2016) and hepatic oxidative stress (Khayyat, 2016; Rincón-Cervera et al., 2016) in addition to damage of bone marrow cells (Evangelista et al., 2004).

A considerable body of evidence reported that chronic consumption of EVOO in the diet has potential therapeutical effects on the inflammatory diseases by pro-inflammatory proteins reduction or downregulation of the pro-inflammatory gene expression that minimizes the total plasma/serum concentration of pro-inflammatory markers in both postprandial and chronic levels (Lucas et al., 2011). The authors also summarized that EVOO could play a vital role in reducing interleukin-6, C-reactive protein and other pro-inflammatory interleukin levels in high cardiovascular risk (Estruch, 2010; Mena et al., 2009). In addition, the effect of EVOO on high-cholesterol-diet-induced dyslipidemia in rats hearts, which increased tumour necrosis factor- α and malondialdehyde levels, have also been studied; the finding was that EVOO could

improve the heart function of rats by decreasing tumour necrosis factor- α and malondialdehyde levels (Katsarou et al., 2016).

2.4.3. Essential Oil of *Ocimum basilicum* (EOOB)

Ocimum basilicum (OB), also known as sweet basil and Rihan (in Arabic), considered an aromatic annual plant, a kitchen herb is belonging to the Lamiaceae family *Ocimum* genus, aromatic spices that is very common in subtropical and tropical areas of African, Asian and South America countries (Kwee and Niemeyer, 2011; Loughrin and Kasperbauer, 2001) as it is one of the essential herbal families of the plants that have biological and medical applications. The species of *Ocimum* genus are well-known among all medicinal plants, The aromatic spices are well-known family members such as basil, mint, rosemary, thyme, oregano, etc. (Bekut et al., 2018). OB has other uses especially in food like pasta sauces such as the Italian pesto, tomato-based dishes and in salads, in addition to perfumes production. As a medicinal plant, nutritional herb, and anti-inflammatory agent, it has been cultivated by local citizens. Since ancient time in folk medicine, different sweet basil extracts are used to cure several health problems such as skin infections, antispasmodic, parasiticide, antiseptic, dysentery, haematuria, inflammation, kidney conditions (Zeggwagh et al., 2007; Duke, 1985). A growing body of reports revealed that OB has beneficial activities against neurological diseases like sedation, anxiety and depression due to antioxidant and anti-inflammatory properties (Sestili et al., 2018).

OB is rich in essential oils that have been studied extensively in chemical studies (Hammer et al., 1999; Grayer et al., 1996). Since the Middle Ages, the essential oil that defined as a volatile and odorous form of the plant after secondary metabolism, is used as a known item in modern cosmetic, food, pharmaceutical industries, and aromatherapy in folk medicine. The therapeutical impacts of OB and its active components like ursolic acid have been carried out in cognitive defects on different animal models (Singh et al., 2018; Liang et al., 2016; Wang et al., 2016). Fandohan. et al. (2007) mentioned that EOOB diluted in corn oil in appropriate concentrations could be considered safe for human consumption (Fandohan et al., 2008).

2.4.3.1. Chemical Composition of EOOB

Essential oil is extracted from the aromatic plant by the hydrodistillation method. The major chemical component of essential oil is oxygenated minor products (terpenoids), terpenes, aliphatic and aromatic products that obtained from phenol (Abad et al., 2012). The literature states that some commercial form of essential oils rich in terpenes has shown antioxidant capacity (Aazza et al., 2011). Besides, other report investigates the chemical constituents of EOOB and states that the most dominant constituents of EOOB are phenylpropanoids (methyl chavicol, methyl cinnamate), acyclic alcoholic monoterpenes (nerol, linalool, geraniol), acyclic aldehyde monoterpene (geranial, neral), cyclic ether monoterpenes (1, 8-cineole), and hydrocarbon bicyclic sesquiterpene (caryophyllene) (Karar and Kuhnert, 2017; Kavooosi and Amirghofran, 2017).

2.4.3.2. Biological Activities of EOOB

The significant number of evidences reported several biological properties of OB, including antioxidant, antimicrobial, antimalarial activities (Flanigan and Niemeyer, 2014; Ntonga et al., 2014; Zheng and Wang, 2001). These protective properties might refer to essential oil constituents, such as methyl chavicol, methyl cinnamate, eugenol, camphor, and linalool. There is a growing body of in vivo and in vitro studies to prove that EOOB has radical scavenging and antioxidant activities in treat oxidative damage associated with some inflammatory cases (Kavooosi and Amirghofran, 2017; H. Li et al., 2017; Rodrigues et al., 2016; Tshilanda et al., 2016). However, it's ameliorative effect as a neuroprotective agent to combat several CNS disorders has been reported; it enhanced memory after ischemic stroke (Singh et al., 2018), it cured Multiple sclerosis (Garabadu and Singh, 2020), antiepileptic effect (Tsyvunin and Shtrygol, 2015; Oliveira et al., 2009; Ismail, 2006).

Besides, EOOB could combat with various disorders such as hypertension and type 2 diabetes (Ademiluyi et al., 2016), liver fibrosis (Ogaly et al., 2015), acute otitis media (Kristinsson et al., 2005). A previous study revealed that EOOB could use as an

enhancer to promote skin drugs absorption (Fang et al., 2004). EOOB extracted from leaf shown antinociceptive effects in rodents (Venâncio et al., 2011).

A relevant report to the current study had stated that EOOB, and its major component (-) -Linalool showed anti-excitability activity on the sciatic nerve after trauma (Venancio et al., 2016). Moreover, another *Ocimum* species is *Ocimum sanctum* has been investigated to assess its effect on sciatic nerve transaction, which induce neuropathy in rats model, and the authors summarized that it has a potential curable impact and improve the regeneration (Muthuraman et al., 2008).

2.5. Stereology

2.5.1. Basic Concepts of Stereology

Over the last 30 years, a substantial advance has been made about introducing stereology in the biomedical field (Geuna, 2005). The term stereology is derived from the Greek word stereo, which means something solid. Thus, stereology is defined as a morphometric (multidisciplinary) science that allows actual measurement of the objects with 3D features based on the data obtained from their 2D biological structure images or sections, despite size, shape, orientation, by using several techniques in addition to statistical sampling strategies (Kaplan, 2018; Deniz et al., 2018; Yurt et al., 2018; Onger et al., 2017). Accordingly, the planar sections passing through the biological structures such as organs can represent all components with different orientations and size in the organ as sectional profiles (Altunkaynak et al., 2012) (Figure 2.7).

In old decades, quantitative techniques such as assumption-based counting have been employed in studies about the estimation of cells number inside the organs. Moreover, it is essential to mention that some information about an object, like real dimensions, would be lost during the sectioning stage. Based on this, the scientific knowledge available indicates that these approaches have a wide range of bias due to lack of accuracy and lack of matching with new techniques such as 3D counting. Therefore, stereology in quantitative studies would be highly recommended (Altunkaynak et al., 2012; West, 1999).

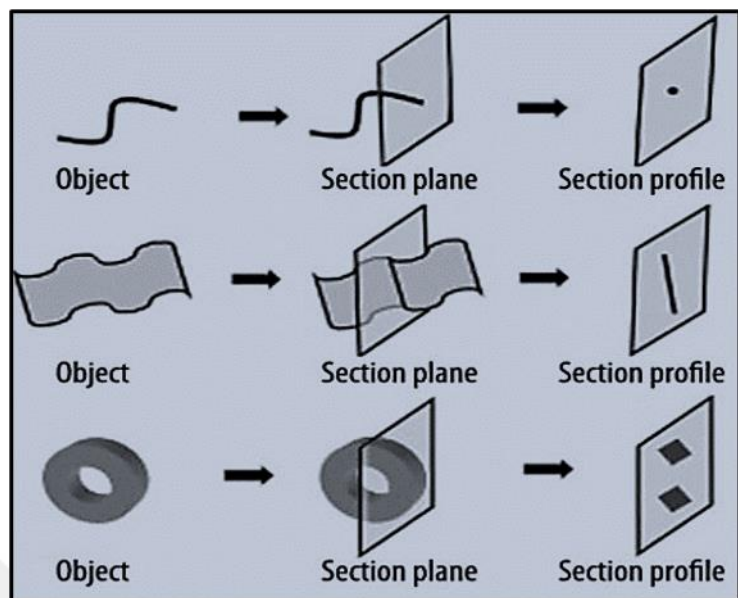


Figure 2.7. The sectional planes passed through the different dimensional objects to represent the objects as a sectional profile with respect to changed dimensions. Modified image from (Altunkaynak et al., 2012)

Since the starting of morphological studies, several types of methods were used. Furthermore, a considerable percentage of results errors in each method have raised. Nowadays, for correction purposes of these method-based errors, new approaches have been explored. Special attention has been given to stereology methods due to their widespread application and reliability in quantization (Gundersen et al., 1999; West, 1993; Gundersen et al., 1988). Moreover, in this manner, Computers and auxiliary equipment are needful to achieve the reliable and rapid use of stereological methods, that a new creative aid to researchers (Canan et al., 2004; Kaplan et al., 2001).

In clinical studies, to extract quantitative data, a stereological analysis was performed. In recent years, a quantitative analysis of the degeneration and regeneration of PNS and CNS has attracted the attention of several authors (Ehrenberg et al., 2017; Ardalan et al., 2016).

2.5.2. The Nucleator

For estimating the volume of small particles such as organelles of the cell, another stereological tool is used known as the nucleator; the two steps method is employed to calculate the volume. For instance, the nucleator used in peripheral nerves studies to

estimate the myelin thickness and axonal area (Onger et al., 2017) (Figure 2.8). Furthermore, in the first step, dissector sampling is used. Then, the centre of the determined object is pointed to recognize the nucleator algorithm. It is worth mentioning that this unique tool should be applied in stereological computer-workstation (Kaplan, 2018; Altunkaynak et al., 2012).

2.5.3. Fractionator

The fractionator is defined as a design to select the samples linked to any different stereological assessments to get unbiased results when estimating the object. There are two types of fractionator; optical and physical (Gundersen et al., 1988).

2.5.3.1. Optical Fractionator

This particular fractionator type is a useful method of obtaining the total quantity of layered structure such as nervous tissue by using optical dissector counting and the systematic random sampling method. Hence, the total amount can be estimated by multiplying the sampling ratio with counted particles (Turgut et al., 2007).

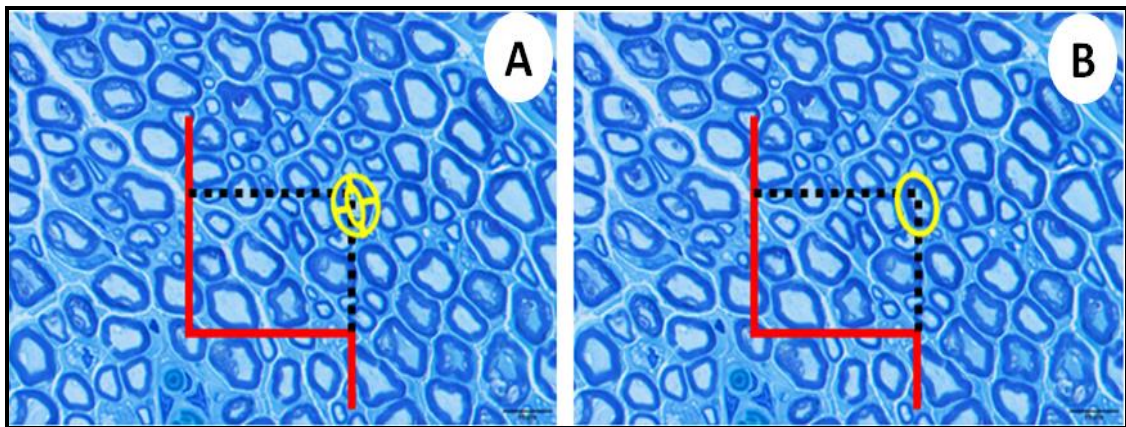


Figure 2.8. Shows Nucleator tool in estimating myelin sheath thickness (A) and axonal area (B)

3. MATERIALS AND METHODS

3.1. Animals of the Experimental Study

At Ondokuz Mayıs University (Samsun city, Turkey), this study was conducted in the Animal Experimental Research Centre. The Ethical Committee and Animal Care of the institute of Health Science in the Ondokuz Mayıs University approved this study on 14/01/2019 with the number 2019/01. According to the regulations and guidelines of the ethical committee, the experimental process was conducted carefully. In this study, experimental animals were provided by the Animal Experimental Research Centre of the Ondokuz Mayıs University. Sixty-four healthy adult male *Wistar albino* rats, their weight was 260 ± 30 g with 12 to 14-weeks-old were used in this study. Rats were housed in stainless steel cages with a 12-h dark-light cycle at room temperature (22 ± 2 °C) and humidity (50-60%). Animals were free to reach food and water (Figure 3.1).



Figure 3.1. Animals of our experimental study in the stainless-steel cages (male Wister albino rats)

3.2. Animals Groups Design

Sixty-four healthy adult male *Wistar albino* rats employed in this study. The rats were divided randomly into eight groups, with eight rats in each group. The group was designed as follow:

Group 1: Control group (Cont) (n= 8)

Group 2: Injury group (Inj) (n= 8)

Group 3: Inj + AD group (n= 8)

Group 4: Inj + EVOO group (n= 8)

Group 5: Inj + EOOB group (n= 8)

Group 6: AD group (n= 8)

Group 7: EVOO group (n= 8)

Group 8: EOOB group (n= 8)

3.3. Procedure of Crush Surgery

The surgical procedure has performed in standard condition, after anaesthesia with an intraperitoneal route of administration of 50 mg/kg ketamine (Ketalar[®], Eczacibasi, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun[®], Bayer, Istanbul, Turkey); the hair was shaved from the back of the right thigh. The medial side of the right thigh was shelved, and a longitudinal incision was applied on the back of the thigh. The right sciatic nerve was exposed from the sciatic notch to the site of bifurcation. The nerve freed from the surrounding tissue then crush injury was performed using standardized clamp forceps (Bahadır, Samsun, Turkey); the crash site was 5 mm distal to the sciatic notch, the crush force was 50 Newton, and the crush duration was 60 seconds (sec) (Torul et al., 2018) (Figure 3.2).

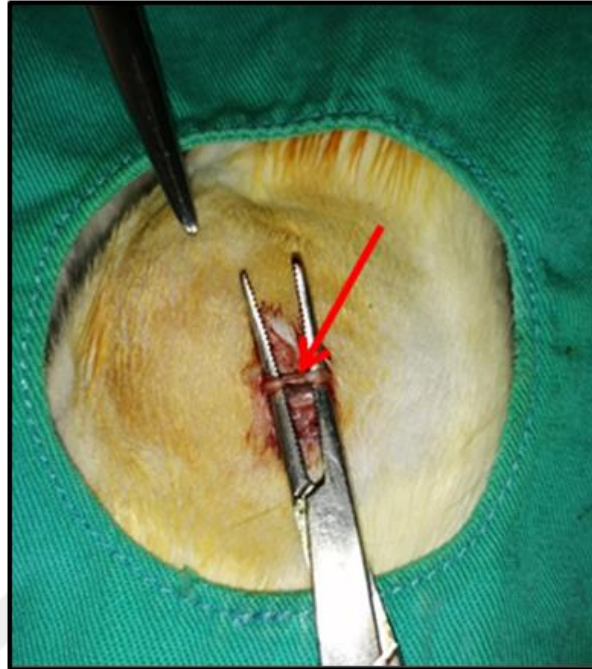


Figure 3.2. Shows the sciatic nerve after applying crush injury by using clamp forceps

3.4. Postoperative Care

After application of crush surgery, the skin was sutured back, and the experimental rats were treated immediately with antibacterial spray Pederipra (Hipra, Amer, Girona, Spain) that was applied directly to the incision area for prevention of wound infection purposes. Moreover, animals were treated with an intramuscular injection of antibiotic (Sefazolin sodium Eqizolin 1g IM/IV) for three days after surgery; the doses were 4mg/kg, 2mg/kg and, 1mg/kg, respectively for infection management (Figure 3.3).

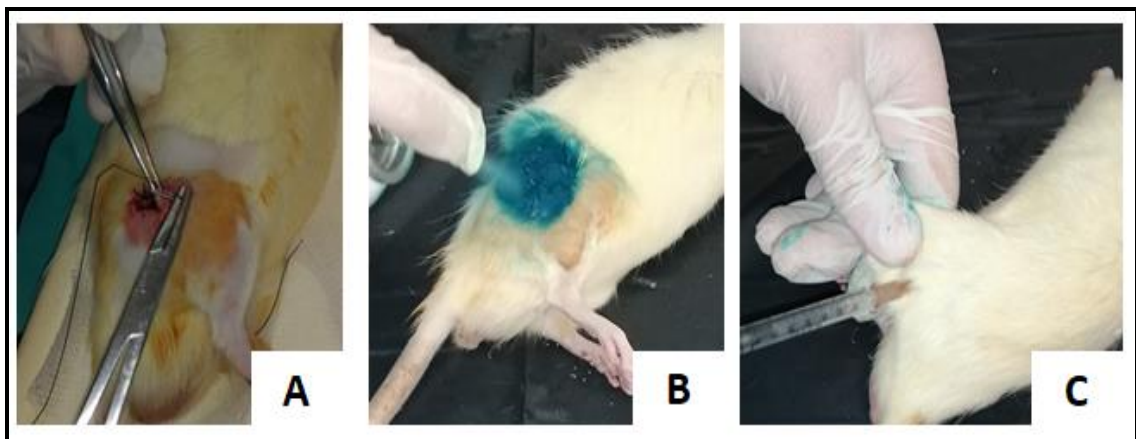


Figure 3.3. A. Skin suturing, B. Antiseptic spray applied directly on the site of surgery

3.5. Experimental Animals Groups

The experimental animals were separated into eight groups equally; each group was kept in a separate cage; the 50 Newton force of crush model of injury with duration of 60 sec was applied to injury groups. After the study's duration (21 days) and under anesthesia, sciatic nerves were harvested. The details of the experimental animals are given in (Table 3.1.).

Group 1: Cont group

The experimental animals of this group were not exposed to any particular protocol; they were left in the cage over 21 days; the experiment's duration and the animals were free to reach food and water.

Group 2: Inj group

The right sciatic nerve of this group's experimental animals was exposed from the sciatic notch to the site of bifurcation; crush injury was applied at force 50 Newton for 60 sec (Torul et al., 2018). Then, the skin sutured back, and the animals were left in their cage for 21 days.

Group 3: Inj + AD group

In this group, the right sciatic nerve was exposed, and crush injury was applied. The duration of the crush injury was 60 sec at force 50 Newton. After that, animals were treated with AD at dose of 400 mg/kg dissolve in 1 ml of distilled water via oral gavage every day for 10 days (Suliman et al., 2020).

Group 5: Inj + EVOO group

In this group, the right sciatic nerve was showed, and crush injury was achieved; the crush's duration was 60 sec at force 50 Newton. This group's animals were then treated with 2 mL/kg EVOO through oral gavage every day for 10 days (Khayyat, 2016).

Group 6: Inj + EOOB group

In this group, the right sciatic nerve of the experimental animals was exposed. Crush injury was made for 60 sec at force 50 Newton, and then the animals were treated

with EOOB at dose of 1500 mg/kg of EOOB diluted in 2 ml/kg of corn oil through oral gavage every day for 10 days (Fandohan et al., 2008).

Group 7: AD group

Animals in this group were treated with AD 400 mg/kg dissolve in 1 ml of distilled water through oral gavage every day for 10 days. Animals of this group were not exposed to any surgical operation of crush injury.

Group 8: EVOO group

The experimental animals in this group were treated with EVOO at dose of 2 mL/kg with oral gavage every day for 10 days. Neither surgical operation nor crush injury was performed in this group.

Group 9: EOOB group

In this group, the experimental animals were not exposed to any surgical operation or crush injury; the animals only were treated with 1500 mg/kg of EOOB diluted in 2 ml/kg of corn oil via oral gavage every day for 10 days.

3.6. Treatment Preparation, Doses and Route Of Administration

AD fruit pulp powder was purchased from the local market, Khartoum, Sudan (Figure 3.4). The plant was authenticated and identified by a specialist in the Food Engineering department, Engineering Faculty, Ondokuz Mayıs University. The Aqueous extract of AD was prepared every week; each 400 mg/kg of the AD fruit pulp fine powder was accurately weighed, then immersed in 1 ml of distilled water for 1 hour, then filtrated by Cotton Gauze Swabs and kept in the refrigerator (4°C) (Bakare et al., 2019) till administration time to experimental animals, it was orally administrated via oral gavage (Suliman et al., 2020). EVOO was obtained from the local market, Samsun, Turkey (Trade and Industry Kontuvarı TAŞ- Crystal oils®). It was orally administrated via oral gavage (4 ml/kg/day) for 10 days (Khayyat, 2016) (Figure 3.5). EOOB was also purchased from the local market, Samsun, Turkey (Botalife®), it was orally administrated via oral gavage each 1500mg/kg diluted in 2 ml/kg of corn oil (Evin®, Turkey) per day for 10 days (Fandohan et al., 2008) (Figure 3.6).



Figure 3.4. AD fruit pulp powder



Figure 3.5. A. shows oral administration via oral gavage, B. shows EVOO substance

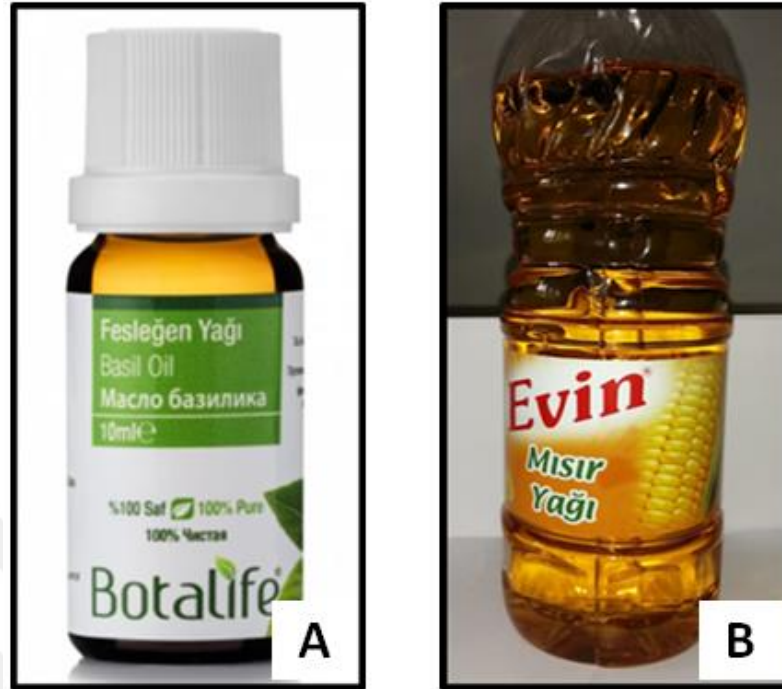


Figure 3.6. A. Shows EOOB substance, B. shows corn oil

Table 3. 1. Details of the experimental groups

Group	Details of the group
Cont	This group was not exposed to any particular protocol.
Inj	The right sciatic nerve was exposed to crush injury at force 50 Newton for 60 sec.
Inj+AD	The right sciatic nerve was exposed to crush injury at force 50 Newton for 60 sec, then treatment with AD (400 mg/kg) dissolve in 1 ml of distilled water through oral gavage every day for 10 days.
Inj+EVOO	The right sciatic nerve was exposed to crush injury at force 50 Newton for 60 sec, then treatment with EVOO (2 mL/kg) with oral gavage every day for 10 days.
Inj+EOOB	The right sciatic nerve was exposed to crush injury at force 50 Newton for 60 sec, then treatment with EOOB (1500 mg/kg) diluted in 2 ml/kg of corn oil via oral gavage every day for 10 days.
AD	Treatment with AD (400 mg/kg) dissolves in 1 ml of distilled water through oral gavage every day for 10 days.
EVOO	Treatment with EVOO (2 mL/kg) with oral gavage every day for 10 days.
EOOB	Treatment with EOOB (1500 mg/kg) of diluted in 2 ml/kg of corn oil via oral gavage every day for 10 days.

3.1. Functional Evaluations

3.7.1. Sciatic Functional Index (SFI) and Walking Track Analysis

On the day of 21th, walking track analysis was applied to assess the SFI in all groups. This approach preformed to evaluate the hind limbs' motor activity recovery. The hind limbs of the rats were stained with blue ink, and the animals were allowed to walk along a corridor, A 3 papers were placed to receive the footprint of the hind limbs. The distance between the second toe to the fourth toe (middle toe spread) (IT), the distance from the first toe to the fifth toe (toe spread) (TS), and the distance from the heel to the middle toe (print length) (PL) were measured. In addition, parameters were measured for each rat's experimentally injured right foot (E) and normal left foot (N). The data collected were used to calculate the SFI using the formula created by (Bain et al., 1989) (Figure 3.7).

$$SFI = -38.3 \times \left(\frac{EPL - NPL}{NPL} \right) + 109.5 \times \left(\frac{ETS - NTS}{NTS} \right) + 13.3 \times \left(\frac{EIT - NIT}{NIT} \right) - 8.8$$

SFI and walking track analysis are used to examine the nerve injury severity and restoration of the rat's sciatic nerve; the score zero is deemed normal, whereas -100 demonstrates the nerve's motor activity loss.



Figure 3.7. Walking track and footprint paper of functional sciatic index

3.7.2. Electromyographic Evaluation (EMG)

We applied the EMG test as another functional examination of the sciatic nerve recovery. At the end of the study and before the sacrifice of the animals, rats were anaesthetized with an intraperitoneally using ketamine-xylazine (5-2 mg/kg) (Ketalar[®], Eczacibasi, Istanbul, Turkey) (Rompun[®], Bayer, Istanbul, Turkey) respectively. The right sciatic nerve of all groups was exposed from the sciatic notch to the bifurcation site. The surrounding connective tissue was freed to perform electromyography, which indicates the electrical potential of the skeletal muscles and motor recovery of the sciatic nerve. Power Lab4SP (AD Instruments, Sydney, Australia) and EMG Scope software (ver. 3.7.2, AD Instruments) were used. A signal voltage of 0.01 mV to 10 mV was used to measure the gastrocnemius muscle's action potential. On the sciatic nerve 10 mm proximal to the injury site, the bipolar stimulating electrode was positioned, three mV was the strength of the stimulus used. Gastrocnemius muscle was used to record compound muscle action potential (CMAP); the recording electrode was positioned at the muscle. CMAP peak amplitude, CMAP latency of onset and velocity of the nerve conduction were recorded and measured; the procedure was achieved three times, and the average was calculated and statistically analysed. The amplitude is defined as the

strength of the nerve impulse that reaches the muscle; on the other hand, the latency is the nerve impulse's time to get the muscle across the nerve.

3.8. Tissue Samples Processing Stage for Microscopic Examinations

On the sacrifice day, each animal was weighed, and the average weight of the animals was 260 ± 30 g. Then animals were anaesthetized with intra-peritoneal injection of 5 mg/kg ketamine (Ketalar[®], Eczacibasi. Istanbul, Turkey) and 2 mg/kg xylazine (Rompun[®], Bayer, Istanbul, Turkey). The medial side of the right thigh was shaved. A longitudinal incision was made on the back of the right thigh; the sciatic nerve was exposed from the sciatic notch to the site of bifurcation and freed from the surrounded connective tissue.

The nerve segment, including the crush injury site, was taken from the right sciatic nerve; before taking the animal's nerve, it was overwhelmed with glutaraldehyde 0.5%, then the nerve segment was removed by a sharp blade; immediately nerve segment was put in a tissue container containing fixative solution; glutaraldehyde 0.5%. Because of blood in the fixative solution, three hours later, the fixative solution was renewed by a new solution. Moreover, the renewal of the fixative solution was every four days during the whole fixation period; during that time, the nerve tissues were placed in the refrigerator at a temperature of 4 °C and left in the fixative solution for two weeks.

When the fixation has completed, nerve tissues were washed using 0.1-mole Phosphate Buffered Saline (PBS) (pH 7.4) four times 15 minutes in each; phosphate buffer was changed in every step. For wrinkling prevention, the tissues were washed in 0.2% glycine, and then the nerve segments were put in PBS again for four minutes. After completing of fixation step, nerve tissues were put in a solution of osmium tetroxide 2% osmium tetroxide (Sigma-Aldrich Co. LLC., St. Louis, USA), and the tissue was left in the solution overnight and kept in the dark area away from the light until the tissues became hard and dark black. Then nerve segments were washed using PBS (pH 7.4) (0.1) mole in four steps 15 minutes for each; in every step, PBS was changed. After washing, tissues dehydration was done using acetone in different concentrations 50% for 15 minutes, then 70% for 15 minutes, after that 95% for 15 minutes and finally 100% for 20 minutes two times. Following dehydration, the tissue

was put in propylene oxide for 20 minutes two times, and then the tissues were placed in a solution of propylene oxide 50% and Araldite epoxy resin 50% for one hour. Before blocking, the connective tissue surrounding the nerve was carefully removed using fine forceps; also the tissues were coded; therefore, the stereological assessments would be achieved blindly. At the last step, tissues were kept in the desiccator for one night, and then resin moulds were put in the oven at a temperature of 50°C; after that, the temperature was adjusted to escalate every 30 minutes until it finally reached 62 °C, then the blocks have kept in the oven for two days until blocks became ready for sectioning stage (Figure 3.8).

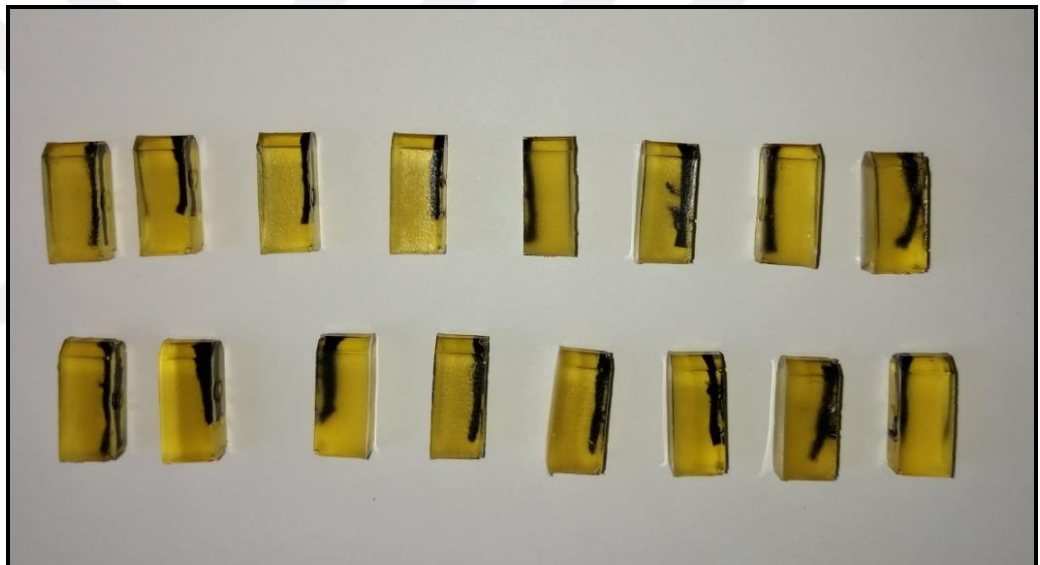


Figure 3.8. Right sciatic nerve tissue blocks

3.8.1. Sectioning Step

The nerve tissue blocks have been sectioned using the ultra-microtome; (Leica Ultra cut UCT, Leica Microsystems GmbH; Germany) (Figure 3.9.), and the glass blade was used to take semi-thin sections 500 nanometer (nm) for light microscope (LM) examination. Thin sections 70-80 nm for electron microscope (EM) evaluation, one section was taken from each animal for staining in preparation for stereological analysis. Semi-thin sections for LM evaluation were put in a slide and kept in the laboratory to dry.



Figure 3.9. Ultra-microtome (Leica Microsystems GmbH)

3.8.2. Staining Process

The toluidine blue was used to stain the obtained semi-thin sections. First, 1% solution of toluidine blue stain was prepared by dissolving 2g of sodium borate in 100 ml of deionized water; after that, 1g of toluidine blue was added, then the solution stirred until dissolved. The solution then was filtered using filter paper (pore size: 11 μm), and the solution was kept in an opaque bottle for two weeks at room temperature. Next, a drop of toluidine blue was added to the nerve tissue using a plastic pipette and left for 20-30 seconds; after that, the slide was dipped into a deionized water jar, and the procedure was repeated 3-4 times until the section was cleared. (Figure 3.10). Uranyl acetate and lead citrate were used to stain the thin sections for EM examination (JEOL STEM JSM-7001F, JEOL Ltd., Tokyo, Japan) applied in the research centre (KITAM) Ondukuz Mayıs University.

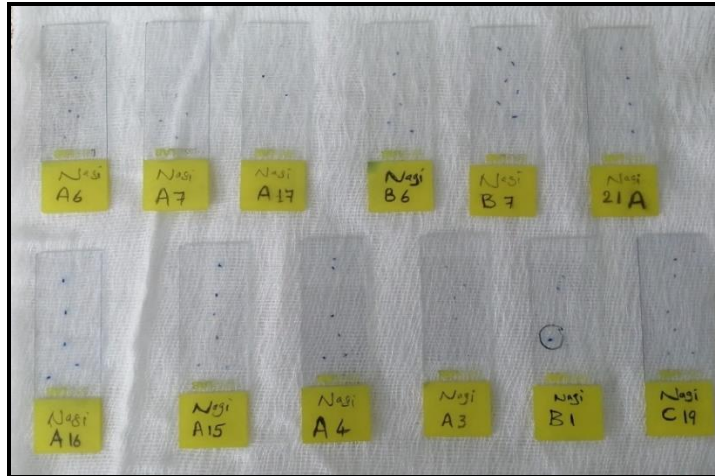


Figure 3.10. Sciatic nerve sections stained with toluidine blue

3.9. Stereological Analysis

Tissue sections of the sciatic nerve stained with toluidine blue were used for stereological analysis. Stereological analysis was applied to estimate the total number of the myelinated nerve axons through the light microscope using the fractionator method.

Semi-thin sections ($0.5\ \mu\text{m}$) underwent stereological analysis in the stereology work station at the Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayıs University, images obtained from the light microscope with magnification $\times 100$ assisted by camera attachment light microscope (Olympus, BX43, Center Valley, PA, USA) with a computer-aided cellSens Entry microscope software program (Olympus, Center Valley, PA, USA) (Figure 3.11).

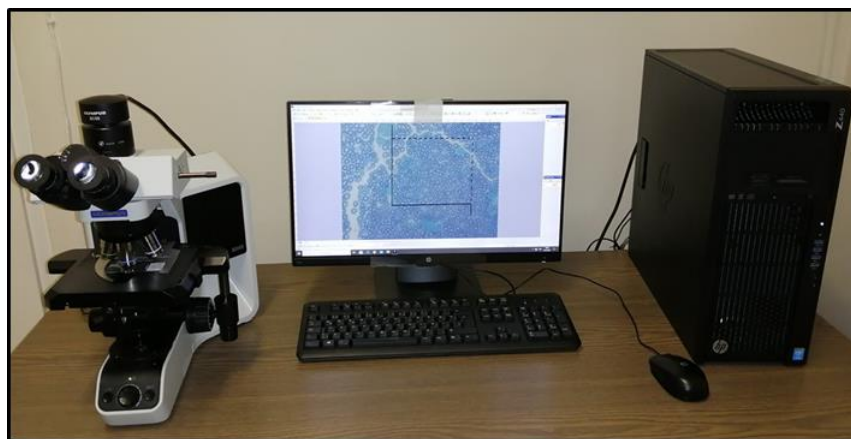


Figure 3.11. The system of stereological analysis

In the current study, the fractionator technique was applied to estimate the total number of the myelinated nerve axons; on the other hand, myelin sheath thickness and axonal area were measured using a nuclear probe (Nucleator). Counting of the myelinated axon number was done with the magnification of x100. The unbiased counting frame was used to count the myelinated nerve axons number (Figure 3.12). Nerve axons inside the counting frame or hitting the inclusion line were counted. Those who hit the exclusion line or located outside the counting frame were not counted; the axon at the upper right corner was selected to measure the axonal area and myelin sheath thickness. The unbiased counting frame has four boundaries: two inclusion lines and two exclusion lines with extension. The number of the myelinated axons counted was used to calculate the total number of the axons of the nerve with the application of the following formula:

$$\text{Total number of the myelinated nerve axons} = \frac{\text{Step size area (9469.43)}}{\text{Counting frame area}} \times \text{number of axons counted}$$

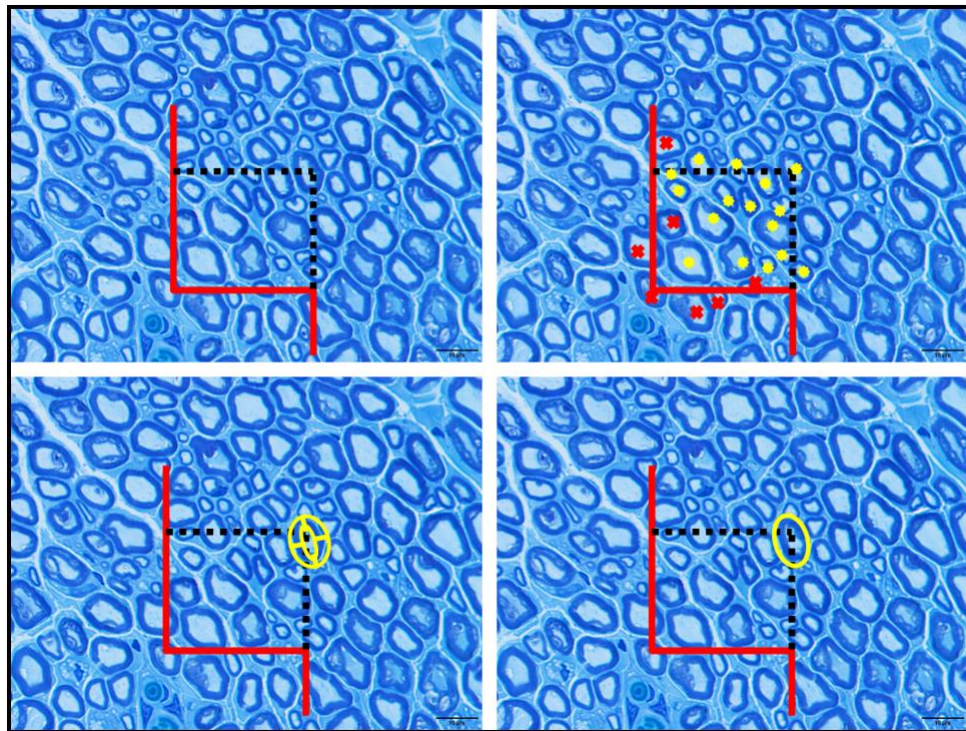


Figure 3.12. Stereological analysis using unbiased counting frame

3.10. Statistical Analysis

SPSS software program (version 22.0) was applied to analyse the data obtained from the recent study. Before analysis, a normality test was applied to select suitable statistics to analyse the current study's data. After performing the normality test, we found that the data for a mean of myelinated axon number, myeline thickness, SFI and amplitude were normally distributed; thus, we applied a One-way ANOVA test (Post-Hoc Test) to detect the degree of significance between groups. Whereas the data of the axonal area and latency mean were abnormally distributed, we employed the nonparametric test (Kruskal-Wallis Test) to explore the significant differences among the groups. P-value less than 0.05 was appreciated significant.

4. RESULTS AND DISCUSSION

4.1. Results

Our experimental study was conducted to explore the effectiveness of AD, EVOO and EOOB on the rat sciatic nerve healing after crush injury model. The crushing force was 50 Newton for 60 sec. The current study data has many parameters collected from stereological analyses. Regarding results of stereological techniques, we obtained numerical data on myelinated axons number, the myelin sheath thickness and the axonal area. After that, we analysed this data to examine the nerve regeneration after employed crush injury.

Furthermore, the data obtained from the physiological tests, which are the EMG and SFI, were analysed to evaluate motor recovery. In addition, the AD, EVOO and EOOB effect on the injured nerves was also examined using both light and electron microscopy in terms of cross-section images. The tables and graphs below were showed all the stereological and functional results of the current work.

4.1.1. Stereological Findings

The 2D fractionator technique was employed to estimate the total number of myelinated axons of the sciatic nerve. In comparison, the nucleator technique was applied to measure the axonal area and the myelin sheath thickness of myelinated axons. These three parameters were applied on the sciatic nerve of the Cont group, which was not exposed to injury or received treatment, the Inj group in which the sciatic nerve was crushed for 60 sec, in the Inj+AD that was exposed to AD following the crushing of the sciatic nerve for 60 sec, the Inj+EVOO group which was treated with EVOO after sciatic nerve was crushed for 60 sec, in the Inj+EOOB group that was exposed with EOOB after sciatic nerve was crushed for 60 sec, in the AD group which was only treated with AD, in the EVOO group which was only treated with EVOO and in the EOOB group which was only exposed to the EOOB.

4.1.1.1. Myelinated Axon Numbers Estimation

The myelinated axons mean number and the standard deviation (SD) are shown below in Table 4.1 and Figure 4.1.

Table 4. 1. Reveals the mean of myelinated axons number, the standard deviation (SD)

Groups	Mean Number of The Myelinated Axons \pm SD
Cont	6130.470 \pm 84.483
Inj	7203.671 \pm 145.016
Inj+AD	6646.356 \pm 209.693
Inj+EVOO	6434.958 \pm 147.017
Inj+EOOB	6476.859 \pm 272.368
AD	6107.782 \pm 105.474
EVOO	6172.885 \pm 109.961
EOOB	6136.388 \pm 72.138

The data obtained from the counting of the myelinated axons of the nerve was statistically analysed. Higher significant differences were detected between the Cont and Inj groups ($p < 0.01$), Cont and Inj+AD groups ($p < 0.01$). In addition, a significant difference was also found between Cont and Inj+EVOO groups ($p < 0.05$). Moreover, higher significant differences were observed among the Inj+AD and the Inj ($p < 0.01$), Inj+EVOO and the Inj ($p < 0.01$) in terms of myelinated axons number. In addition, the myelinated axons mean number of the Inj+EOOB group was significantly decreased than the Inj group ($p < 0.05$). Likewise, the Inj group was high significantly differed from AD, EVOO and EOOB groups ($p < 0.01$). On the contrary, no significant differences were found between the Cont and AD ($p > 0.05$), Cont and EVOO ($p > 0.05$), Cont and EOOB ($p > 0.05$) groups, respectively (Table 4.2).

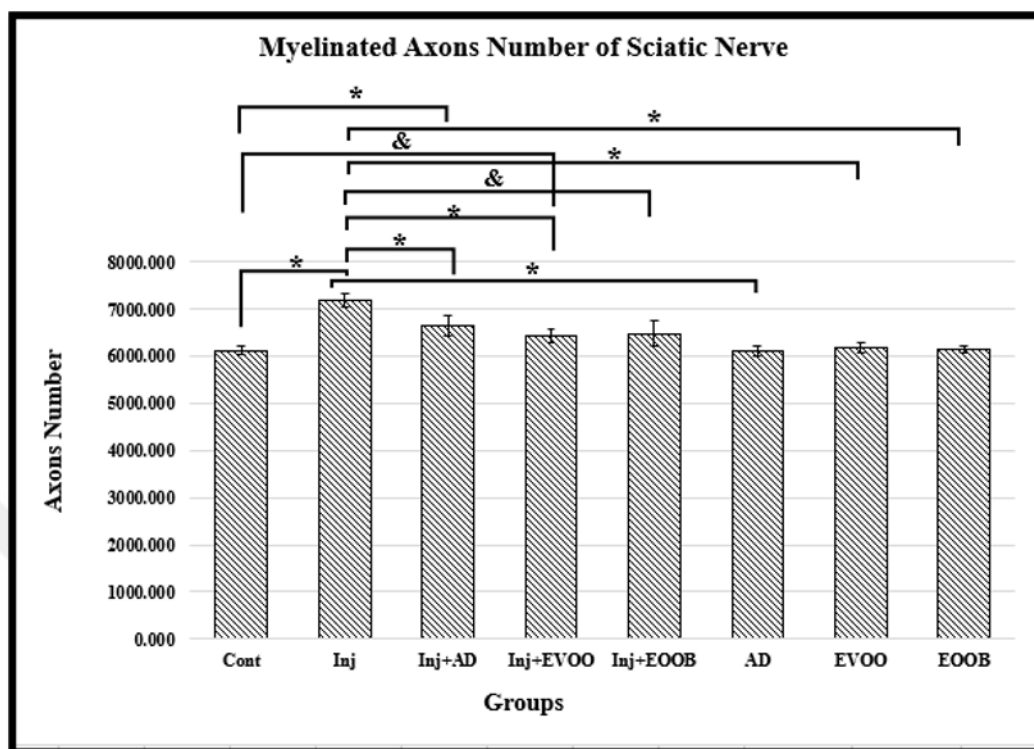


Figure 4.1. Exhibits the difference among the groups in term of myelinated axons mean number of the sciatic nerve. The level of significance at $p < 0.01$ is indicated by (*), while the significance level at $p < 0.05$ is indicated by (&)

Table 4. 2. Shows degree of significance among groups in terms of numbers of myelinated axon

Comparison between groups	Degree of Significance
Inj with Cont	0.000
Inj with Inj+AD	0.005
Inj with Inj+EVOO	0.000
Inj with Inj+EOOB	0.014
Cont with AD	1.000
Cont with EVOO	1.000
Cont with EOOB	1.000
Cont with Inj+AD	0.009
Cont with Inj+EVOO	0.012
Inj with AD	0.000
Inj with EVOO	0.000
Inj with EOOB	0.000

4.1.1.2. The Myelin Thickness Measurement

The myelin sheath thickness average of the sciatic nerve and the standard deviation (SD) are shown below in Table 4.3 and Figure 4.2.

Table 4. 3. The mean value of myelin thickness (μm) and the standard deviation (SD)

Groups	Mean Value of The Myeline Thickness (μm) \pm SD
Cont	1.869 \pm 0.269
Inj	0.592 \pm 0.057
Inj+AD	0.844 \pm 0.107
Inj+EVOO	0.469 \pm 0.143
Inj+EOOB	0.705 \pm 0.124
AD	1.935 \pm 0.052
EVOO	1.388 \pm 0.108
EOOB	1.558 \pm 0.309

Based on statistical analysis, which was employed to measure the myelin thickness of the myelinated axons. It was observed that the high significant alterations were appreciated among Cont and Inj ($p < 0.01$), Cont and Inj+AD ($p < 0.01$), Cont and Inj+EVOO ($p < 0.01$), Cont and Inj+EOOB ($p < 0.01$) groups. Furthermore, a significant increase was detected in the Inj+AD group compared to the Inj group ($p < 0.05$). The high significant differences were also found in comparing between Inj with AD ($p < 0.01$), Inj with EVOO ($p < 0.01$) groups. In contrast, the alteration was significantly different between Inj and EOOB ($p < 0.05$). On the other hand, no significant difference was found Inj+EVOO and Inj+EOOB groups compared to the Inj group ($p > 0.05$). Likewise, no significant difference was appreciated in comparison between the Cont and AD, EVOO, and EOOB groups in terms of mean myelin thickness ($p > 0.05$) (Table 4.4).

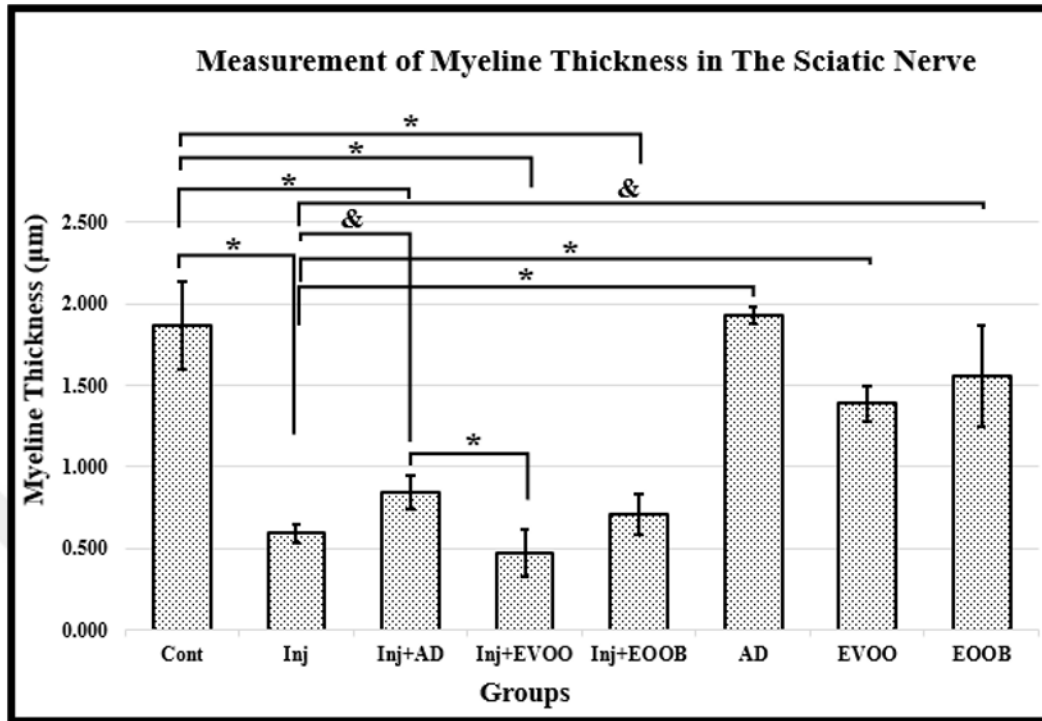


Figure 4.2. Shows the difference of myeline thickness mean in the sciatic nerve among the groups. The level of significance at $p < 0.05$ is indicated by (&), while the significance level at $p < 0.01$ is indicated by (*)

Table 4. 4. Shows degree of significance among groups in terms of the thickness of myeline sheath

Comparison between groups	Degree of Significance
Inj with Cont	0.001
Inj with Inj+AD	0.010
Inj with Inj+EVOO	0.783
Inj with Inj+EOOB	0.908
Cont with AD	1.000
Cont with EVOO	0.142
Cont with EOOB	0.936
Cont with Inj+AD	0.003
Cont with Inj+EVOO	0.000
Cont with Inj+EOOB	0.001
Inj+AD with Inj+EVOO	0.002
Inj with AD	0.000
Inj with EVOO	0.000
Inj with EOOB	0.014

4.1.1.3. Axonal Area Measurement

The mean axonal area in the sciatic nerve and the standard deviation (SD) are displayed below in Table 4.5 and Figure 4.3.

Table 4. 5. Presents the mean the axonal area and the standard deviation (SD)

Groups	Axonal Area Mean (μm^2) \pm SD
Cont	59.213 \pm 8.850
Inj	16.748 \pm 1.215
Inj+AD	16.731 \pm 1.962
Inj+EVOO	6.013 \pm 0.732
Inj+EOOB	15.968 \pm 2.526
AD	71.855 \pm 3.063
EVOO	59.676 \pm 7.806
EOOB	41.422 \pm 6.845

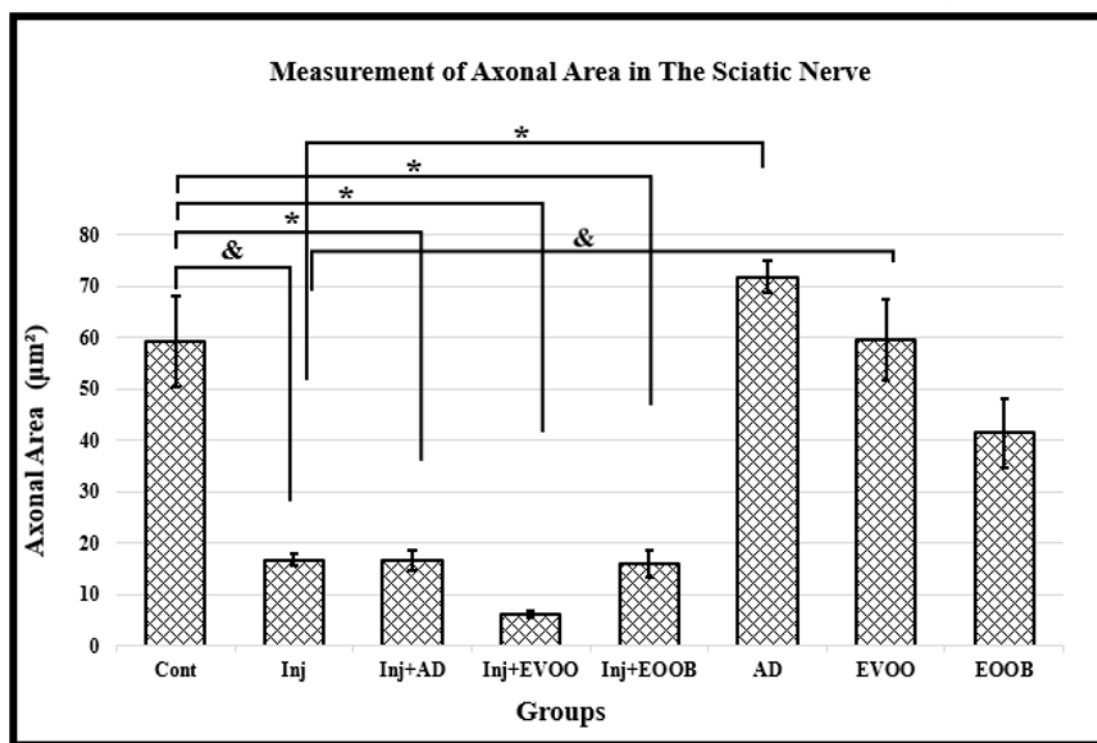


Figure 4.3. Display the differences of the axonal area mean (μm^2) across groups. The significance level at $p < 0.05$ is indicated by (&)

Table 4.6. Shows degree of significance between groups in terms of axonal area

Comparison between groups	Degree of Significance
Inj with Cont	0.014
Inj with Inj+AD	0.947
Inj with Inj+EVOO	0.074
Inj with Inj+EOOB	0.816
Cont with AD	0.426
Cont with EVOO	0.938
Cont with EOOB	0.268
Cont with Inj+AD	0.009
Cont with Inj+EVOO	0.000
Cont with Inj+EOOB	0.007
Inj with AD	0.001
Inj with EVOO	0.011

After the statistical analysis applying to the stereological data obtained from the axonal area measurement in the myelinated axons, it was seen that the mean axonal area in the Inj group was significantly decreased when compared to the mean axonal area in the Cont group ($p < 0.05$), also, highly significant differences were detected among Con and Inj+AD ($p < 0.01$), Con and Inj+EVOO ($p < 0.01$), Con and Inj+EOOB ($p < 0.01$) groups. However, no significant differences were observed regarding the mean axonal area in the Inj group comparing to the Inj+AD and Inj+EOOB groups ($p > 0.05$). On the other hand, the Inj group was appreciated high significant alteration when compared to the AD group ($p < 0.01$), while a significant difference was found between Inj and EVOO ($p < 0.05$) groups. Moreover, the comparison between the Inj group and Inj+EVOO in terms of the axonal area mean show no statistically significant difference ($p > 0.05$). Furthermore, the significant difference was not appreciated in the Cont group when compared to the AD, EVOO and EOOB groups in terms of the mean axonal area ($p > 0.05$) (Table 4. 6).

4.1.2. Functional Assessments of Sciatic Nerve Healing

4.1.2.1. Sciatic Functional Index Results

The mean of the SFI and SD are shown below in Table 4.7 and Figure 4.4.

Table 4. 7. Displays the mean value of SFI and the standard deviation (SD)

Groups	Mean value of SFI ± SD
Cont	-4.112 ± 0.421
Inj	-33.364 ± 2.642
Inj+AD	-21.863 ± 3.053
Inj+EVOO	-17.721 ± 3.840
Inj+EOOB	-23.450 ± 3.096
AD	-3.318 ± 0.779
EVOO	-6.625 ± 1.912
EOOB	-4.988 ± 0.832

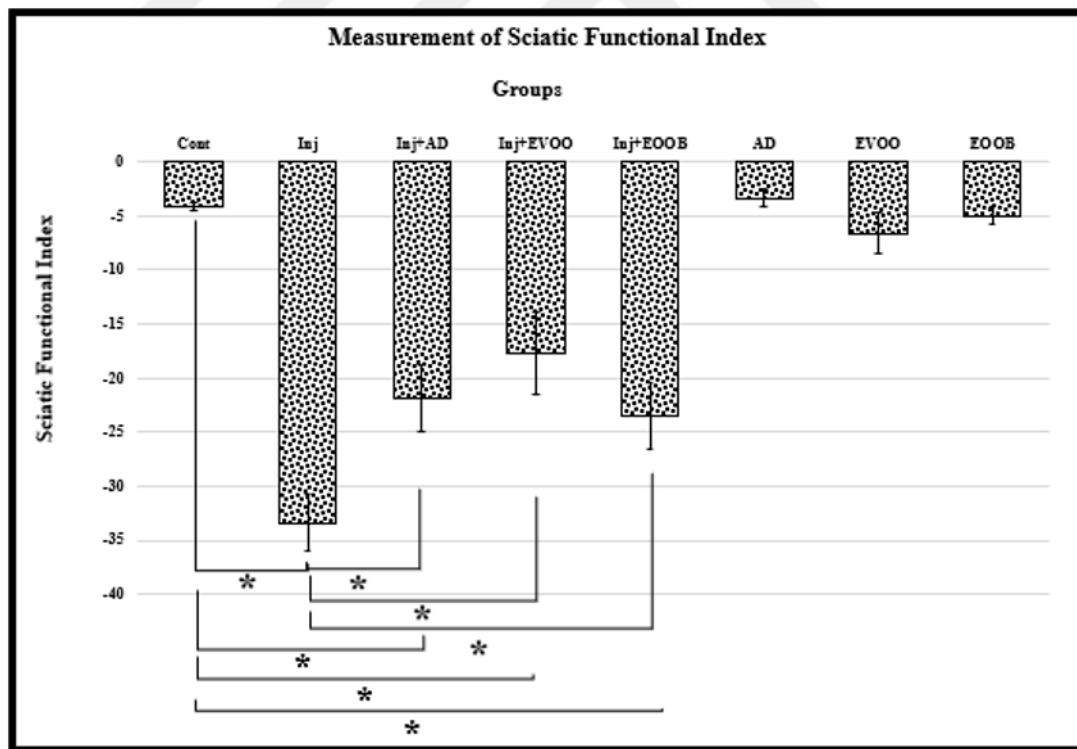


Figure 4.4. Display the differences of mean SFI across groups. The significance level at $p < 0.01$ is indicated by (*)

According to statistical analysis of the SFI data, it was found that the mean of SFI in the Cont group was significantly higher than the Inj group ($p < 0.01$). Likewise, high significant alterations were detected among Cont and Inj+AD ($p < 0.01$), Cont and Inj+EVOO ($p < 0.01$), Cont and Inj+EOOB ($p < 0.01$) groups, respectively. On the other hand, a high significant difference was observed among Inj+AD and Inj groups ($p < 0.01$), Inj+EVOO and Inj groups ($p < 0.01$), Inj+EOOB and Inj groups ($p < 0.01$) regarding the mean of SFI. In contrast, no significant differences were appreciated in terms of mean SFI between Cont and AD groups ($p > 0.05$), Cont and EVOO groups ($p > 0.05$), Cont and EOOB groups ($p > 0.05$) (Table 4.8.).

In summary, the results of the SFI proved that motor nerve fibre recovery is improved. Thus, there is no noticeable difference in the damaged right lower limb's movement compared to the standard left lower limb.

Table 4.8. Demonstrates the degree of significance among groups in terms of the SFI mean

Comparison between groups	Degree of Significance
Inj with Cont	0.000
Inj with Inj+AD	0.000
Inj with Inj+EVOO	0.000
Inj with Inj+EOOB	0.004
Cont with AD	0.889
Cont with EVOO	7.005
Cont with EOOB	2.677
Cont with Inj+AD	0.000
Cont with Inj+EVOO	0.001
Cont with Inj+EOOB	0.000

4.1.2.2. Electromyography Findings, Amplitudes of CMAP

The average amplitudes and the standard deviation (SD) of the muscle compound action potentials are shown below in Table 4.9 and Figure 4.5.

Table 4.9. Displays the mean value of amplitude and standard deviation (SD) across-groups

Groups	Mean amplitude (mV) \pm SD
Cont	44.269 \pm 2.860
Inj	22.388 \pm 2.179
Inj+AD	27.411 \pm 4.563
Inj+EVOO	54.421 \pm 8.619
Inj+EOOB	24.354 \pm 4.403
AD	56.481 \pm 10.446
EVOO	32.252 \pm 5.943
EOOB	30.896 \pm 2.945

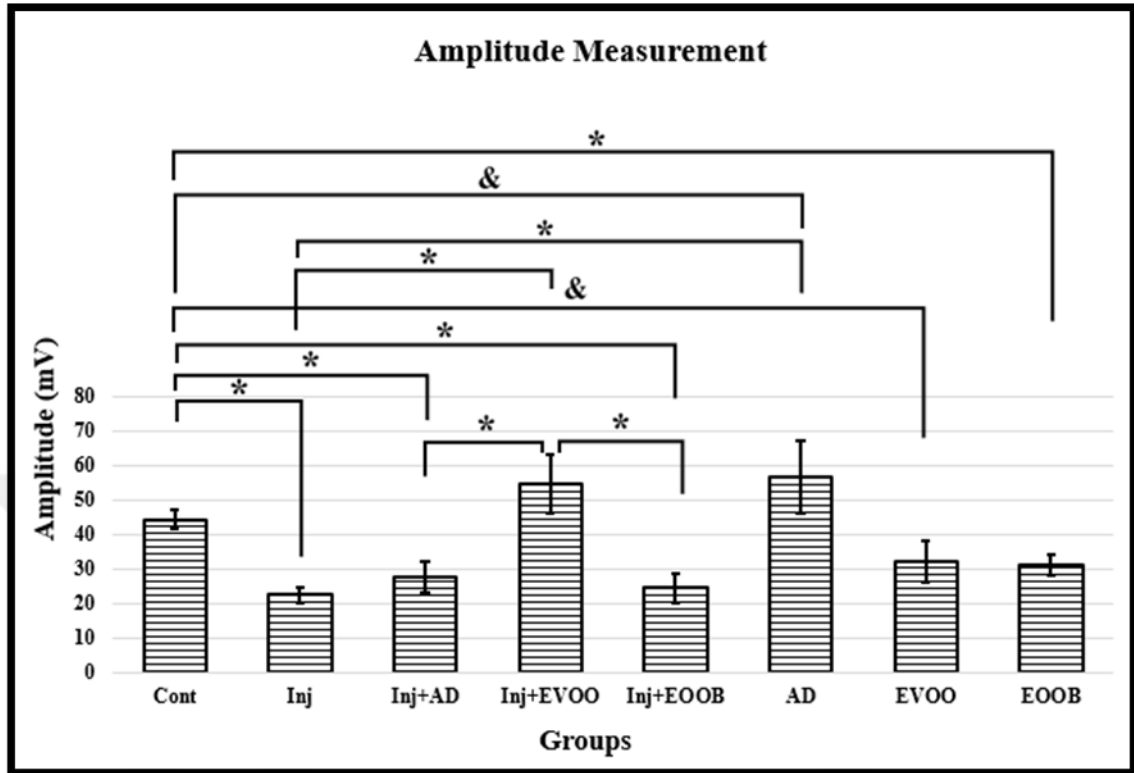


Figure 4.5. Shows the differences among the groups in terms of the mean value of amplitude. The level of significance at $p < 0.05$ is indicated by (&), while the significance level at $p < 0.01$ is indicated by (*)

The statistical analysis was employed for the data collected in terms of mean amplitude values. As a result, the Cont group was significantly higher than the Inj group ($p < 0.01$), as well a highly significant alteration was seen among Inj and AD groups ($p < 0.01$). Similarly, there were highly significant differences observed between the Inj+EVOO and Inj group ($p < 0.01$), Cont and Inj+AD ($p < 0.01$), Cont with Inj+EOOB ($p < 0.01$). In contrast, no significant differences were observed when comparing Inj+AD and Inj groups ($p > 0.05$), Inj+EOOB and Inj groups ($p > 0.05$). Moreover, the significant differences were appreciated among the following groups; Cont and AD groups ($p < 0.05$), Cont and EVOO groups ($p < 0.05$), Cont and EOOB groups ($p < 0.01$) (Table 4.10).

Table 4.10. Shows the degree of significance among groups in terms of the amplitude mean

Comparison between groups	Degree of Significance
Inj with Cont	0.000
Inj with Inj+AD	0.807
Inj with Inj+EVOO	0.000
Inj with Inj+EOOB	0.999
Inj with AD	0.000
Cont with AD	0.022
Cont with EVOO	0.026
Cont with EOOB	0.009
Cont with Inj+AD	0.000
Cont with Inj+EOOB	0.000
Inj+EVOO with Inj+AD	0.000
Inj+EVOO with Inj+EOOB	0.000

4.1.2.3. Electromyography Findings, Latencies of CMAP

The mean of latency and the standard deviation (SD) are shown below in Table 4.11 and Figure 4.6.

Table 4. 11. Demonstrates the mean latency and standard deviation (SD) of the groups

Groups	Mean latency (sec) ± SD
Cont	0.209 ± 0.010
Inj	0.256 ± 0.018
Inj+AD	0.191 ± 0.018
Inj+EVOO	0.199 ± 0.016
Inj+EOOB	0.260 ± 0.018
AD	0.177 ± 0.034
EVOO	0.176 ± 0.035
EOOB	0.197 ± 0.023

After the statistical analysis applied for collected data regarding the mean latency values of groups, no significant difference was observed among the Cont and Inj groups ($p>0.05$). Moreover, highly significant differences were shown among the Inj+AD and the Inj groups ($p<0.01$), Inj+EVOO and the Inj groups ($p<0.01$), Inj and AD groups ($p<0.01$), Inj and EVOO groups ($p<0.01$), Inj and EVOO groups ($p<0.01$). In contrast, no significant alteration was detected among Inj+EOOB with Inj groups ($p>0.05$). Also no significant differences were found when compared the Cont group to the AD, EVOO or EOOB groups ($p>0.05$) in terms of mean latency values (Table 4.12).

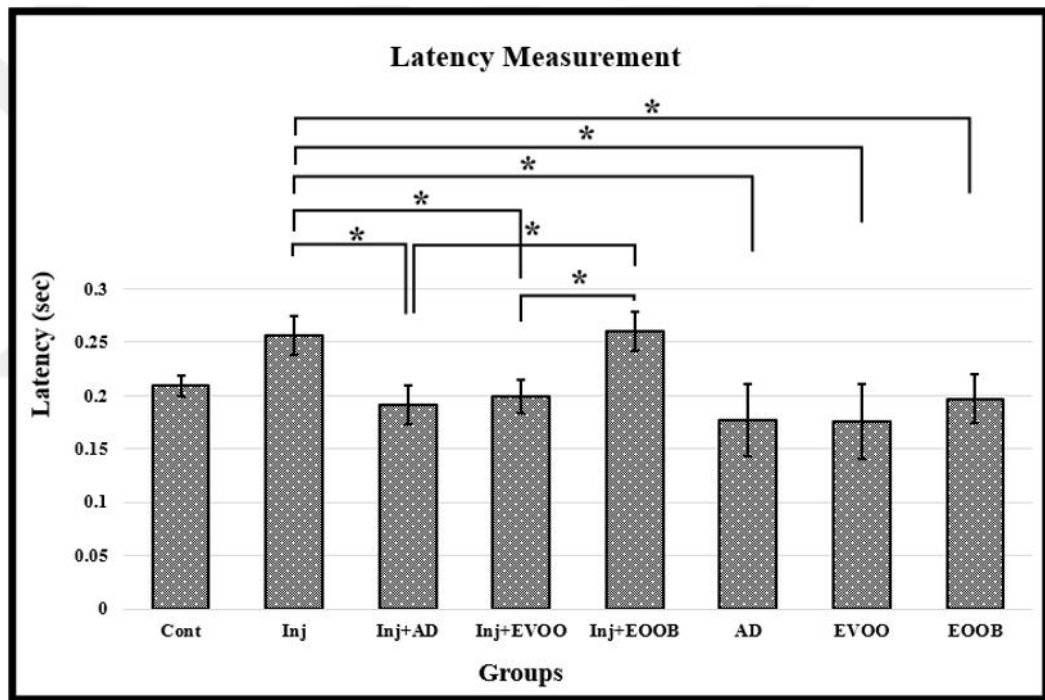


Figure 4.6. Shows the differences between the groups regarding the mean of latency. The of significance level at $p<0.01$ is indicated by (*)

Table 4. 12. Indicates the significant differences of latency mean among the groups

Comparison between groups	Degree of Significance
Inj with Cont	0.051
Inj with Inj+AD	0.001
Inj with Inj+EVOO	0.003
Inj with Inj+EOOB	0.984
Inj with AD	0.000
Inj with EVOO	0.000
Inj with EOOB	0.005
Cont with AD	0.111
Cont with EVOO	0.106
Cont with EOOB	0.403
Inj+AD with Inj+EOOB	0.001
Inj+EVOO with Inj+EOOB	0.003

Table 4. 13. Regarding evaluated parameters, the table shows the mean values of the data from higher to lower manner among the three substances. Although it is observed that some substances have a beneficial effect of increasing (myeline thickness etc.) or decreasing (latency etc.) the parameters, they could not reach the statistical levels as mentioned in the third column

Parameter	Mean Value	Significant Differences (p value) among substances	Statistical Graph Number
Axon Number	AD > EOOB > EVOO	NO	Figure 4.1.
Thickness	AD > EOOB > EVOO	Inj+AD and Inj+EVOO (p=0.002).	Figure 4.2.
Area	AD > EOOB > EVOO	NO	Figure 4.3.
SFI	EVOO > AD > EOOB	NO	Figure 4.4.
Amplitude	EVOO > AD > EOOB	Inj+EVOO and Inj+AD (p=0.000). Inj+EVOO and Inj+EOOB (p=0.000).	Figure 4.5.
Latency	EOOB > EVOO > AD	Inj+AD and Inj+EOOB (p=0.001). Inj+EVOO and Inj+EOOB (p=0.003).	Figure 4.6.

4.1.3. Light Microscopical (LM) Evaluation Results

The sciatic nerve semi-thin cross-sections were taken and stained by toluidine blue, then evaluated by LM. The obtained results are shown below.

4.1.3.1. LM Findings of the Cont Group

LM evaluation was employed in semi-thin sections taken from the Cont group; the general histological architecture appeared normal. Epineurium surrounds the whole nerve looks intact. The nerve bundles are surrounded by thin perineurium that containing blood vessels normally. Variable-sized of myelinated axons were observed normal and enclosed by a thick myelin sheath. The nerve fibres coherence is deemed normal. The myelin sheath of a few axons showed a physical impairment appearance such as cleft, vacuolization and tubulisation due to poor fixation, unproper tissue processing, or unknown reason. Furthermore, some physiological impairments of the myeline coat were also seen, such as Schmidt-Lanterman clefts. The unmyelinated axons were also seen. Moreover, the nucleus of Schwann cells was appeared very clear in some sections. The mast cells were also found among the connective tissue of the nerve. Blood cells inside the blood vessel were also observed (Figures 4.7- 10).

4.1.3.2. LM Findings of the Inj Group

Semi-thin sections of the sciatic nerve were collected from the Inj group and examined by LM. A weak observation of the nerve structures consistency was seen. The general histological structure of the nerve looks partially normal. The adipose cells in the connective tissue around the nerve were shown healthy with intact perineurium. There was some tissue loss in the nerve cross-section; it might result from improper tissue processing. Most axons showed undergoing WD with thinner myelin sheath and tiny size. Some of the normal thin myelinated axons are scattered among damaged fibres. Many macrophages with foamy appearance were seen in-between the degenerated nerve fibres to phagocytise the myelin debris. In the connective tissue between myelinated axons, the aggregation of mast cells is frequently seen. A large number of Schwann cells were found around the newly formed myelinated axons to

stimulate remyelination. Moreover, some blood vessels with premature endothelial cells were also observed (Figures 4.11- 14).

4.1.3.3. LM Findings of the Inj+ AD Group

LM investigated the cross-sections collected from Inj+ AD Group. The general histological structure of neural tissue was well protected. The connective tissue, epineurium and perineurial cells forming perineurium all were looks intact. A thin myelin sheath enclosed the degenerated axons, and small-sized myelinated fibres were observed. The SCs were seen closed to axons due to their function. Many macrophages which remove the degenerated fibres and myeline debris and thicker blood vessels with normal blood cells were also found. Some findings, such as protected thicker myelinated axons, were seen due to the positive effect of treatment AD in the terms of axon number and myelin thickness. Additionally, unmyelinated axons were also shown. On the other hand, some myelin sheath defects were also found; this might be due to improper tissue processing steps (Figures 4.15- 18).

4.1.3.4. LM Findings of the Inj+ EVOO Group

LM examination was employed for semi-thin cross-sections of the sciatic nerve collected from the Inj+ EVOO group. The general structural view of the nerve was healthy. The intact epineurium delimits the nerve fascicle, and normal perineurium around the nerve bundles was seen. Adipose cells were also found normally. The normal blood vessels with blood cells were abundant as one of the features following crush surgery. The degenerative observations were found; the small-sized axons myelinated by thin sheath were dominant. The degenerated axons were also observed. The SCs nuclei are shown close to the newly formed small axon due to their function. The mast cells were seen frequently in the connective tissue of the nerve. In addition, a large number of macrophages were observed to remove the myeline debris. A thick myelin sheath enclosed the protected myelinated axons with some abnormality of the myeline sheath in a few axons (Figures 4.19- 22).

4.1.5.5. LM Findings of the Inj+ EOOB Group

The general structures of the neural tissue were healthy. The connective tissue which surrounds the whole nerve, epineurium, was intact. The nerve bundle was covered by normal perineurium. The adipose cells around the nerve section were also shown. The blood vessels with a thicker wall and normal blood cells were found. A thick myelin sheath enclosed most of the different sized myelinated axons; besides, a few have a thin myeline sheath. Some of the sheath abnormalities were observed. These defects might refer to poor fixation of the tissue processing step. The degenerated axons were also seen, in addition to the considerable number of axons were enclosed by a thin myeline sheath. The macrophage which engulfs the damaged fibres and myeline waste was observed (Figures 4.23- 26).

4.1.3.6.LM Findings of the AD Group

Because of, no crush surgery was performed to this group, all nerve structures were seen healthy. The nerve fascicles are delimited by intact epineurium in addition to healthy perineurium, which encloses each bundle. The majority of myelinated axons were covered by thick myeline sheath were seen. The SCs could be differentiated by their nuclei which were presented close to myelinated axons. Some abnormal myelinated fibres were found. The blood cells within the lumen of blood vessels were observed normal (Figures 4.27- 30).

4.1.3.7. LM Findings of the EVOO Group

The cross-sectioned from EVOO were evaluated using LM. The structural appearance of the nerve tissue was normal. The connective tissue of the epineurium and perineurium was observed intact normally. SCs nuclei were identified and located close to the myelinated axons. Most myelinated axons were surrounded by thick myeline sheath, and some unmyelinated axons and impaired myeline sheath were seen. The blood cells normally appeared within the lumen of the blood vessels (Figures 4.31- 34).

4.1.3.8. LM Findings of the EOOB Group

LM examination was applied for semi-thin sections of the EOOB group. The histological architecture of the nerve was normal. The nerve was surrounded by intact connective tissue, epineurium, besides some adipose tissue was found. Also, each nerve fascicle was enclosed by normal connective tissue, the perineurium. The thick myelin sheath myelinated the majority of axons, as well the unmyelinated axons were also found. Between the connective tissue of the myelinated axons, sometimes the mast cells were observed. The nuclei of SCs were seen closed to the myelinated axons. In addition, the blood cells and vessels were healthy. On the other hand, some impairment of myeline sheath was observed (Figures 4.35- 38).

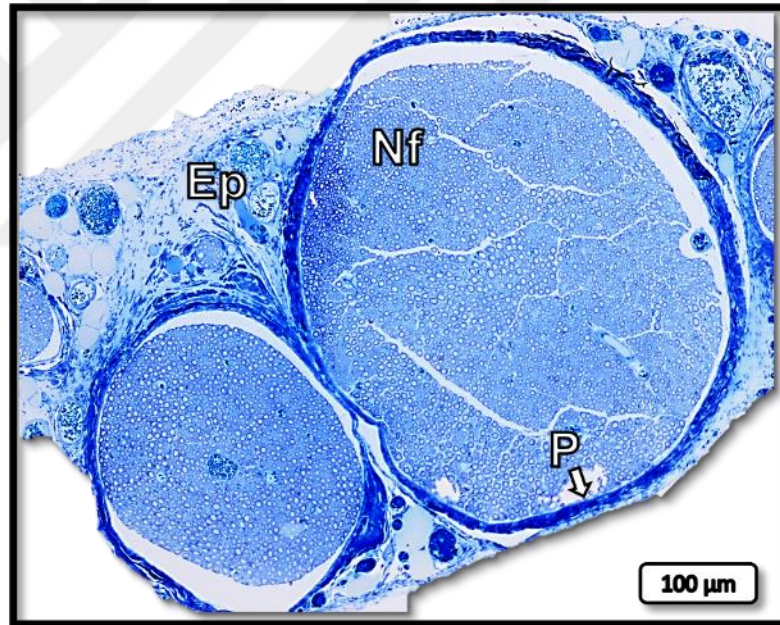


Figure 4.7. A cross section photomicrograph taken from Cont group. The general structure of nerve seen healthy, with intact epineurium (Ep) and perineurium (P) surrounding the nerve fascicle (Nf). Resin section, the thickness was 0.5 micrometer and stained with toluidine blue

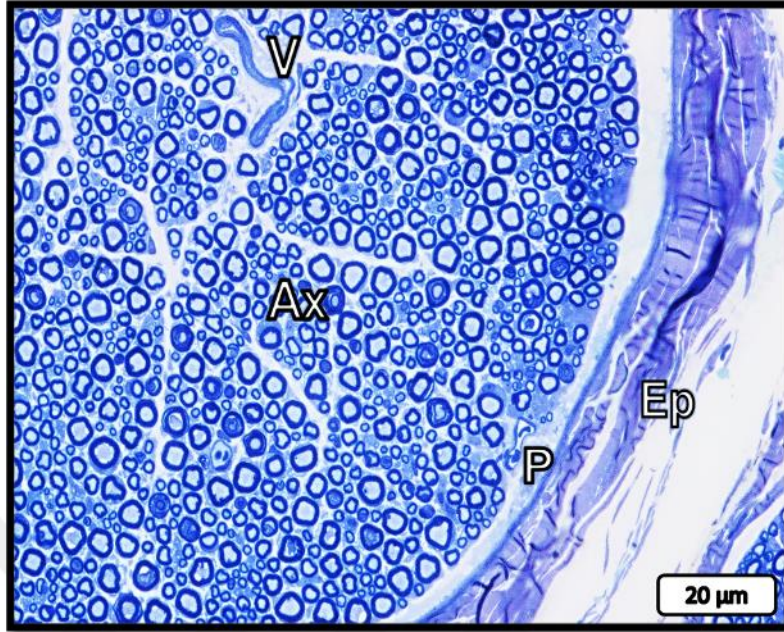


Figure 4.8. An image taken from Cont group is seen. Showed different-sized of myelinated axons (Ax) with healthy connective tissue, perineurium (P) and epineurium (Ep) also blood vessel (V) was preserved and observed normal. Resin section, the thickness was 0.5 micrometer

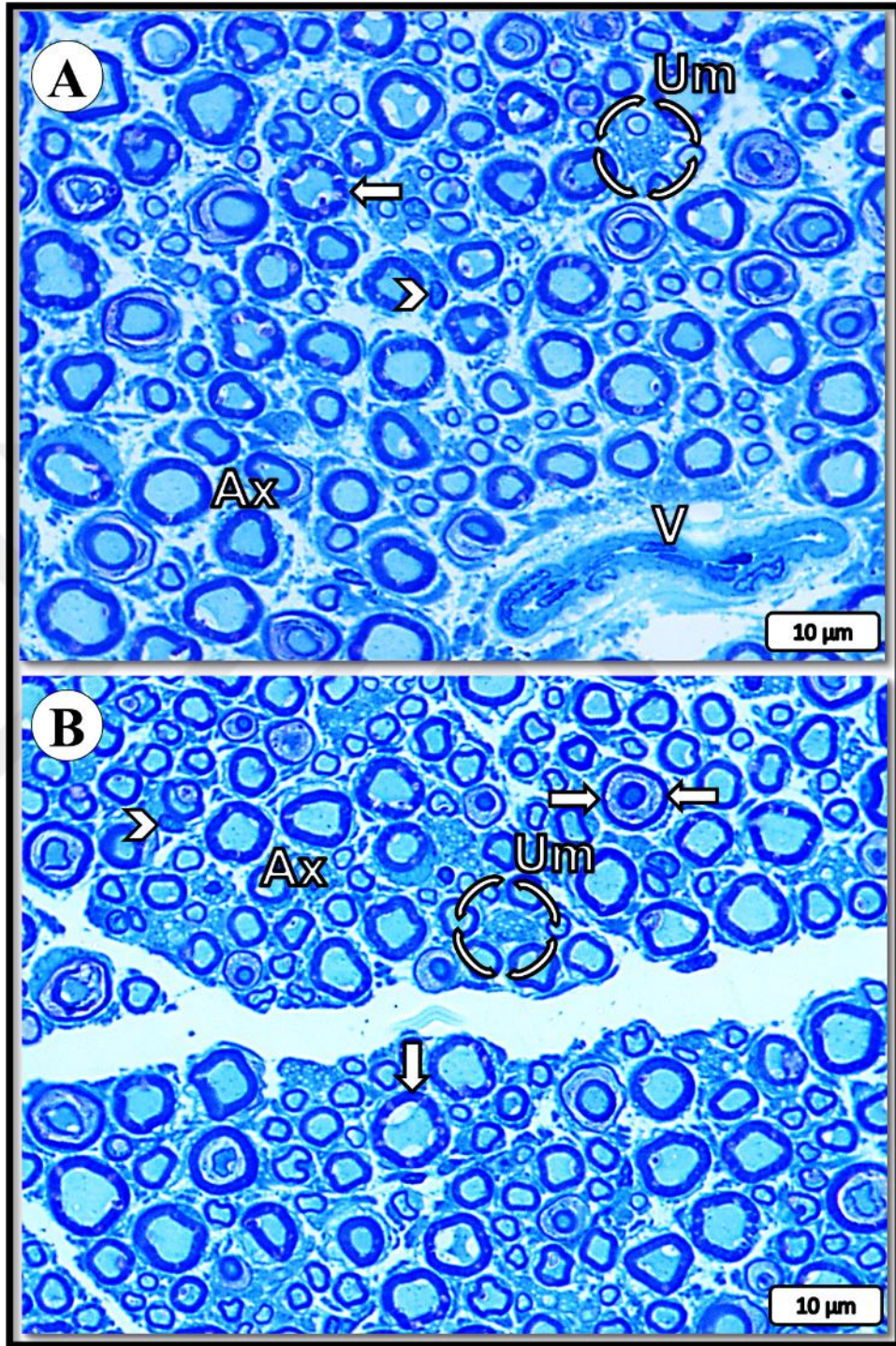


Figure 4.9. (A) and (B) images which taken from Cont group is observed. The majority of axons were myelinated with thick myeline sheath normally (Ax).The Schwann cells nuclei were seen closed to myelinated axons (arrow head). The unmyelinated axons were also indicated by (Um). Some physical impairment of myeline sheath have indicated by (double arrows) for tubulisation or cleft and (single arrow) for myeline sheath vacuolization. The blood vessel (V) has observed with normal appearance and mature endothelial cells. Resin section, the thickness was 0.5 micrometre and stained with toluidine blue

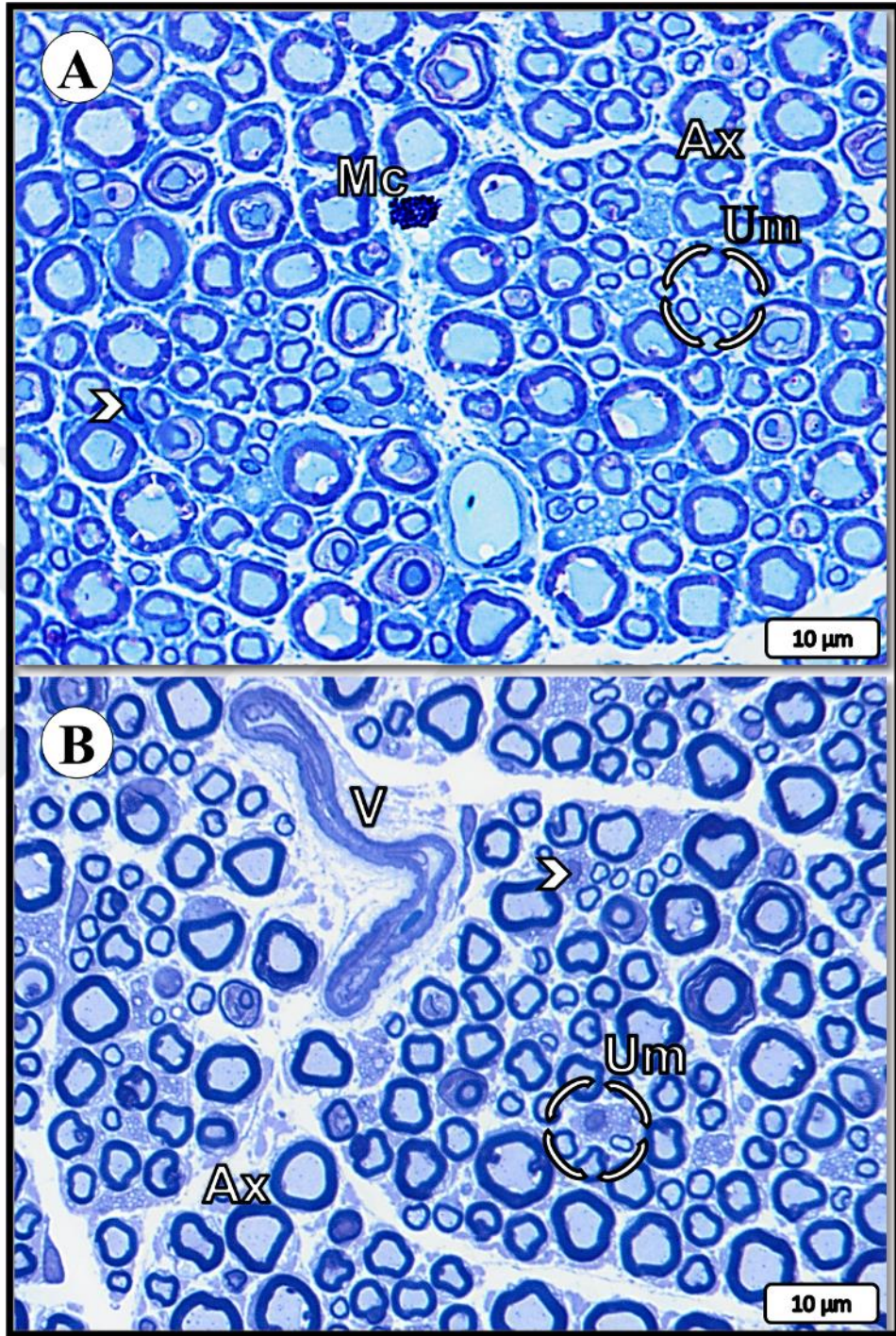


Figure 4.10. (A) and (B) photomicrographs which taken from Cont group is observed. The general features of nerve tissue were seen normal. The healthy axons have indicated by (Ax) as majority. The unmyelinated fibres were observed (Um). The mast cell (Mc) within the connective tissue of the nerve was found. The Schwann cells nuclei were found (arrow head). The healthy blood vessel was found with mature endothelial cells, normal lumen and blood cells inside (V). Resin section, the thickness was 0.5 micrometre and stained with toluidine blue

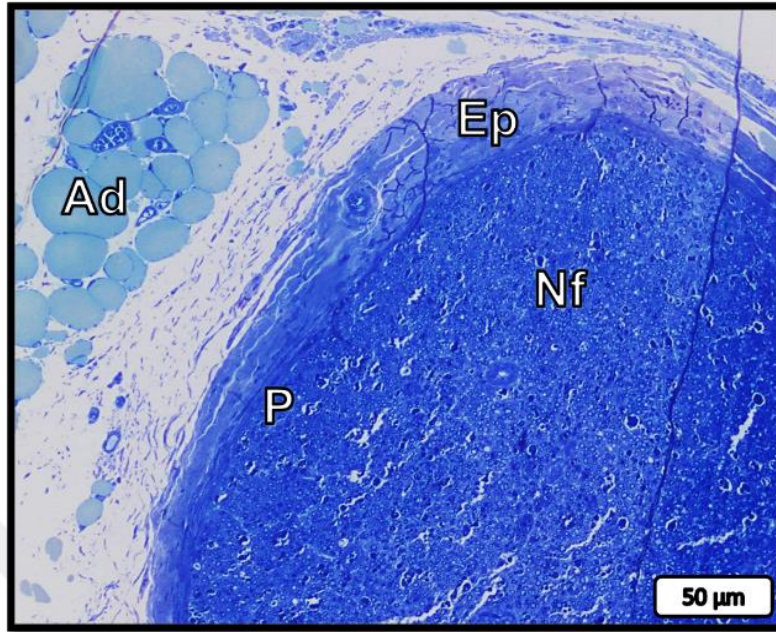


Figure 4.11. Showed photomicrograph of cross-section belongs to Inj group. The structural architecture appeared partially healthy. An irregular connective tissue, epineurium (Ep), while the perineurium was observed intact (P) and enclosed the nerve fascicle (Nf). Ad indicating adipose cells. Resin section, the thickness was 0.5 micrometre and stained with toluidine blue

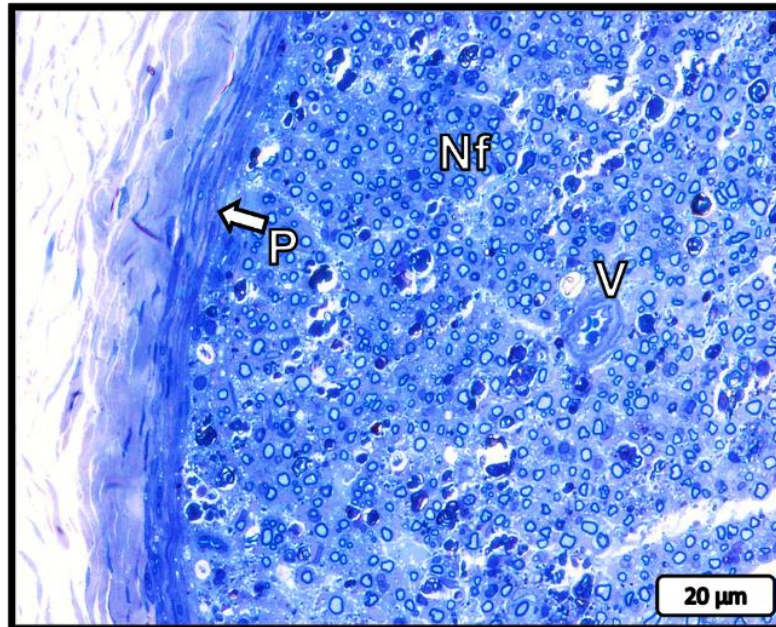


Figure 4.12. An image belongs to the Inj group was showed normal general nerve structures. The nerve fascicle (Nf) was enclosed by intact perineurium (P). The blood vessel (V) looks normal with blood cells. Resin section, the thickness was 0.5 micrometre and stained with toluidine blue

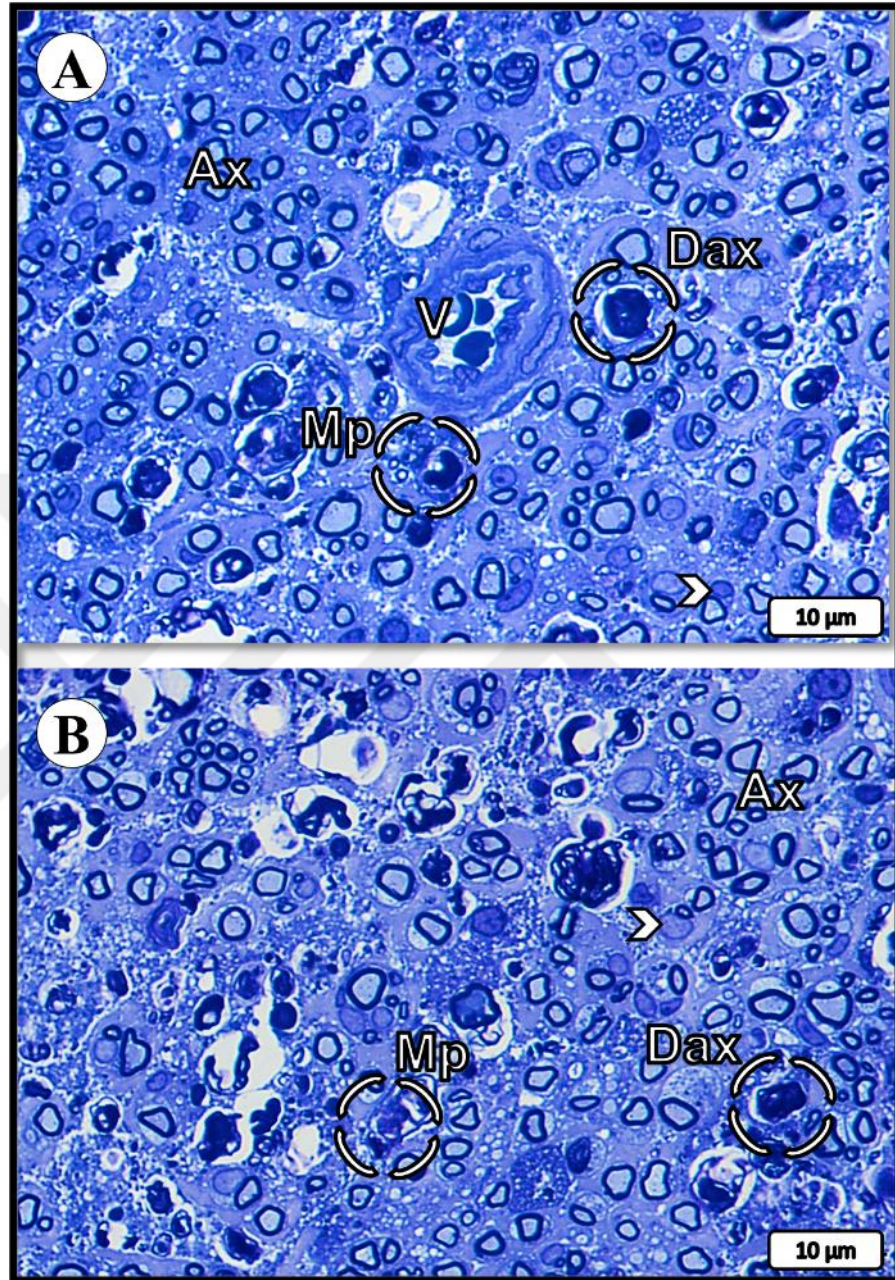


Figure 4.13. (A) and (B) images for the semi-thin section were taken from the Inj group showed normal. Main neuronal structure. Following crush injury, many macrophages were recruited and observed (Mp). Most of the axons are small-sized which enclosed by a thin myeline sheath (Ax). The newly formed fibres were abundant. The nuclei of Schwann cells were observed (arrow head). The degenerated axons were indicated by (Dax). The blood vessel was also found (V). Resin section, the thickness was 0.5 micrometre and stained with toluidine blue

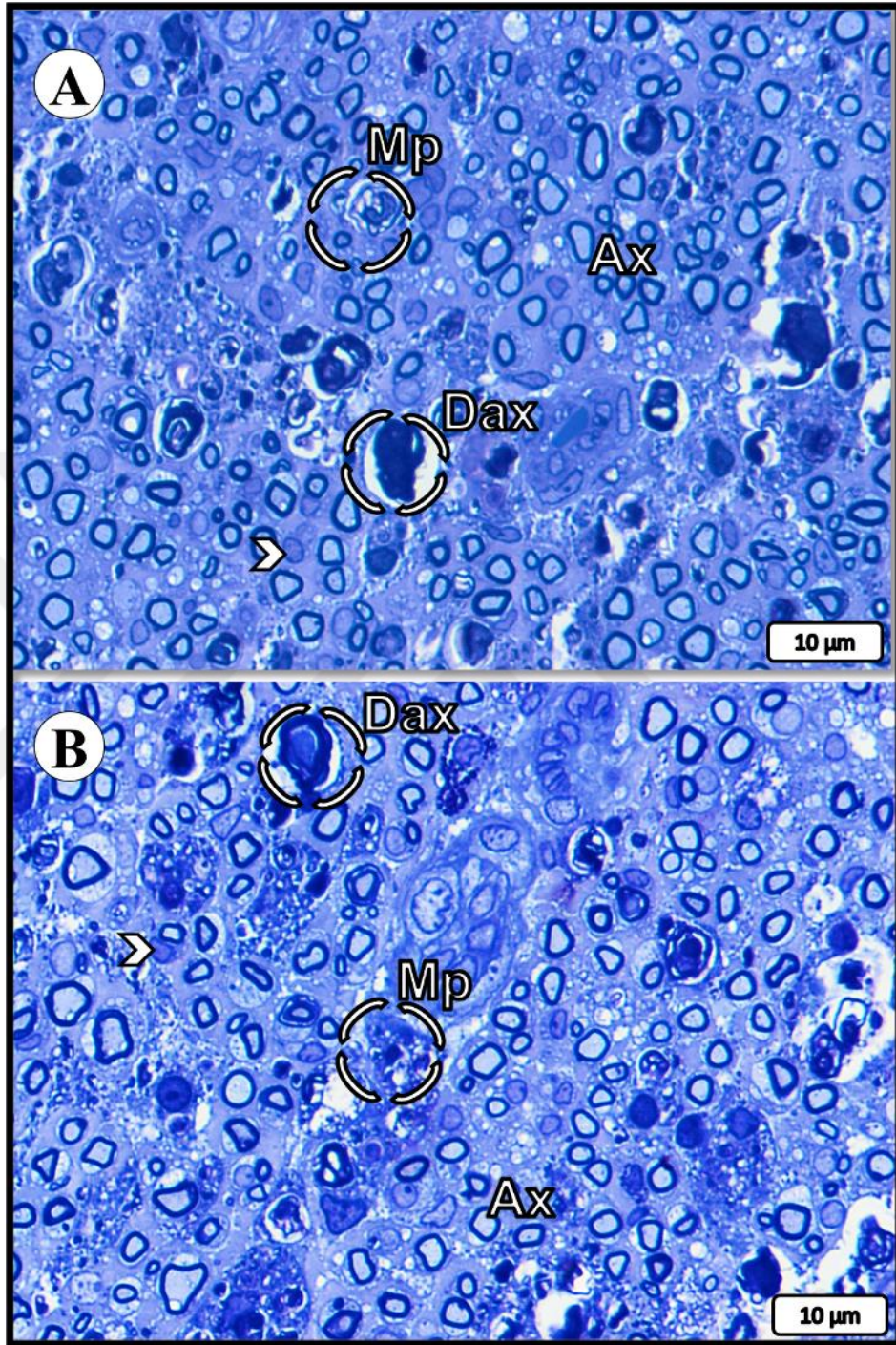


Figure 4.14. (A) and (B) photos for a magnified semi-thin section was taken from the Inj group is seen. Showed degenerative features such as macrophages with foamy appearance (Mp) while clearing myelinated nerve fibres' debris. The degenerated axons (Dax) also were seen. In addition, the Schwann cells nuclei have been found as they myelinate the recently formed small axon and indicated by (arrow heads). Resin section, the thickness was 0.5 micrometre and stained with toluidine blue

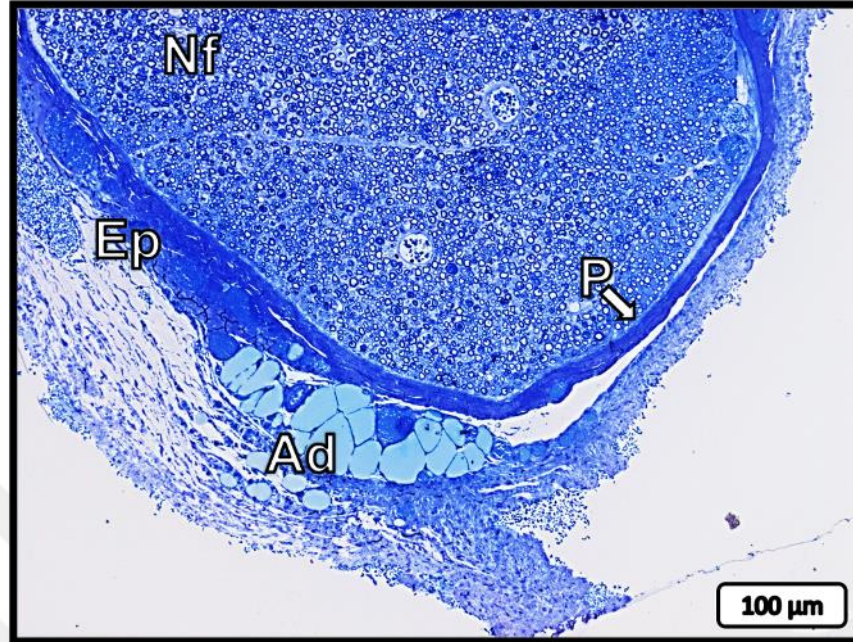


Figure 4.15. A photomicrograph was taken from the Inj+ AD group is seen. It showed the general architecture of neural tissue looks partially healthy. The connective tissue epineurium (Ep) delimits nerve fascicles (Nf) looks normal, as well an irregular connective tissue perineurium (P) appeared intact. The adipose cells are indicated by (Ad). The resin section thickness was 0.5 micrometre and stained with toluidine blue

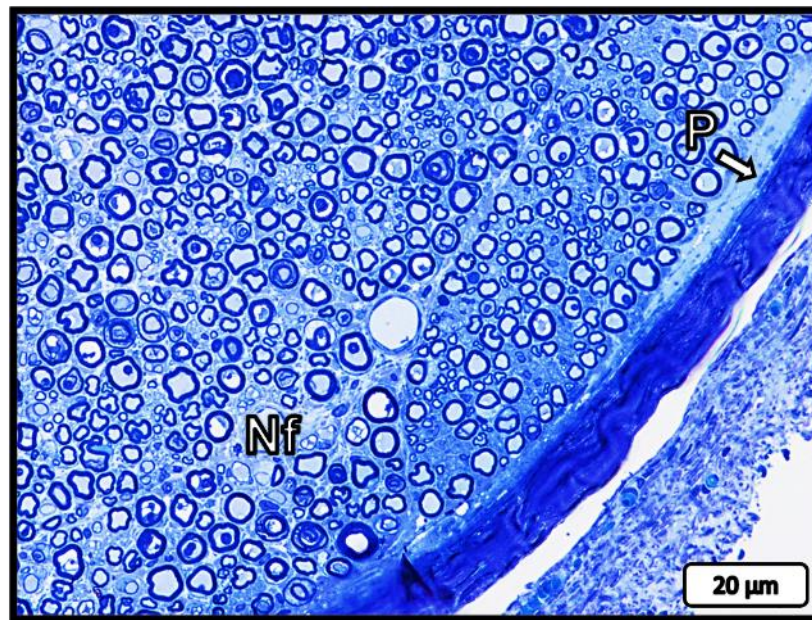


Figure 4.16. A photomicrograph was taken from the Inj+AD group is seen. It showed a partial normal view of the nerve structure. An irregular connective tissue, perineurium (P), covered the nerve fascicle (Nf). The resin section thickness was 0.5 micrometre and stained with toluidine blue

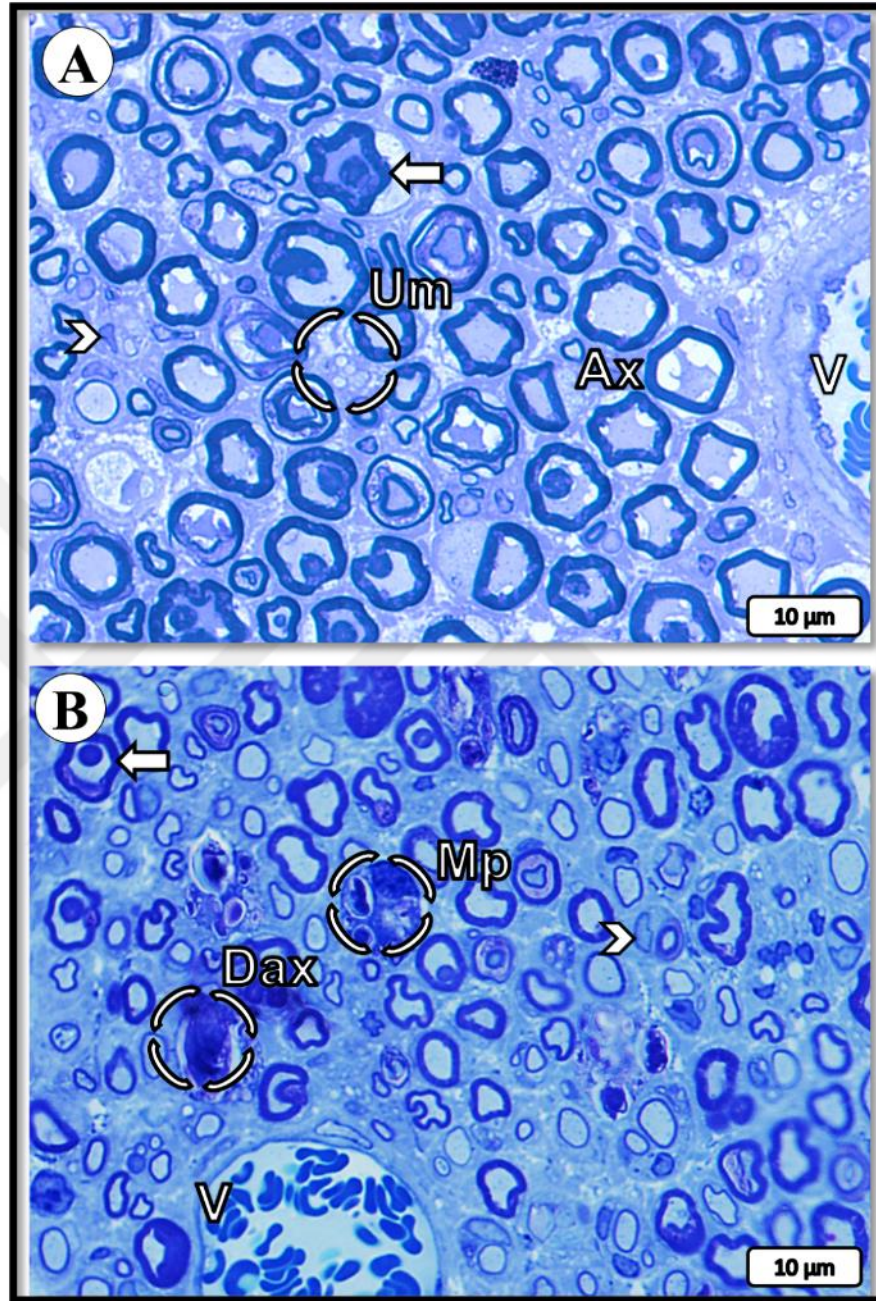


Figure 4.17. (A) and (B) photomicrographs of cross-section which collected from the Inj+AD. group is showed several regenerative features; most of the myelinated axons were normal size (Ax) and delimited by a thick sheath, and macrophages (Mp) clear the myelin droplets, and a remnant of degenerated axons (Dax) is expected to be observed after crush injury. All these features indicate successful regeneration and the positive effect of treatment AD in axon number and myelin thickness. A few axons looked with defects, such as cleft or fragmentation and indicated by arrows. The Um is an indicator for unmyelinated axons. The arrow heads were demonstrated the Schwann nuclei. The mast cell (Mc) was also seen in the connective tissue of the sciatic nerve. The blood vessels were found and indicated by (V). The resin section thickness was 0.5 micrometre and stained with toluidine blue

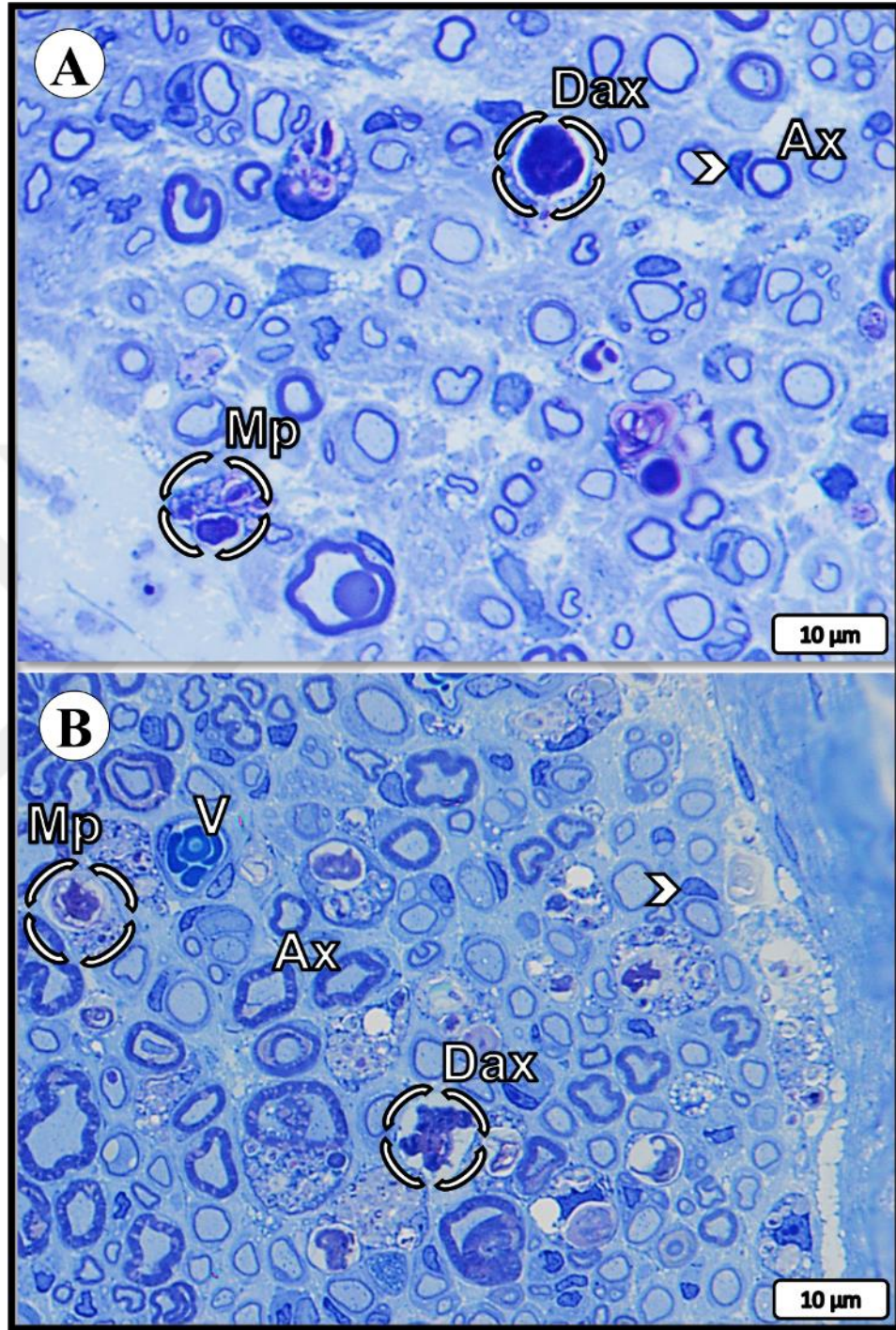


Figure 4.18. (A) and (B) are cross-section photomicrographs from the Inj+ AD group are showed. Several regenerative observations, such as protect the axons and increased myeline thickness (Ax). Mp for macrophages, while Dax for degenerated axons and small-sized axons have appeared following crush surgery. Arrowheads indicate the Schwann cells nuclei. The blood vessel and blood cells were looks normal. The resin section thickness was 0.5 micrometre and stained with toluidine blue

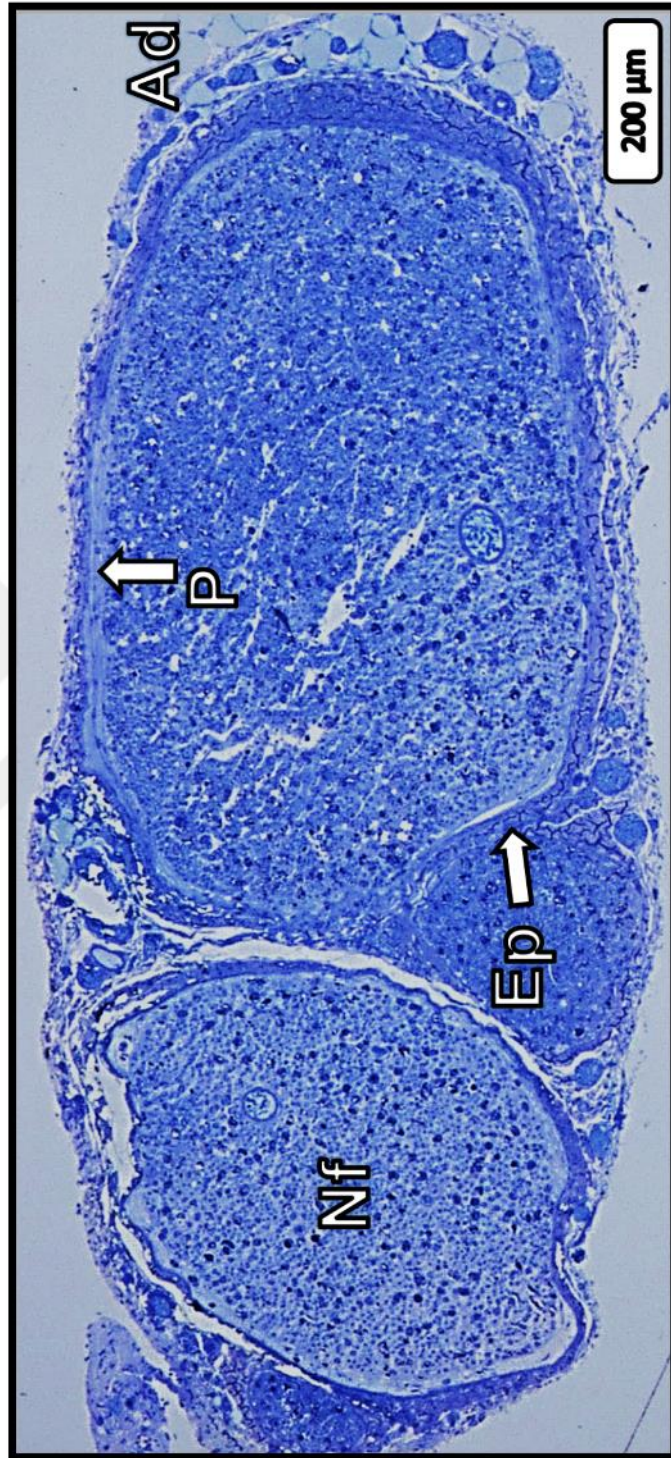


Figure 4.19. The image was taken from the Inj+ EVOO group is observed. The structural view of the nerve was healthy. The intact epineurium (Ep) delimits the fascicle (Nf), and normal perineurium (P) around the bundles of the nerve were seen. Adipose cells (Ad) were also found. The resin section thickness was 0.5 micrometre and stained with toluidine blue

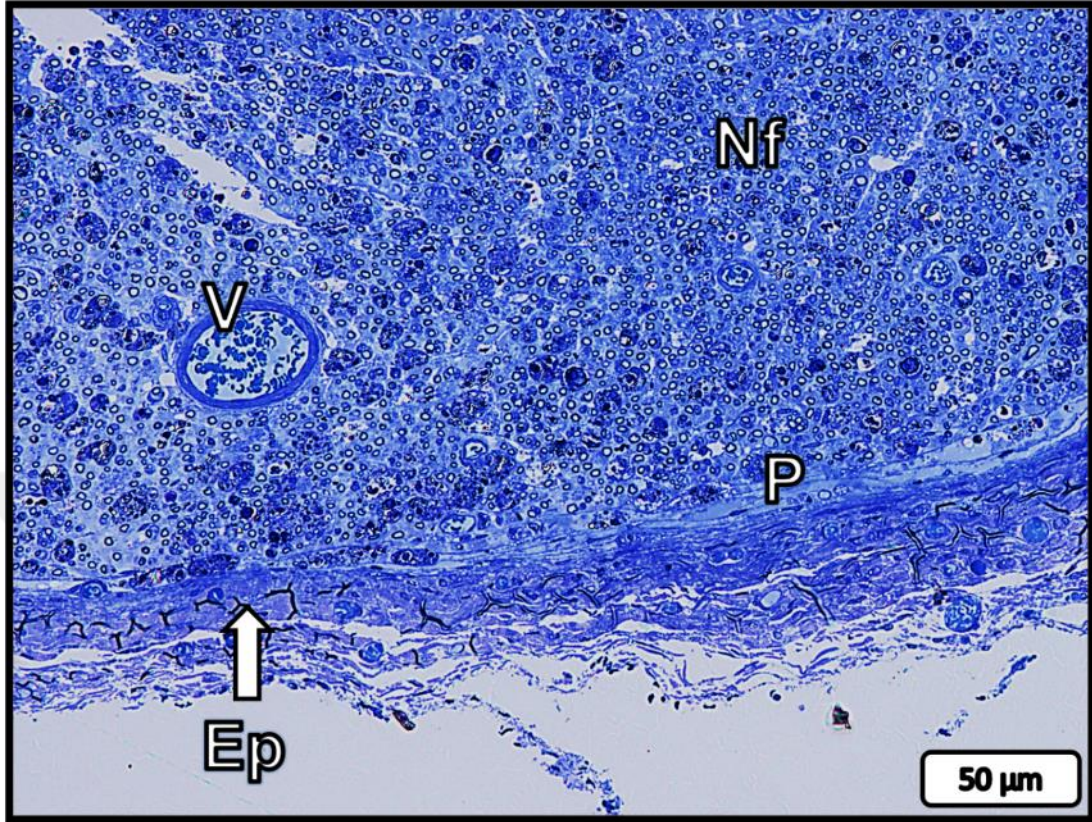


Figure 4.20. The photomicrograph was collected from the sciatic nerve of the Inj+ EVOO group is observed. The connective tissue of the nerve, epineurium (Ep) and perineurium (P), was healthy. V for a blood vessel. Nf is the nerve fascicle. The resin section thickness was 0.5 micrometre and stained with toluidine blue

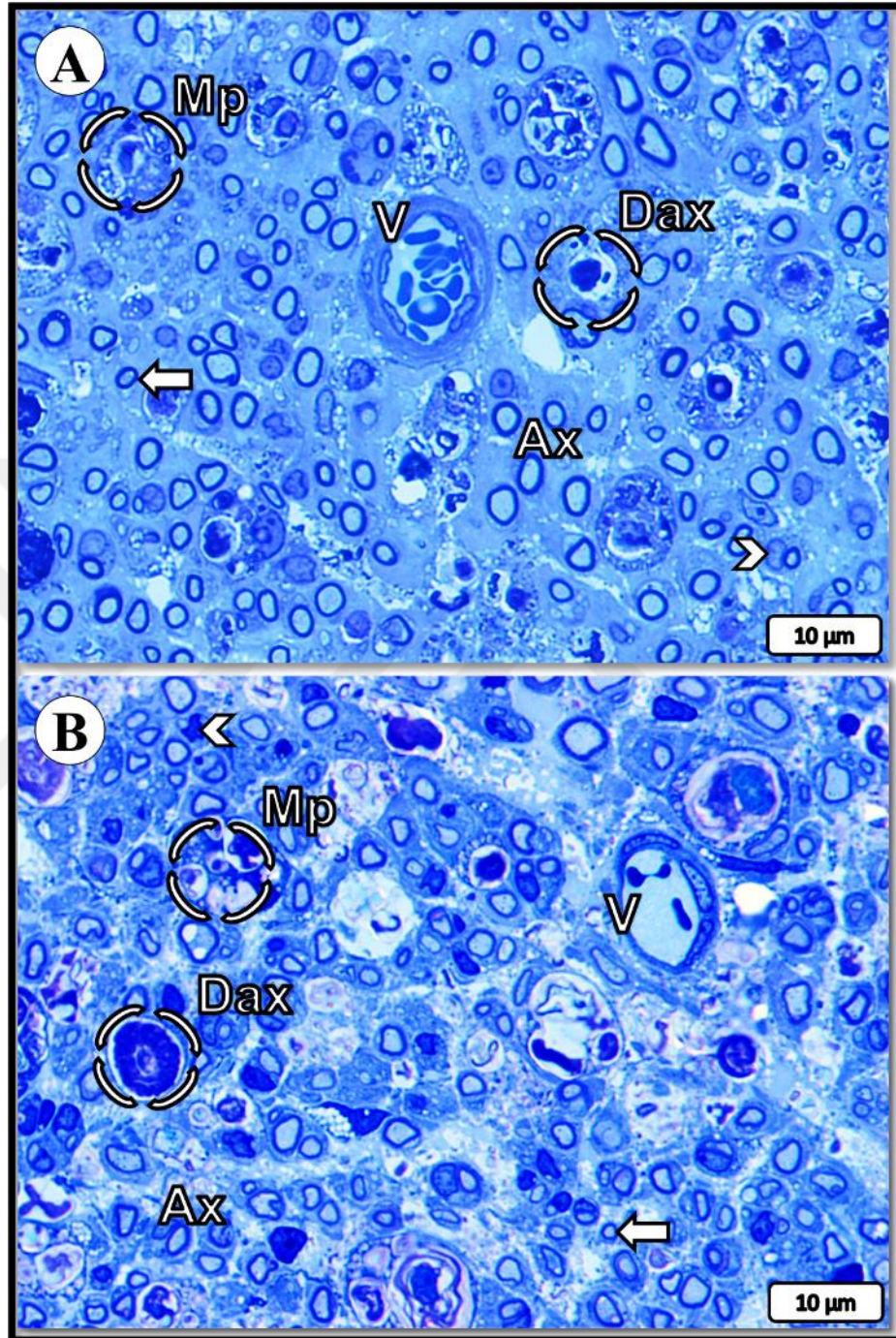


Figure 4.21. (A) and (B) toluidine blue-stained photomicrographs were taken from the sciatic nerve of the Inj+ EVOO group is observed. The degenerated axons (Dax) were seen. The Mp indicator for phagocytosis of myelin droplets by macrophages, the new formed axons were also shown (arrows) and myelinated by Schwann cells (arrow heads). All of these observations follow the crush injury. The blood vessel with its mature endothelial cells (V) was also found. The thickness of the resin section is 0.5 micrometre

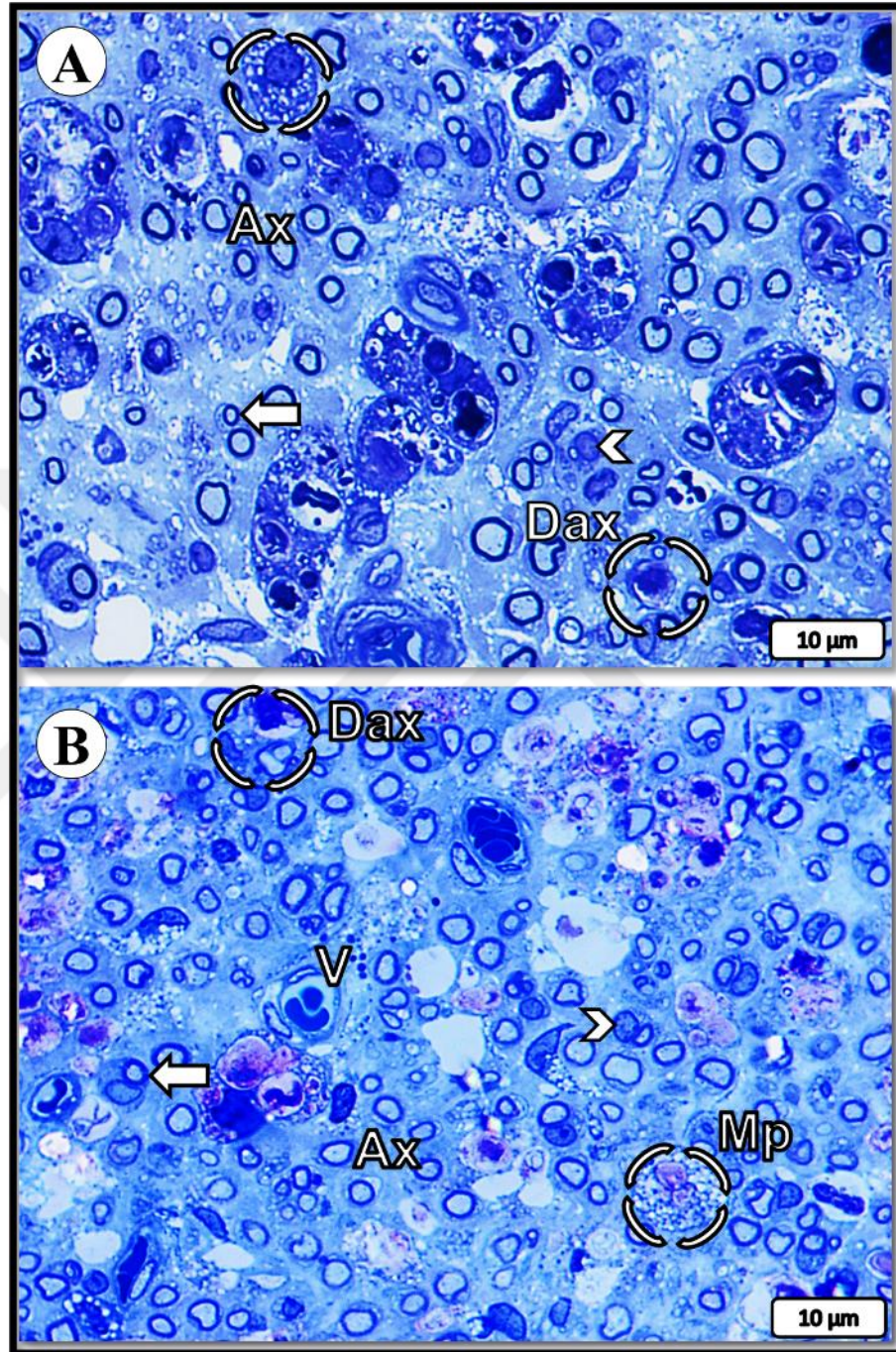


Figure 4.22. (A) and (B) are resin sections with 0.5-micrometre thickness were collected from the sciatic nerve of the Inj+ EVOO group. The macrophages were in large amounts and observed to remove the myeline debris (Mp). The degenerated axons were also observed (Dax). The very small-sized axons (arrows) were also observed. The myelinated axons are protected as an indicator of the regenerative effect of EVOO in terms of axons number. The blood vessels with normal blood cells (V) were abundant as one of the features after degeneration. The section was stained with toluidine blue

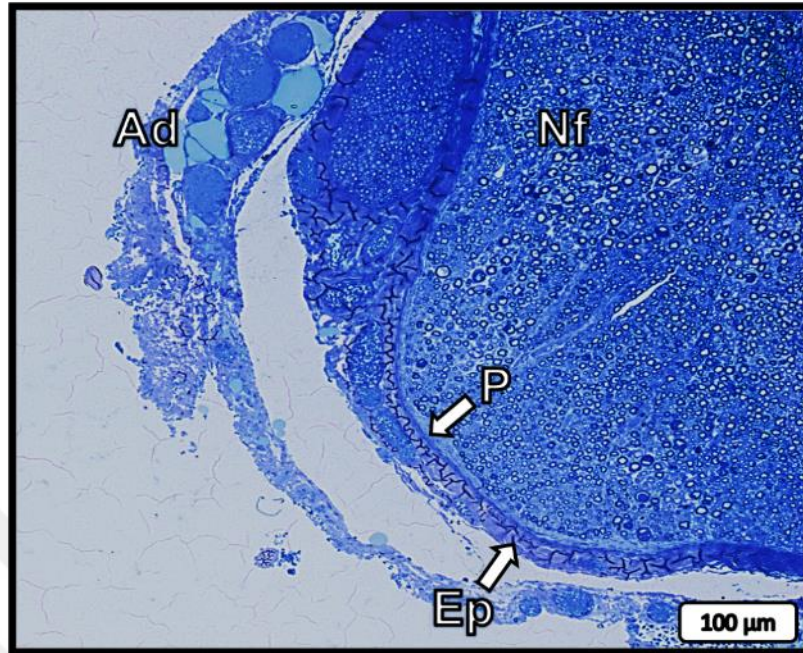


Figure 4.23. An image was collected from the Inj+EOOB group is shown. The general structures of the neural tissue were healthy. The connective tissue which surrounds the whole nerve, epineurium (Ep), was intact. The nerve fascicle (Nf) is covered by normal perineurium (P). The adipose cells (Ad) were also shown. The resin section thickness was 0.5 micrometre and stained with toluidine blue

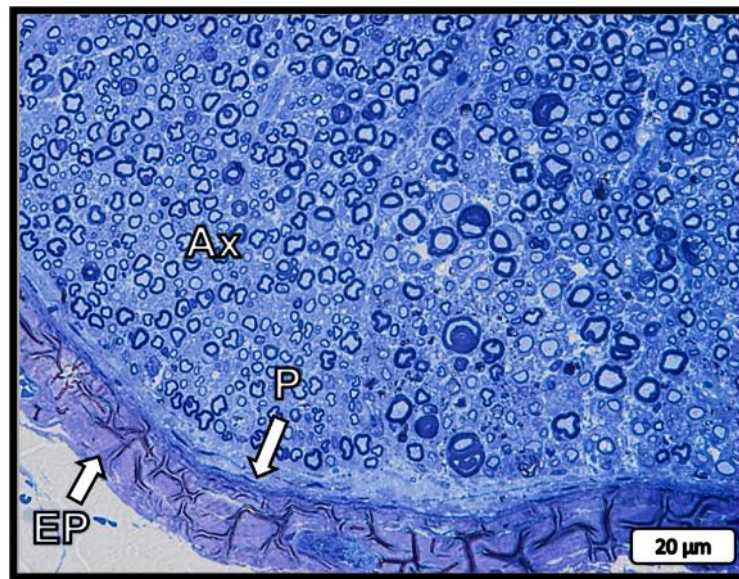


Figure 4.24. The magnified image was taken from the Inj+EOOB group is shown. The general structure of the nerve was normal. The irregular connective tissue, perineurium, was observed intact (P). Ax indicating myelinated axons in the nerve bundle. The resin section thickness was 0.5 micrometre and stained with toluidine blue

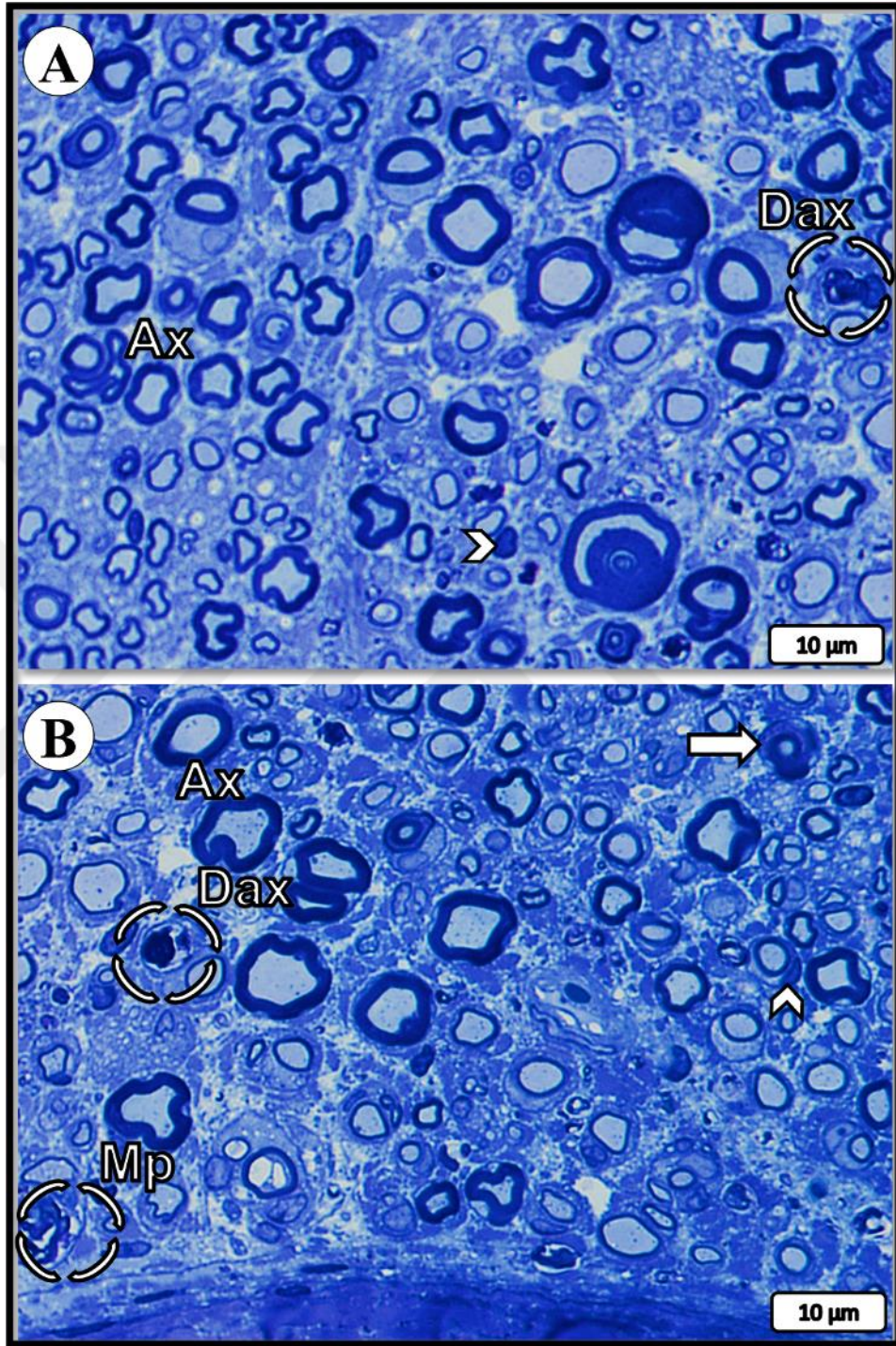


Figure 4.25. (A) and (B) are the 0.5-micrometre thickness of resin sections taken from the sciatic nerve of the Inj+EEOB group. The healthy axons point by Ax. The degenerated axons (Dax) were seen. The macrophage which engulfs the damaged fibres and myelene waste was observed (Mp). Arrows indicated some myelin sheath tubulation and fragmentation; the head arrows pointed to Schwann cells. The images show a reduction of myelinated axons of this group compared to the Inj group. These sections were stained with toluidine blue

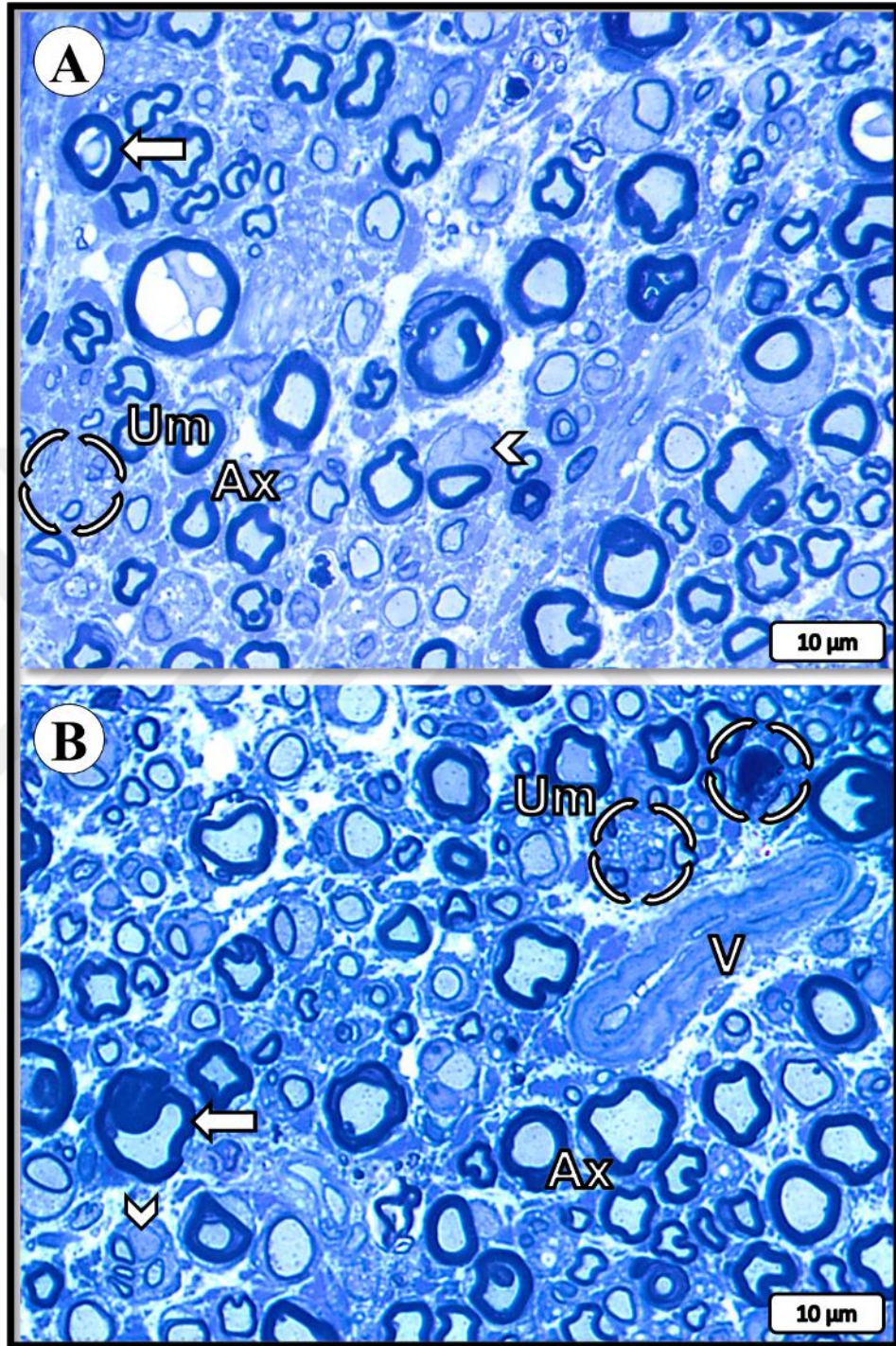


Figure 4.26. (A) and (B) are photomicrographs of resin sections with a 0.5-micrometre thickness taken from the sciatic nerve of the Inj+EOOB group. The normal axons point by Ax. The degenerated axons (Dax) were found. Arrows indicated some myelin sheath tubulation and fragmentation; the head arrows pointed to Schwann cells. The images prove that the EOOB enhances regeneration by decreasing the number of myelinated axons of this group compared to the Inj group. These sections were stained with toluidine blue

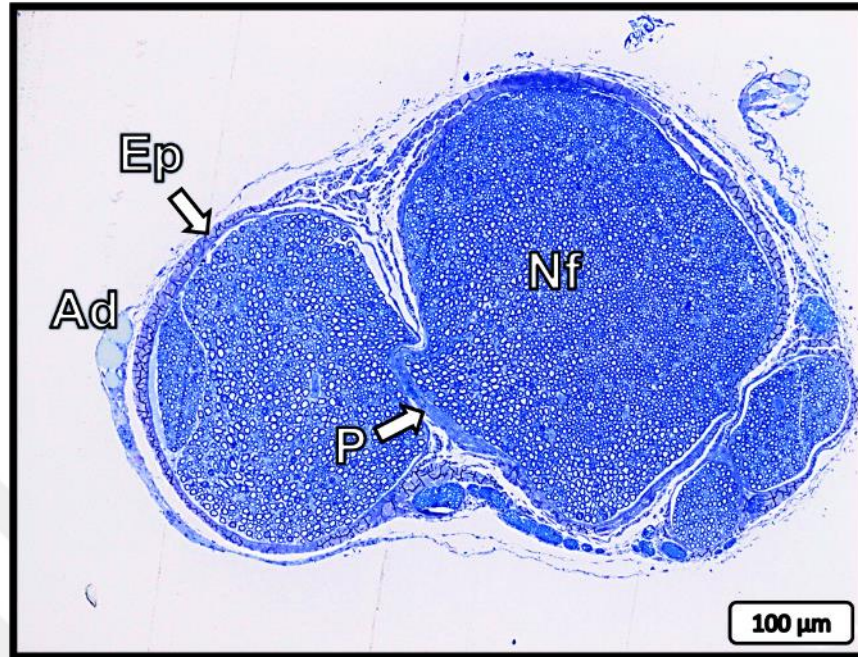


Figure 4.27. A cross-section image was taken from the sciatic nerve of the AD group. The structural view of the nerve was healthy. The intact epineurium (Ep) delimits the fascicle (Nf), and normal perineurium (P) around the bundles of the nerve were seen. Adipose cells (Ad) were also found. The resin section thickness was 0.5 micrometre and stained with toluidine blue

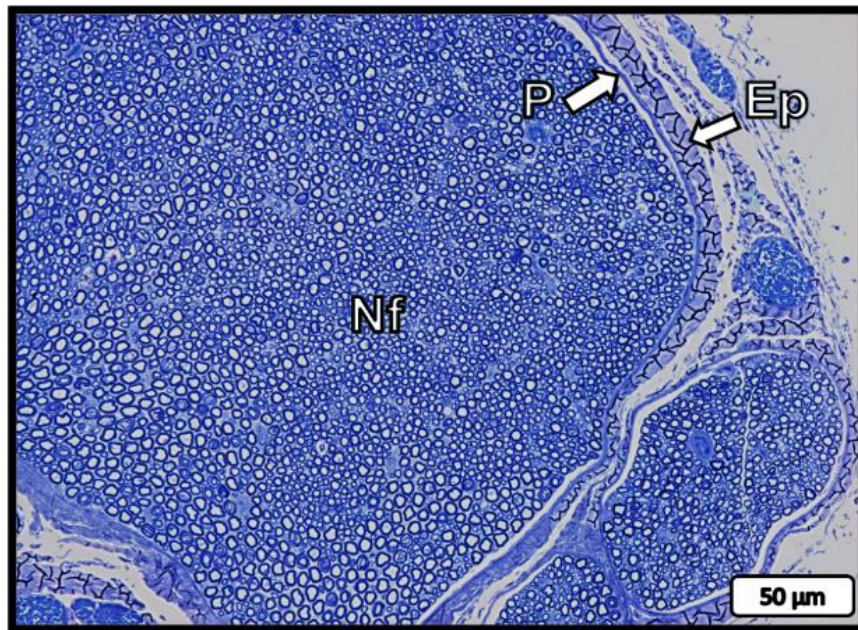


Figure 4.28. Different magnification of photomicrograph was collected from the AD group. It shows the histological structure of the nerve was normal. The irregular connective tissue, perineurium, was observed intact (P) and enclosed the nerve fascicle (Nf). The resin section thickness was 0.5 micrometre and stained with toluidine blue

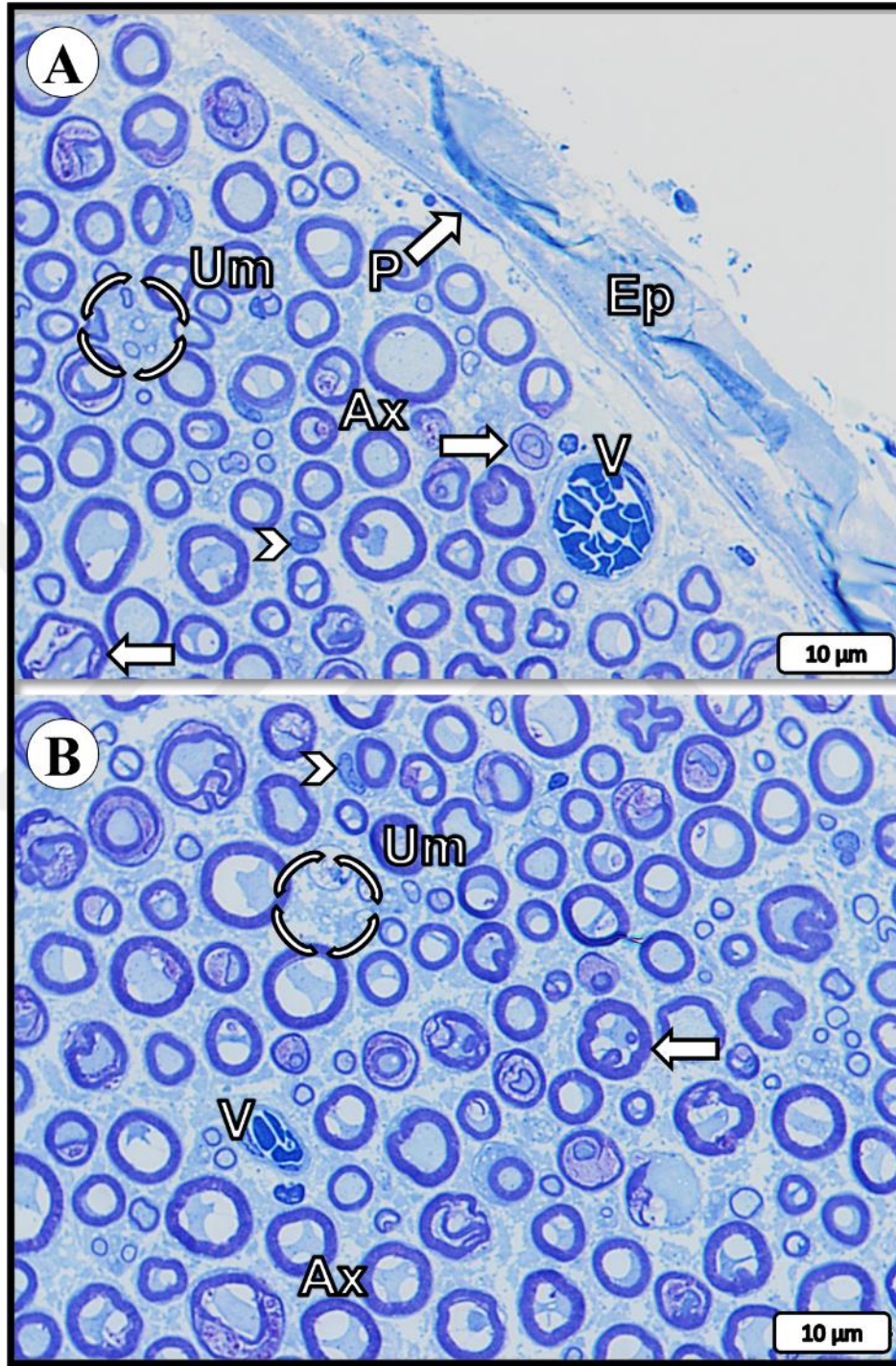


Figure 4.29. (A) and (B) photomicrographs of cross-sections collected from the AD group showed nerve tissue histology; normal different-sized axons (Ax) and delimited by a thick sheath. The connective tissue of the nerve, epineurium (Ep) and perineurium (P) observed intact. A few axons looked with impaired, such as cleft or fragmentation and indicated by arrows. The Um is an indicator for unmyelinated axons. The arrow heads were demonstrated the Schwann nuclei. The blood vessels were found and marked by (V). The resin section thickness was 0.5 micrometre and stained with toluidine blue

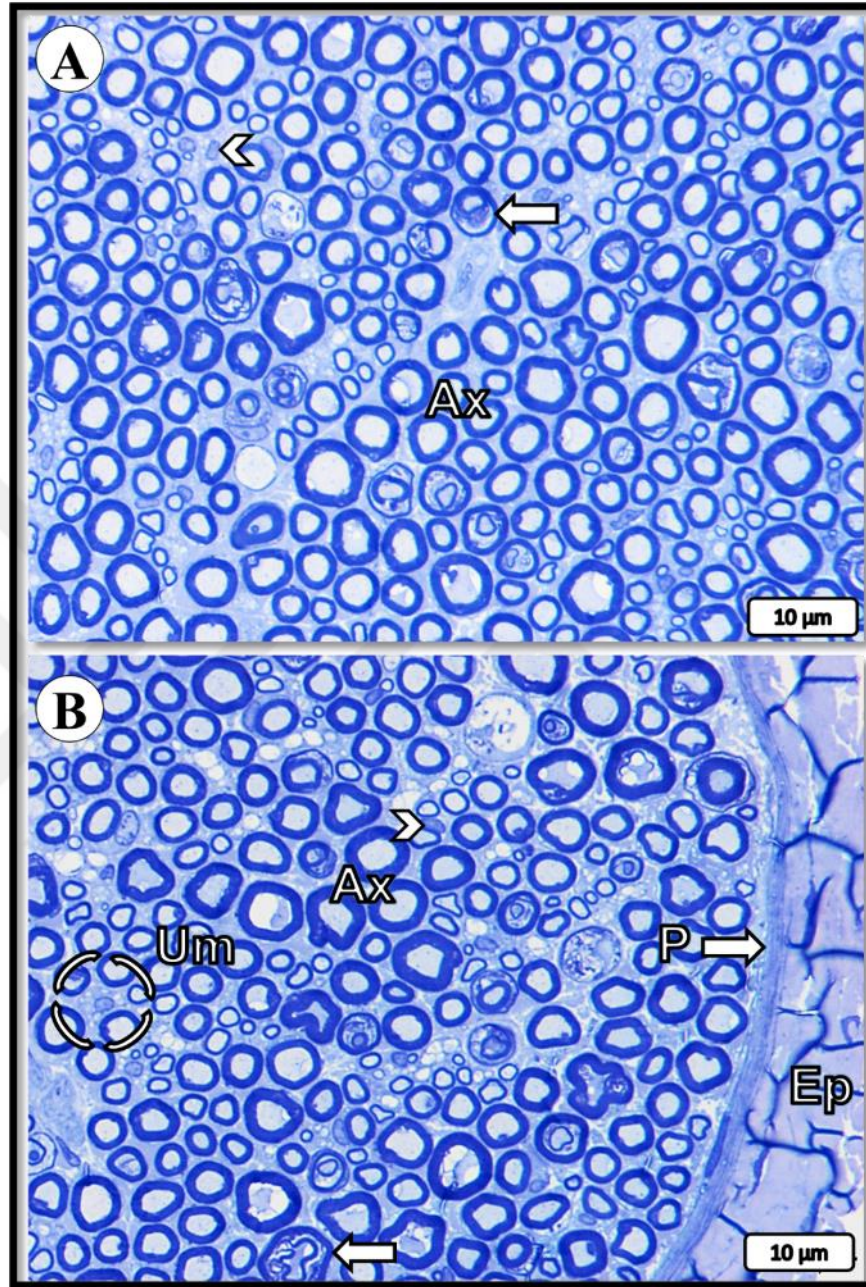


Figure 4.30. (A) and (B) are cross-section images taken from the AD group. They showed nerve tissue features, the connective tissue of the nerve, epineurium (Ep), and perineurium (P) observed intact. A normal different sizes axon (Ax) and delimited by a thick sheath. A few axons looked with impaired, such as cleft or fragmentation and indicated by arrows. The Um is an indicator for unmyelinated axons. The arrow heads were demonstrated the Schwann nuclei. The resin section thickness was 0.5 micrometre and stained with toluidine blue

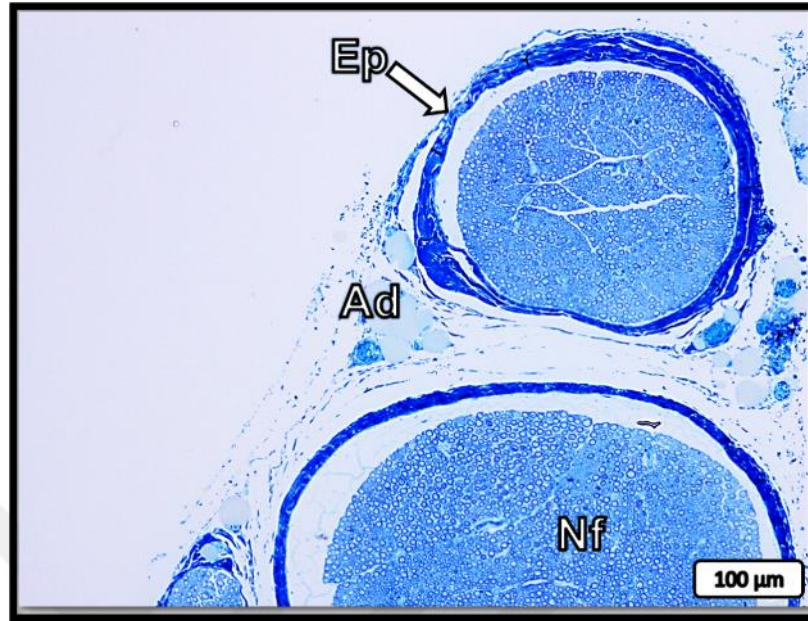


Figure 4.31. This photomicrograph is representing the sciatic nerve of the EVOO group. The general structural view of the nerve was normal. The intact epineurium (Ep) delimits the fascicle (Nf) was seen. Adipose cells (Ad) were also found around the nerve section. The toluidine blue-stained resin section was a 0.5-micrometre thickness

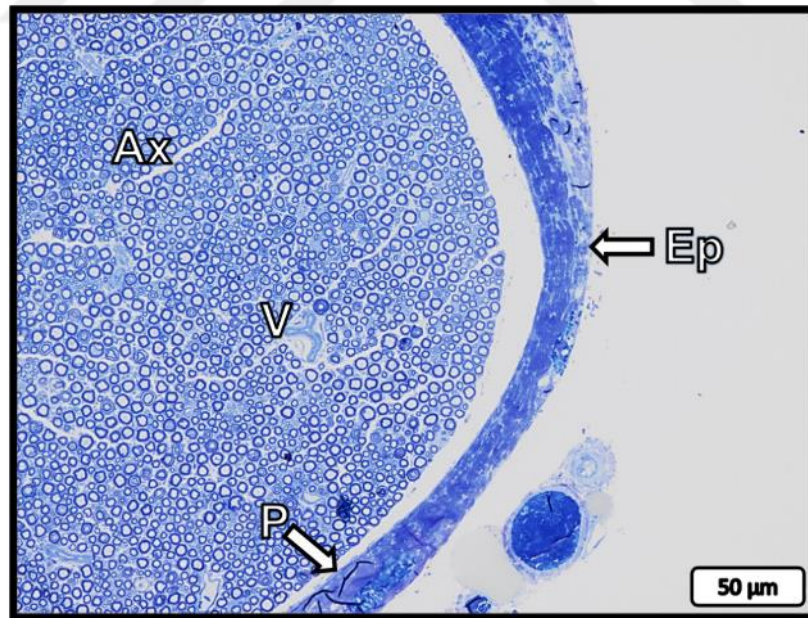


Figure 4.32. This magnified photomicrograph is representing the sciatic nerve of the EVOO group. The general structure of the nerve was normal. The perineurium (P) was observed intact. The normal nerve myelinated fibres (Ax) with different sizes were delimited by a thick sheath. The blood vessels and blood cells were also observed (V). The resin section thickness was 0.5 micrometre and stained with toluidine blue

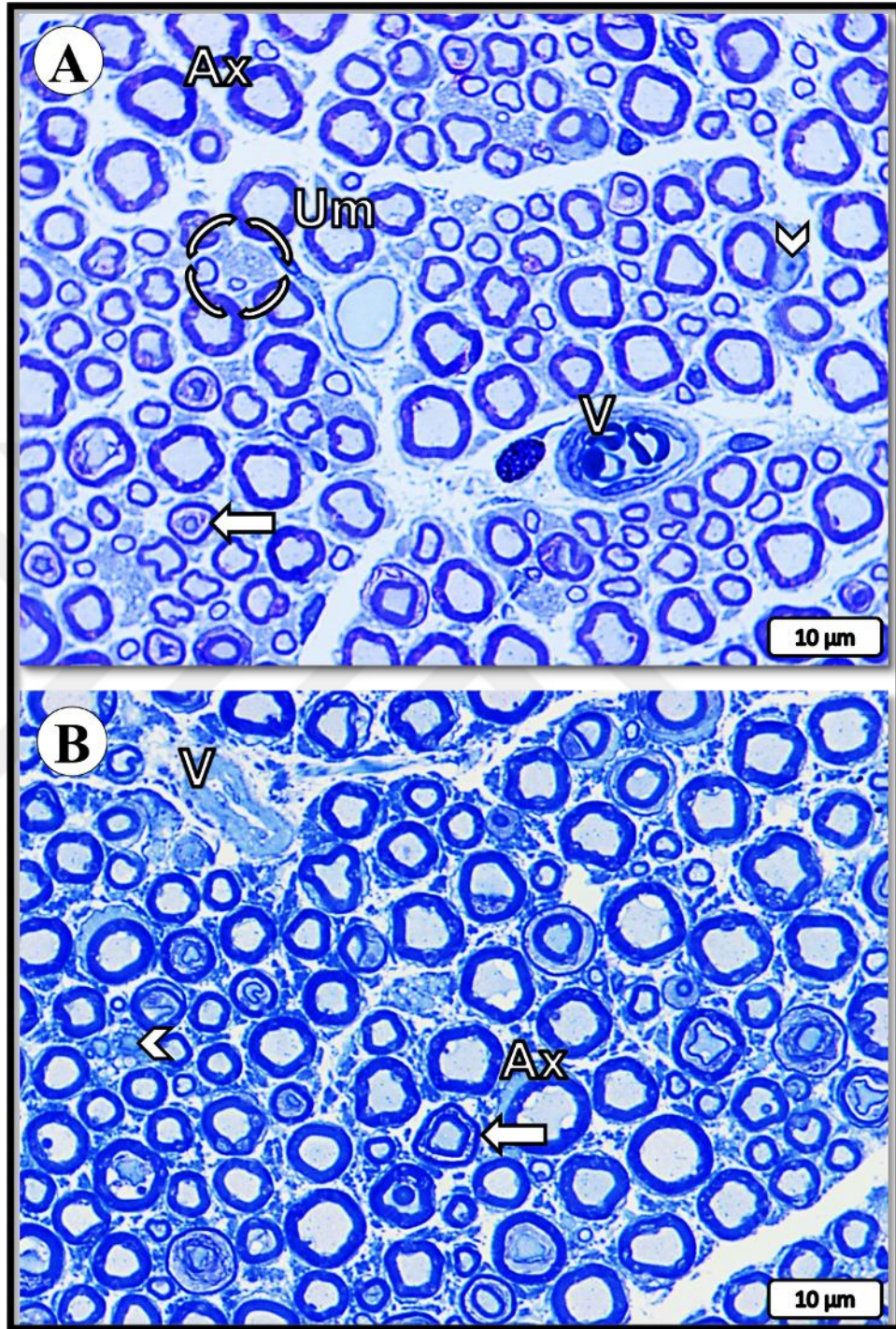


Figure 4.33. (A) and (B) toluidine blue-stained photomicrographs were taken from the sciatic nerve of the EVOO group. Arrowheads indicate Schwann cells. The different sizes normal of myelinated axons (Ax) were seen and enclosed by a thick sheath. The Um indicates an unmyelinated axon. The blood vessels and blood cells were also observed (V). The thickness of the resin section is 0.5 micrometre

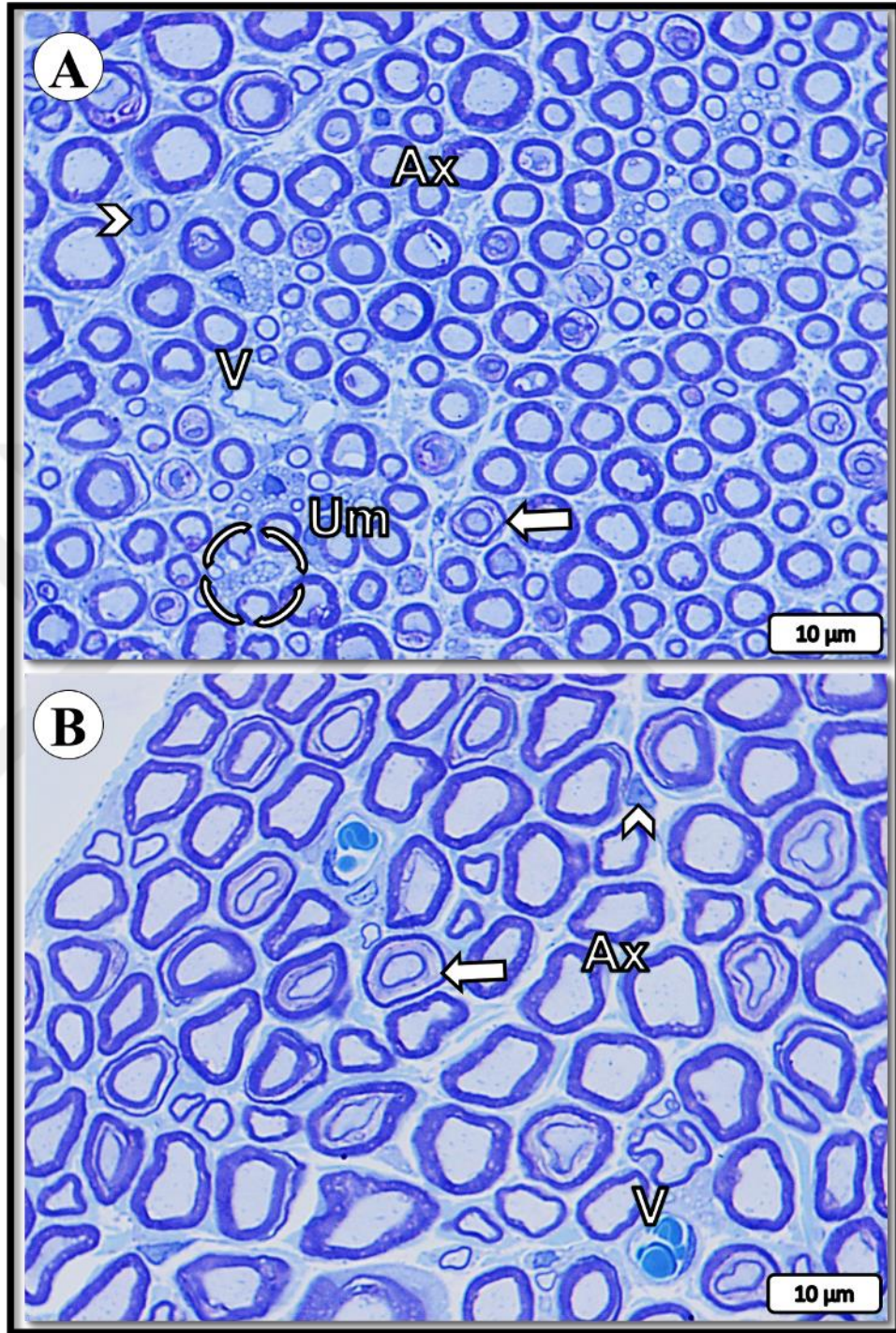


Figure 4.34. (A) and (B) photomicrographs were stained by toluidine blue taken from the sciatic nerve of the EVOO group. The different sizes normal of myelinated axons (Ax) were seen and covered by a thick sheath. Arrowheads indicated the Schwann cells; Arrows were pointed to the myelin cleft/tubulation as myelin sheath impairments. The Um indicates an unmyelinated axon. The blood vessels and blood cells were also observed (V). The thickness of the resin section is 0.5 micrometre

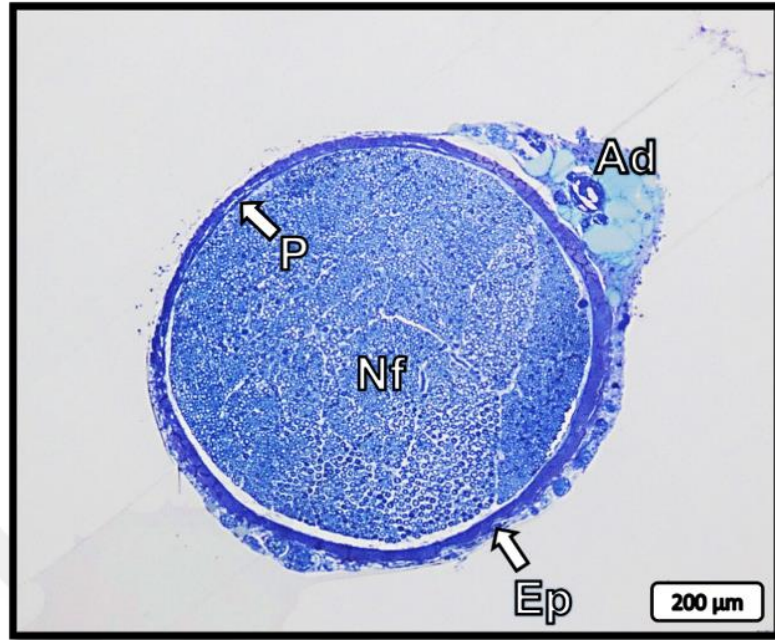


Figure 4.35. A cross-section image was taken from the sciatic nerve of the EOOB group. The structural view of the nerve was healthy. The intact epineurium (Ep) delimits the fascicle (Nf), and normal perineurium (Ep) around the bundles of the nerve were seen. Adipose cells (Ad) were also found. The resin section thickness was 0.5 micrometre and stained with toluidine blue

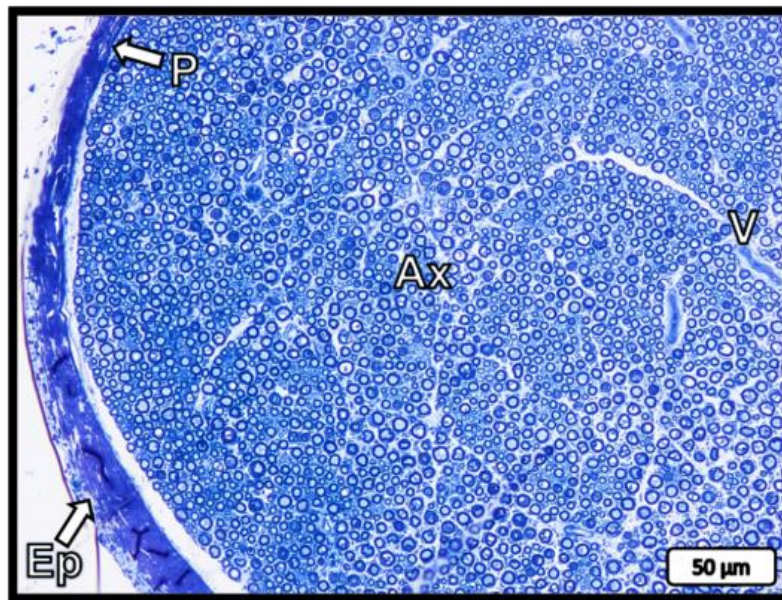


Figure 4.36. The photomicrograph was taken from the sciatic nerve of the EOOB group. The structural view of the neural tissue was normal. The intact epineurium (Ep) delimits the fascicle and axons (Ax), and normal perineurium (Ep) around the bundles of the nerve were seen. The blood vessels (V) were shown normally in the nerve bundle and surrounding connective tissue. The resin section thickness was 0.5 micrometre and stained with toluidine blue

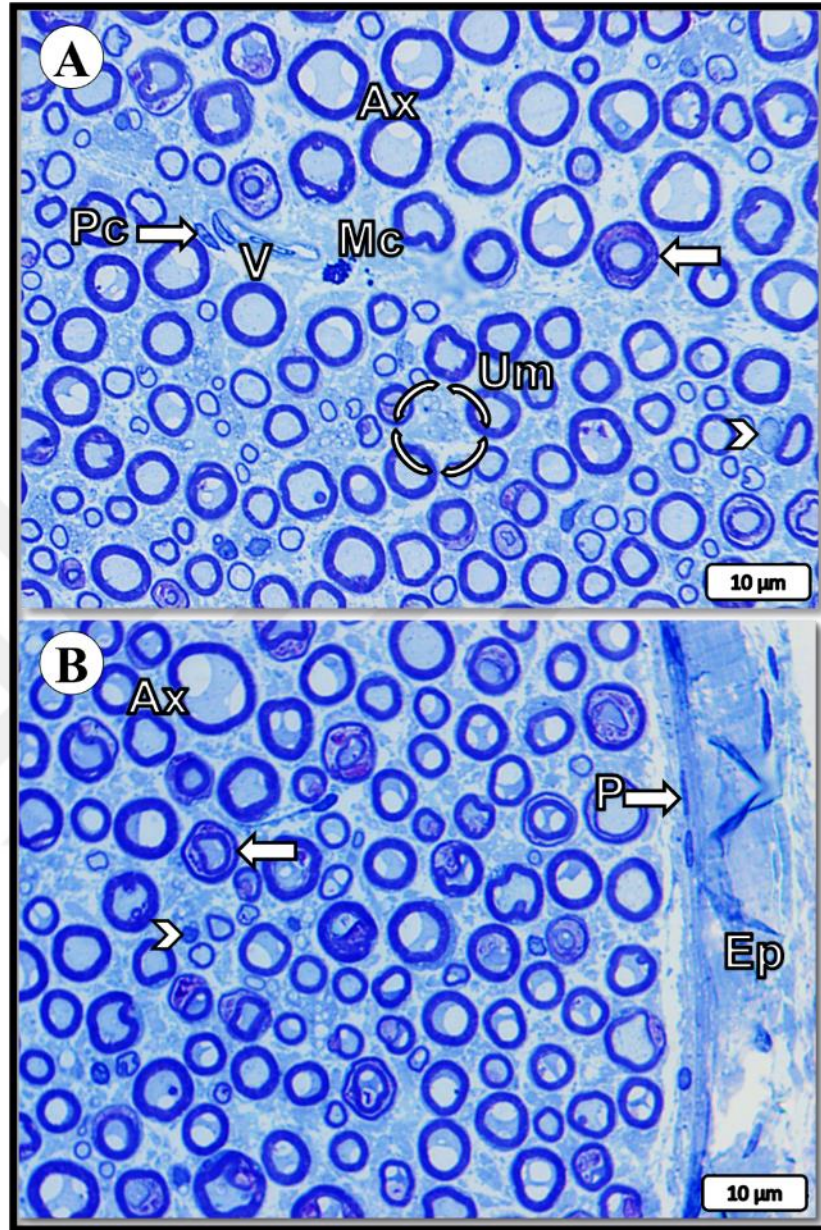


Figure 4.37. (A) and (B) magnified photomicrographs were collected from the EOOB group. P is indicating perineurial cell. Most of the myelinated axons (Ax) with variable sizes were looks normal and surrounded by a thick myeline sheath. There were some of the myeline sheath impairments (arrows), like tubulation. The mast cells (Mc) were found among the connective tissue of the nerve. Pc indicated for pericyte. The resin section thickness was 0.5 micrometre and stained with toluidine blue

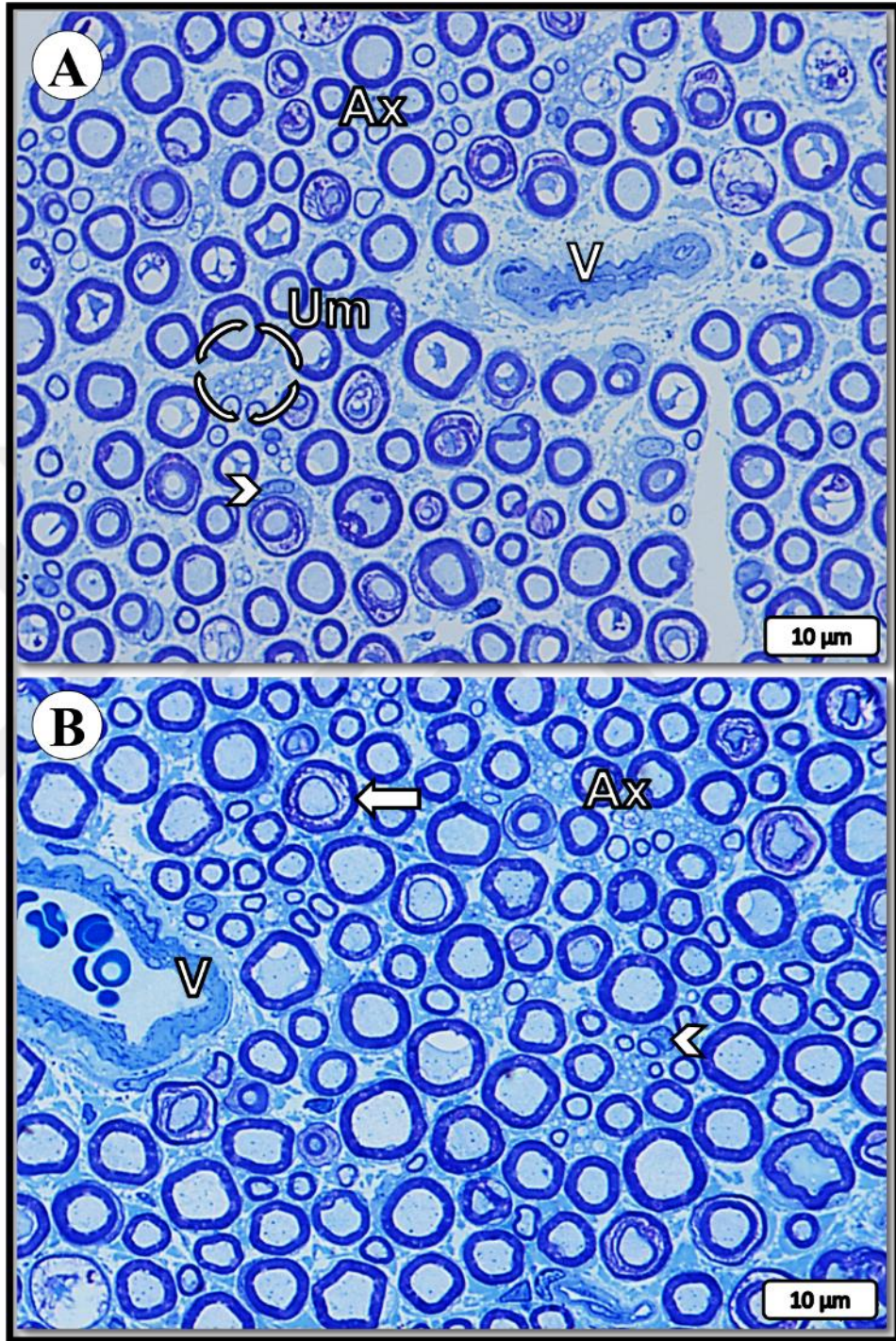


Figure 4.38. (A) and (B) are cross-sectioned images are collected from the EOOB group. It shows the dominance of normal myelinated nerve fibres (Ax), which are surrounded by a thick myeline sheath; the unmyelinated fibres were also observed (Um), the blood vessel appeared normal (V), and the SCs nuclei (black arrow heads) were also found. There were some of the myeline sheath impairments (arrow), like tubulation. The thickness of this resin section was 0.5 micrometre and stained with toluidine blue

4.1.4. Electron Microscopical (EM) Evaluation Results

The sciatic nerve thin cross-sections were taken and stained by lead citrate and uranyl acetate to be evaluated by transmission electron microscope (TEM). The obtained results for each group are seen below.

4.1.4.1. Electron Microscopic Findings of Cont Group

The cross-sections of the sciatic nerve, which belong to the Cont group with thin thickness, were collected and evaluated by TEM. The structural view of nerve tissue appeared healthy. The myelinated nerve fibres were observed normally. Different thicknesses of myeline sheath were seen around the axons. Some vacuoles and clefts of myeline sheath were seen. Besides, the unmyelinated axons groups were observed with obvious limitations. The Schwann cells also were seen very clear. Moreover, the blood vessel was shown with its mature endothelial cells. All of these observations were consistent with the Cont group (Figure 4.33).

4.1.4.2. Electron Microscopic Findings of Inj Group

The sciatic nerve thin cross-sections belong to the Inj group were examined by TEM. The histology of the nerve tissue was affected due to crushing injury application. The following features were shown after applying crush injury. Losing of nerve integrity, the degenerated axons, and macrophages that clean the myeline droplets were abundant. Also, the newly formed baby axons were seen as they started to be myelinated with thin myeline sheath by Schwann cells; therefore, the heterogeneity view of axons size is prominent in this group. The myelin sheath of a few axons showed physical impairment due to tissue processing or unknown reasons. Furthermore, some physiological defects of the myeline sheath were also observed, such as Schmidt-Lanterman clefts. The collection of unmyelinated nerve fibres was seen clearly with defined boundaries. The loose connective tissue between the nerve fibres is seen intact. The premature blood vessel with its premature endothelial cells (angiogenesis), and pericytes (which might be converted to macrophage) were major observations of the Inj group (Figure 4.34-35).

4.1.4.3. Electron Microscopic Findings of Inj+ AD Group

Thin cross-sections of the sciatic nerve were collected from the Inj+AD group and evaluated by TEM. When the injured nerve was treated with AD, it is seen that the general structural view of the nerve fibres looks healthy. The macrophages and degenerated axons were seen clear in the injury site. Schwann cells enclose myelinated axons are observed. Myelin sheath around various-sized myelinated nerve fibres is seen well protected, most of the axons with thick myelene sheath due to the positive impact of AD. Myelene sheath impairments such as tubulation, vacuolization and fragmentation were also seen. A collection of unmyelinated axons was demonstrated. The connective tissue with their fibroblast was intact and observed. The blood vessels were scattered with mature endothelial cells (Figure 4.36-37).

4.1.4.4. Electron Microscopic Findings of Inj+ EVOO Group

A thin cross-section of the sciatic nerve from the Inj+EVOO group was taken and examined by TEM. The histomorphology of the nerve looks affected after applying crush injury. The section recognises the degenerative features such as axons losing, degenerated axons, macrophages that phagocytise the myelin debris of damaged axons and small-sized newly formed axons. Schwann cell nucleus and the blood capillary with its premature endothelial cell were also observed. The protected myelinated axons were observed that prove the positive effect of EVOO. The loose connective tissue of the nerve was seen clearly, as well the collection of unmyelinated axons is also observed (Figure 4.38-39)

4.1.4.5. Electron Microscopic Findings of Inj+ EOOB Group

A thin cross-section of the sciatic nerve belongs to the Inj+EOOB group was collected and evaluated by TEM. The structural view of the nerve looks affected partially after applying crush injury. The heterogeneity of axons size was observed. The degenerative changes were observed in the section, such as loss of the axons; degenerated axons, macrophages that phagocytizing the myelin debris of damaged axons, and small-sized newly formed axons were seen. Schwann cell nucleus and the blood capillary with its premature endothelial cell were also observed. The protected

myelinated axons were observed that prove the positive effect of EOOB. Some impaired myelin sheath, sheath fragmentation and cleft were observed. The loose connective tissue of the nerve was seen clearly, besides the collection of unmyelinated axons. The mast cells were also seen in section (Figure 4.40 -41).

4.1.4.6. Electron Microscopic Findings of AD Group

The sciatic nerve cross-section with a thin thickness micrograph was taken from the AD group and evaluated by an electron microscope. The general nerve elements look healthy. A thick and thin myeline sheath ensheathed the nerve fibres. Some myelin sheath physical impairments such as cleft and fragmentation were observed. The mast cell was seen in the intact connective tissue of the nerve, as well as the blood vessel with mature wall endothelial cells were also shown. The aggregation of unmyelinated axons with well-defined boundaries also appeared (Figure 4.42).

4.1.4.7. Electron Microscopic Findings of EVOO Group

The micrograph of the sciatic nerve cross-section with thin thickness was taken from the EVOO group and evaluated by an electron microscope. The general morphology of the neuronal tissue appeared normal. The nerve fibres had uniform myeline distribution and structure with different thicknesses of myelin sheath were seen. Some vacuoles, clefts and fragmentation of myelin sheath were seen, these physical impairments. The connective tissue of the nerve was intact as the perineurial cells were very clear. The Schwann cells nucleus were also observed. The collection of unmyelinated axons with well-defined borders also appeared (Figure 4.43).

4.1.4.8. Electron Microscopic Findings of EOOB Group

The sciatic nerve thin cross-section belongs to the EOOB group was examined by electron microscope. The histology of the nerve tissue was normal. The general structure view of the nerve looks normal. The connective tissue of the nerve was intact. Different size of myelinated axons was seen. Impaired axons were also seen. The aggregation of unmyelinated fibres was seen. The Schwann cells nucleus were also seen. The blood vessel with mature wall endothelial cells were also observed (Figure 4.44).

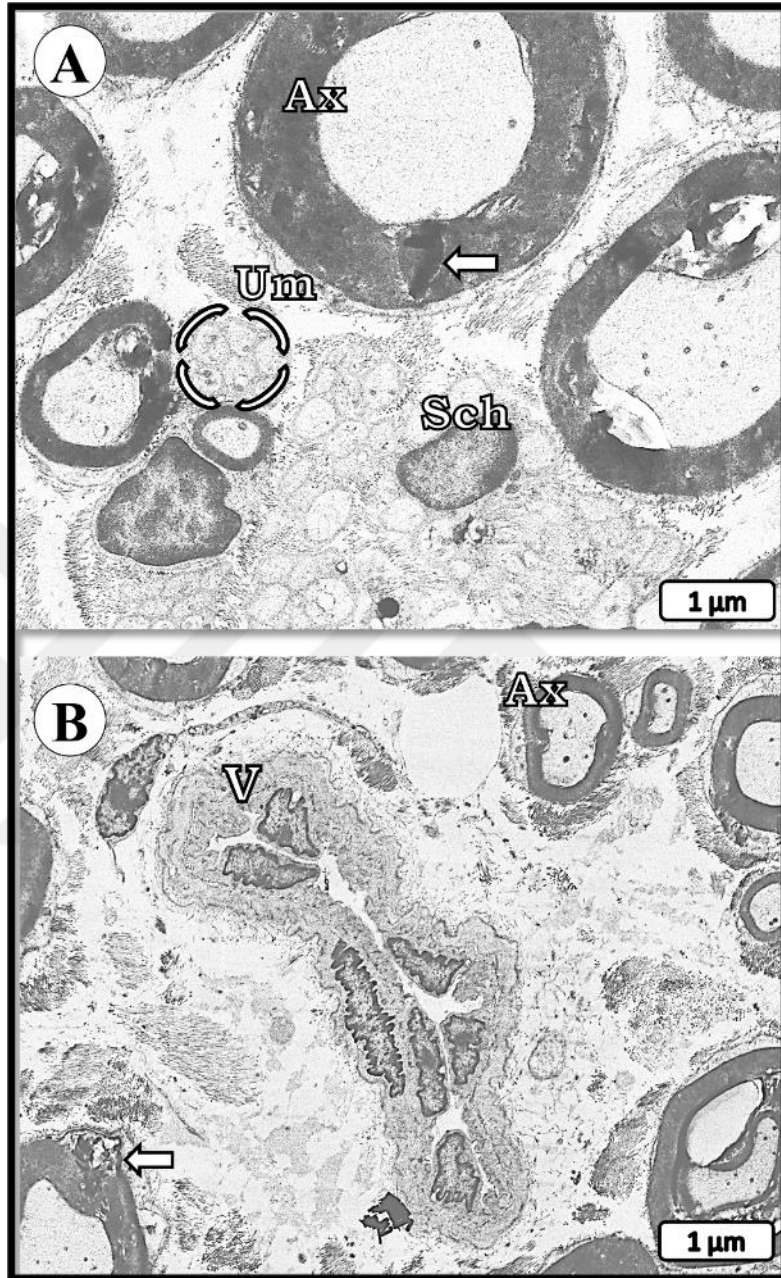


Figure 4.39. (A) and (B) are electron microscopic images of sciatic nerve cross-section were collected from the Cont group. The nerve elements look normal. The nerve fibres were myelinated with thick and thin myelin sheath (Ax). Some vacuoles of myeline sheath were observed (arrow). The unmyelinated axons were seen with clear borders (Um); also, the Schwann cell that myelinates the nerve fiber was very obvious (Sch). The blood vessel looks normal with mature endothelial cell (V)

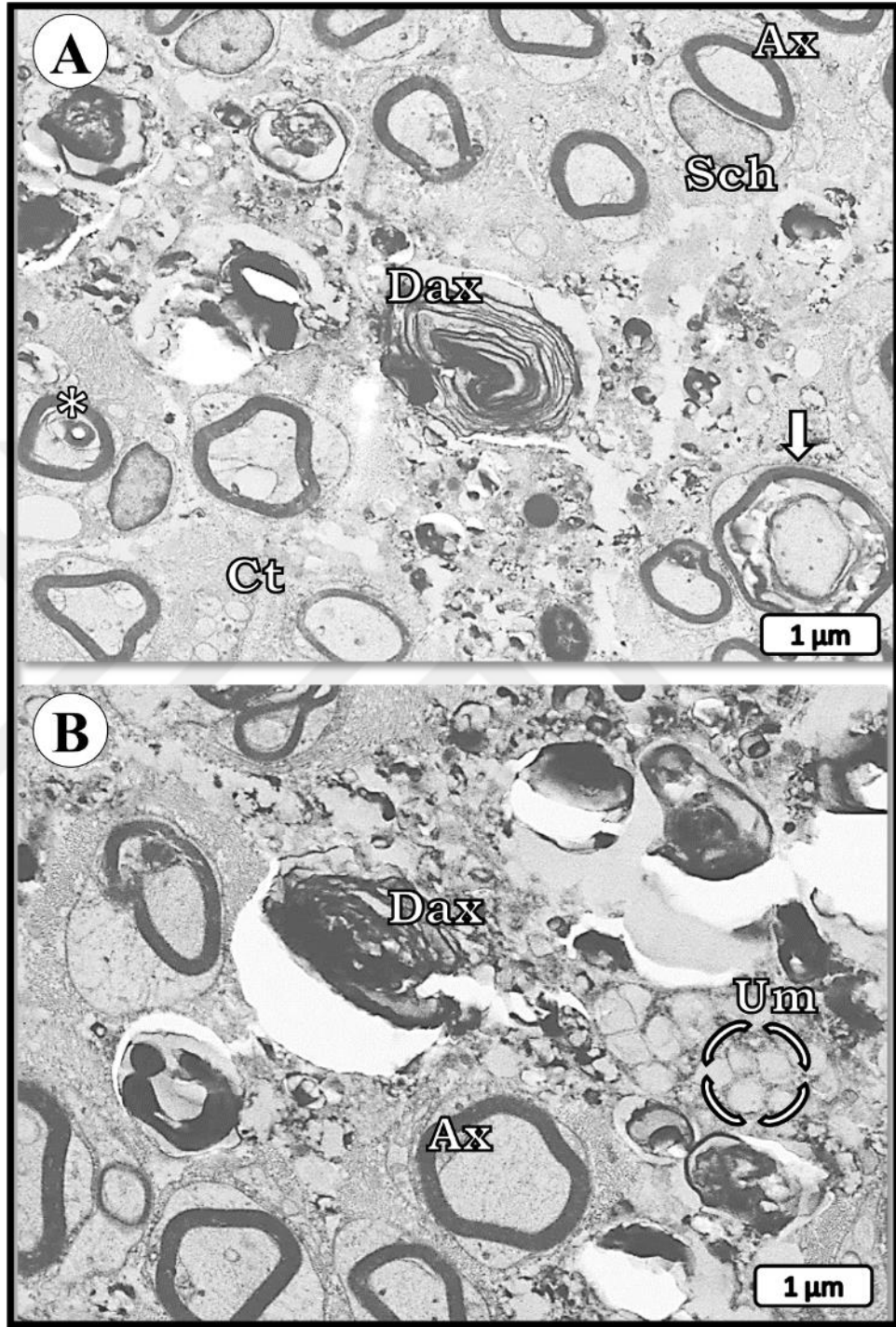


Figure 4.40. (A) and (B) electron microscopic images of sciatic nerve cross-section belonged to the Inj group. The following observed features are shown after applying crush injury. The degenerated axons (Dax), were abundant. Also, newly baby formed axons (Ax) and the Schwann cells (Sch) to myelinate them with thin sheath were appeared. The heterogeneity of axons size was observed. Cleft (arrow) or fragmentation (star) of myeline sheath were seen in large nerve fibres. The loose connective tissue between the nerve fibres is seen intact (Ct). Collection of unmyelinated nerve fibres was observed (Um) obviously

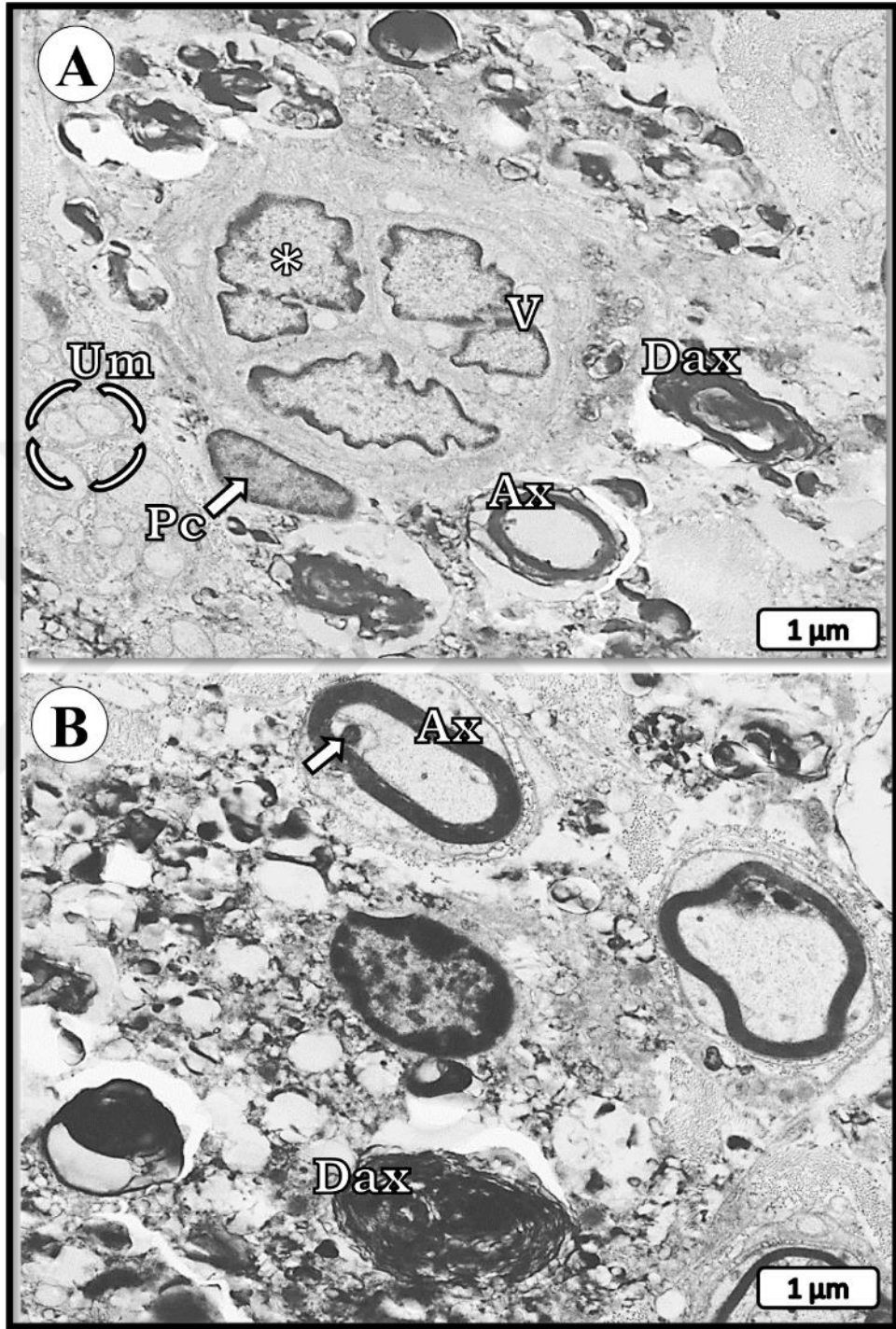


Figure 4.41. (A) and (B) are electron microscopic images of sciatic nerve cross-section belongs to the Inj group. The following observed features are shown after applying crush injury. The degenerated axons (Dax) were abundant. Also, newly formed baby axons (Ax) were seen. The collection of unmyelinated nerve fibres was seen (Um) clearly. The immature blood vessel (V) with its premature endothelial cells (*); angiogenesis was observed, the (Pc) indicates pericyte tend to convert to macrophage. Fragmentation of myeline sheath (arrow) was also seen in large nerve fibres

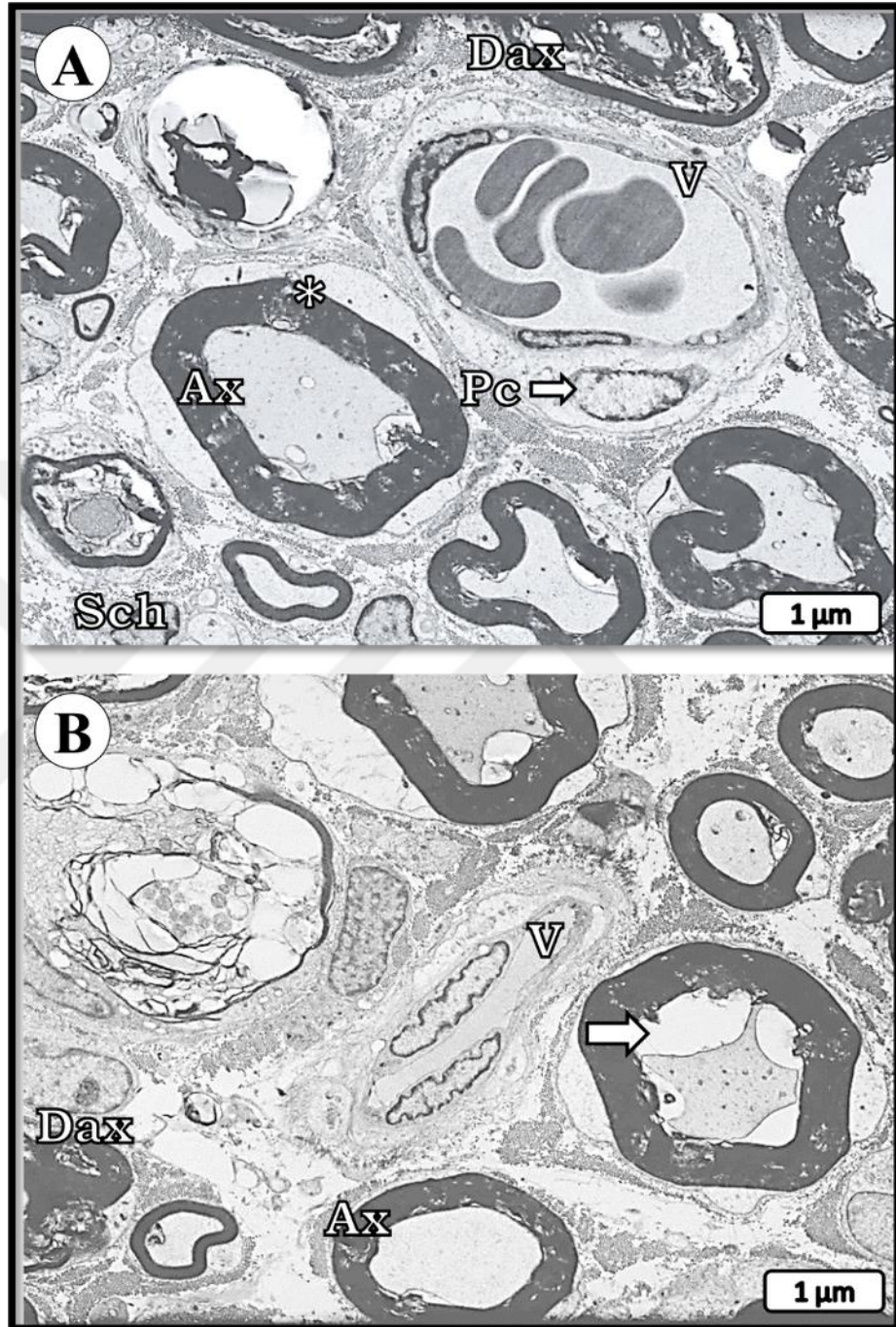


Figure 4.42. (A) and (B) electron microscopic images of sciatic nerve cross-section belong to the Inj+AD group. The general structure of the neuronal tissue was affected due to injury application. The degenerated axons (Dax) were abundant and observed very clear. The myelinated axons are variable in size (Ax), but most of them with thick myelin sheath due to the positive effect of AD. Some sheath impairments were seen, such as vacuolisation (*). Sch indicates Schwann cells. The blood vasculature with mature endothelial cells was seen. Also, (Pc) indicates the pericyte located close to the vessel. Some sheath impairments were seen, such as balloon-shaped vacuolisation (arrow)

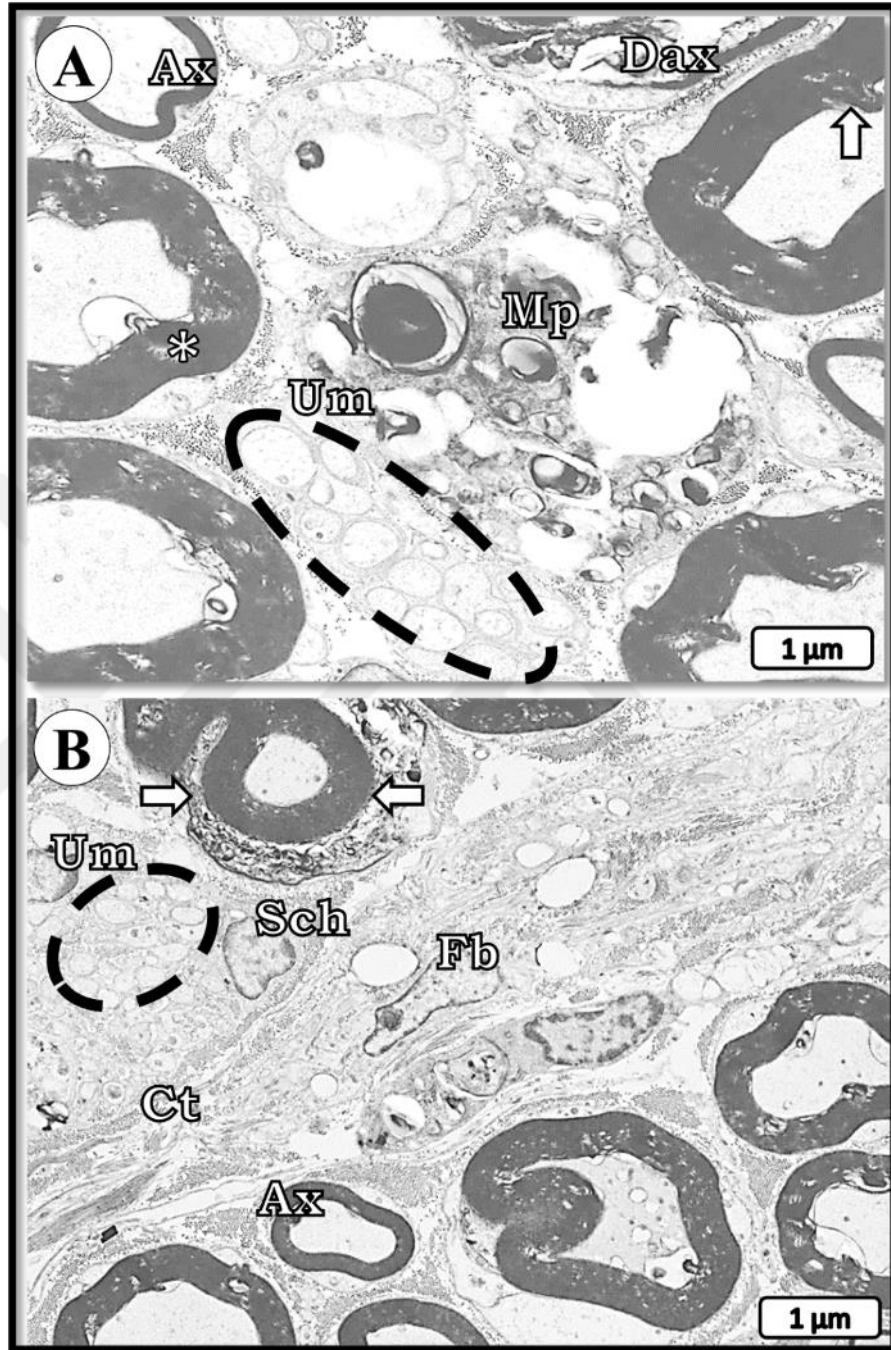


Figure 4.43. (A) and (B) are electron microscopic photos of sciatic nerve cross-sections belong to the Inj+AD group. Substantial changes were observed after crush injury application. Most of the myelinated axons lost their circular shape, the macrophages (Mp), which clean the myeline sheath droplets, and degenerated axons were seen extensively. The myelinated axons vary in size (Ax), but most of them have thick myeline sheath due to the positive impact of AD. Myeline sheath impairments were also seen, such as fragmentation (arrow), vacuolisation (*) tubulation (double arrow). A collection of unmyelinated axons was demonstrated (Um). The connective tissue (Ct) with their fibroblast (Fb) were intact and observed

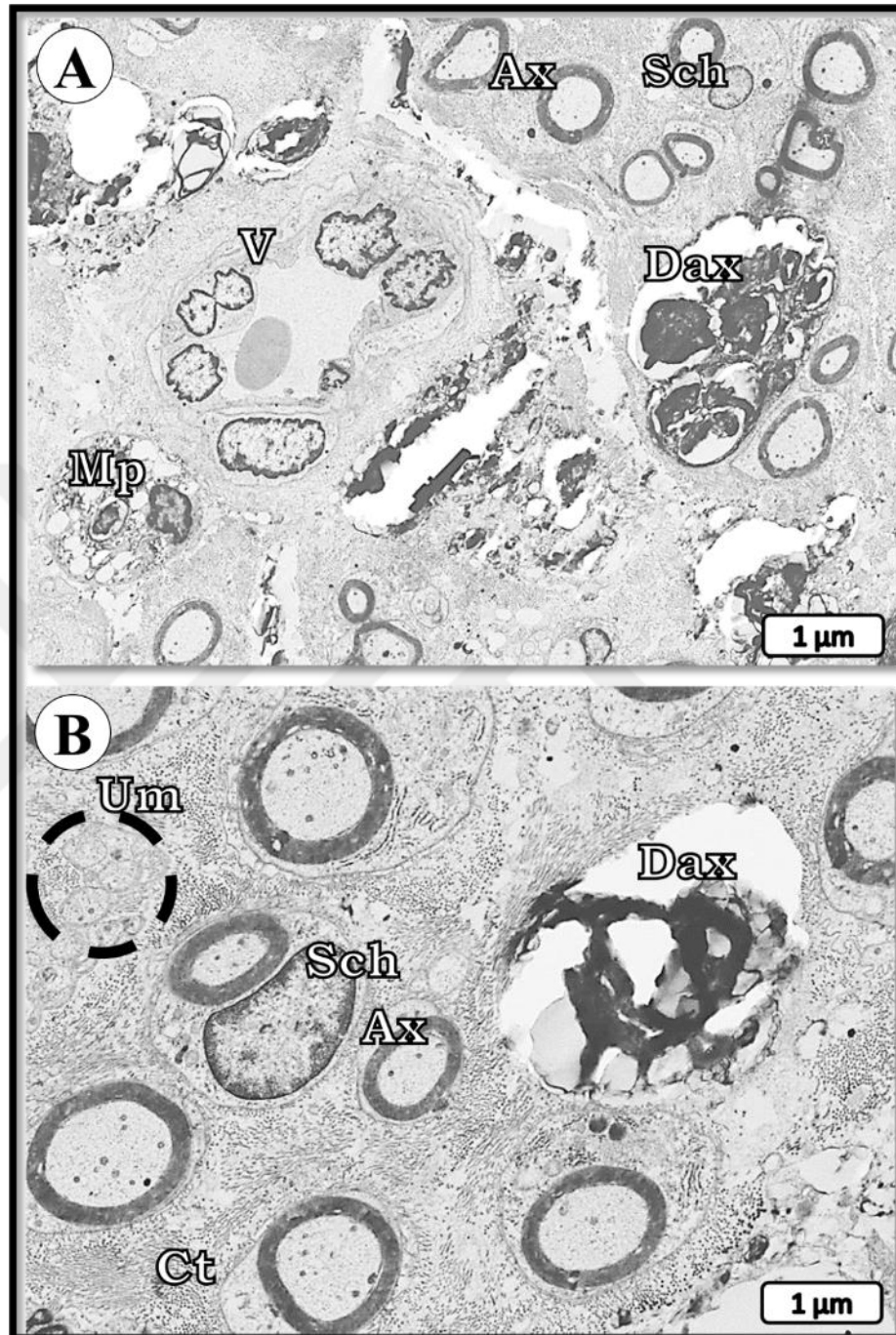


Figure 4.44. EM cross-section images of the sciatic nerve belongs to the Inj+EVOO group. The histomorphology of the nerve looks affected after applying crush injury. The degenerative features were recognized in the section, such as huge space occupied by connective tissue (Ct) between the axons and baby newly formed axons, degenerated axons (Dax) and macrophages (Mp) that phagocytising the myelin debris of damaged axons. Sch indicates Schwann cell. The blood capillary with its premature endothelial cell was also observed. Ax indicates the protected myelinated axons that prove the positive effect of EVOO. Um marks the aggregation of unmyelinated axons

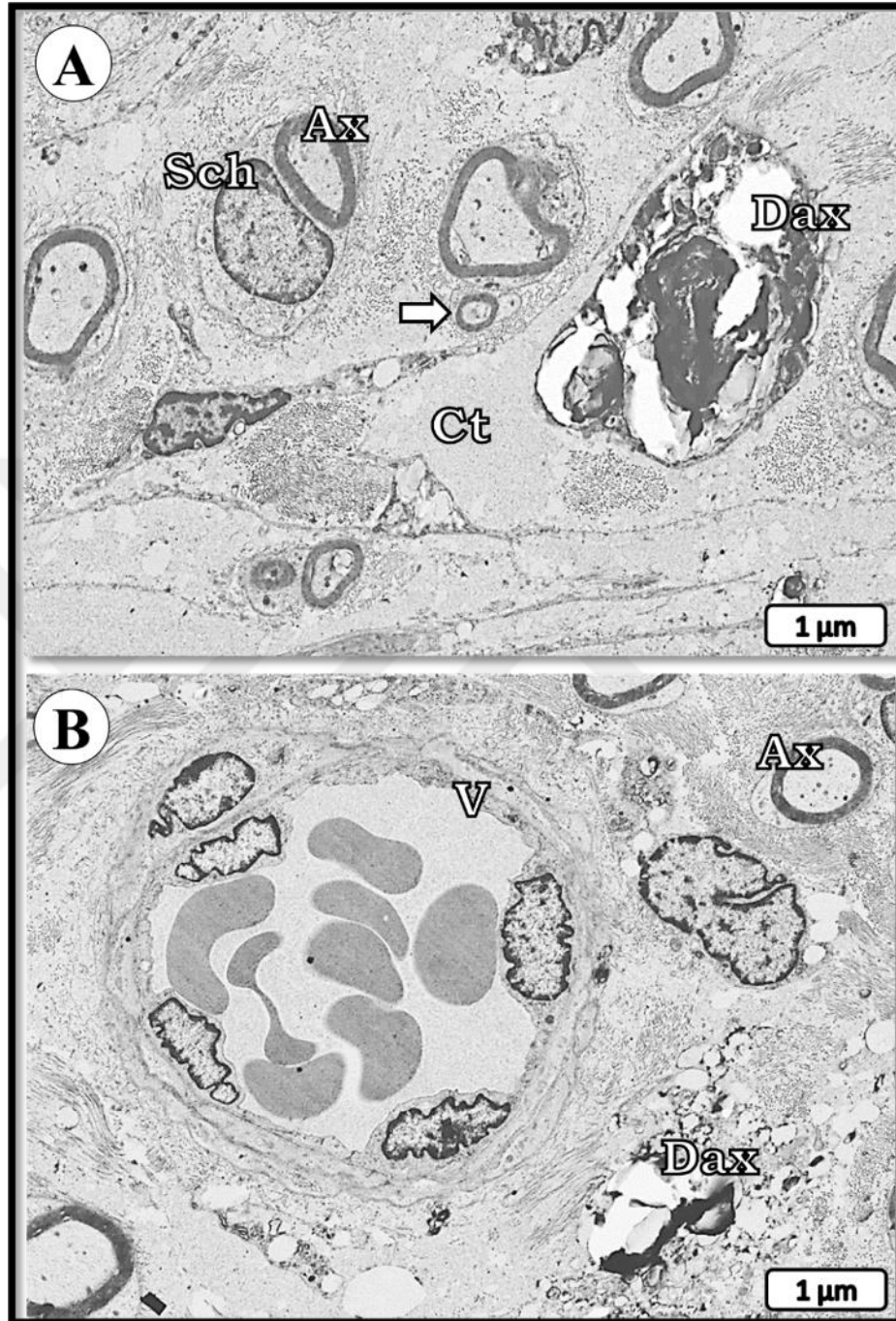


Figure 4.45. EM images of the cross-section of the sciatic nerve were taken from the Inj+EVOO group. The site of crush injury application showed many changes in the nerve tissue. The degenerative features were recognized, such as axons lost and degenerated axons (Dax). Small-sized newly formed axons were also seen (Arrow). Ax indicates the protected myelinated axons, which reflect the positive impact of EVOO. Sch indicates Schwann cell nucleus. The blood capillary with its premature endothelial cell was also observed. (Ct) connective tissue

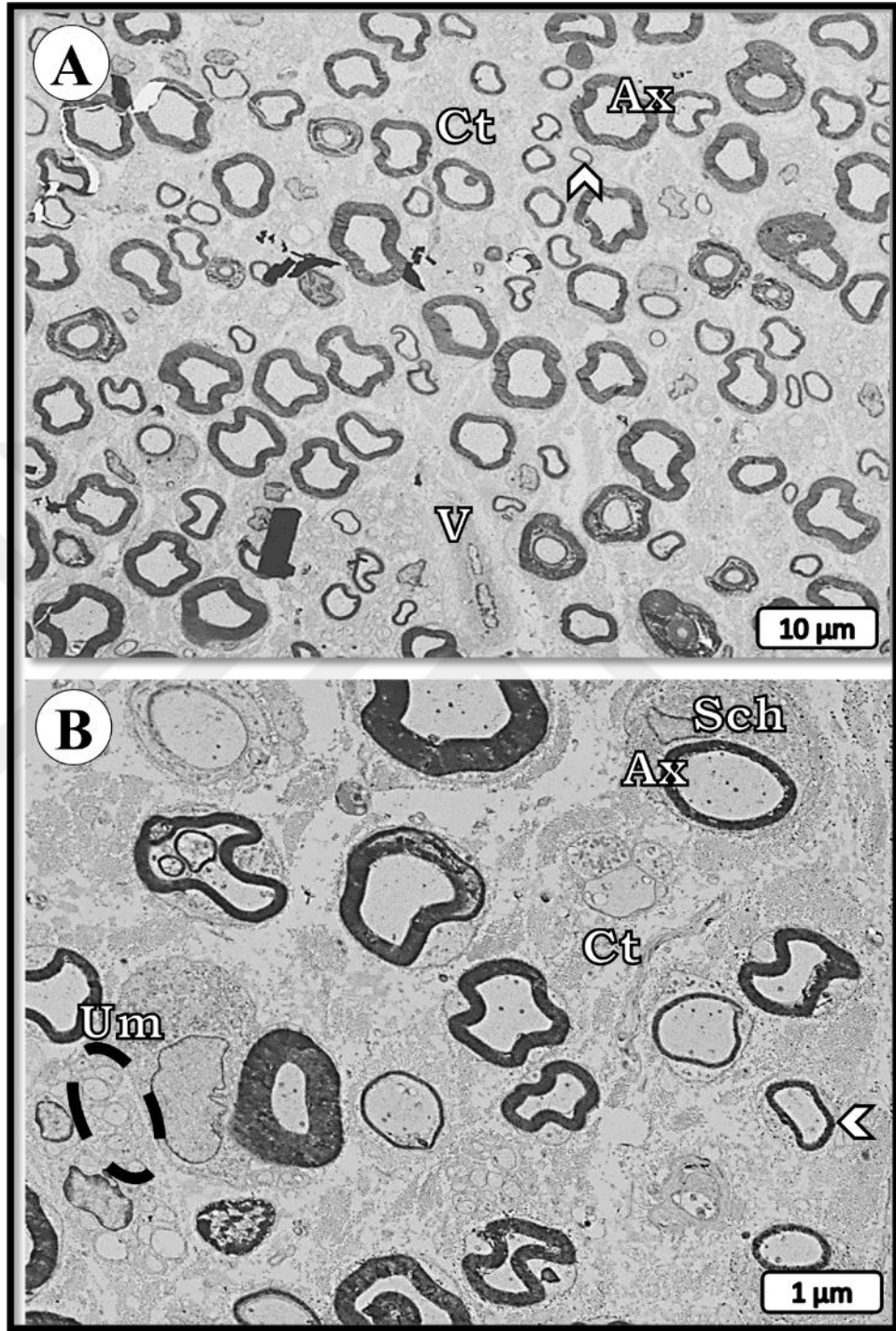


Figure 4.46. (A) and (B) are electron microscopic images of sciatic nerve cross-section belonged to the Inj+EOOB group. The following observed features are shown after applying crush injury. newly baby formed axons (arrowhead) were seen. The heterogeneity of axons size was observed. The protected nerve fibres were also observed (Ax), which proved the therapeutic effect of EOOB regarding axons number. The loose connective tissue between the nerve fibres is seen (Ct). (V) blood capillary. (Sch) Schwann cell. (Um) collection of unmyelinated fibres

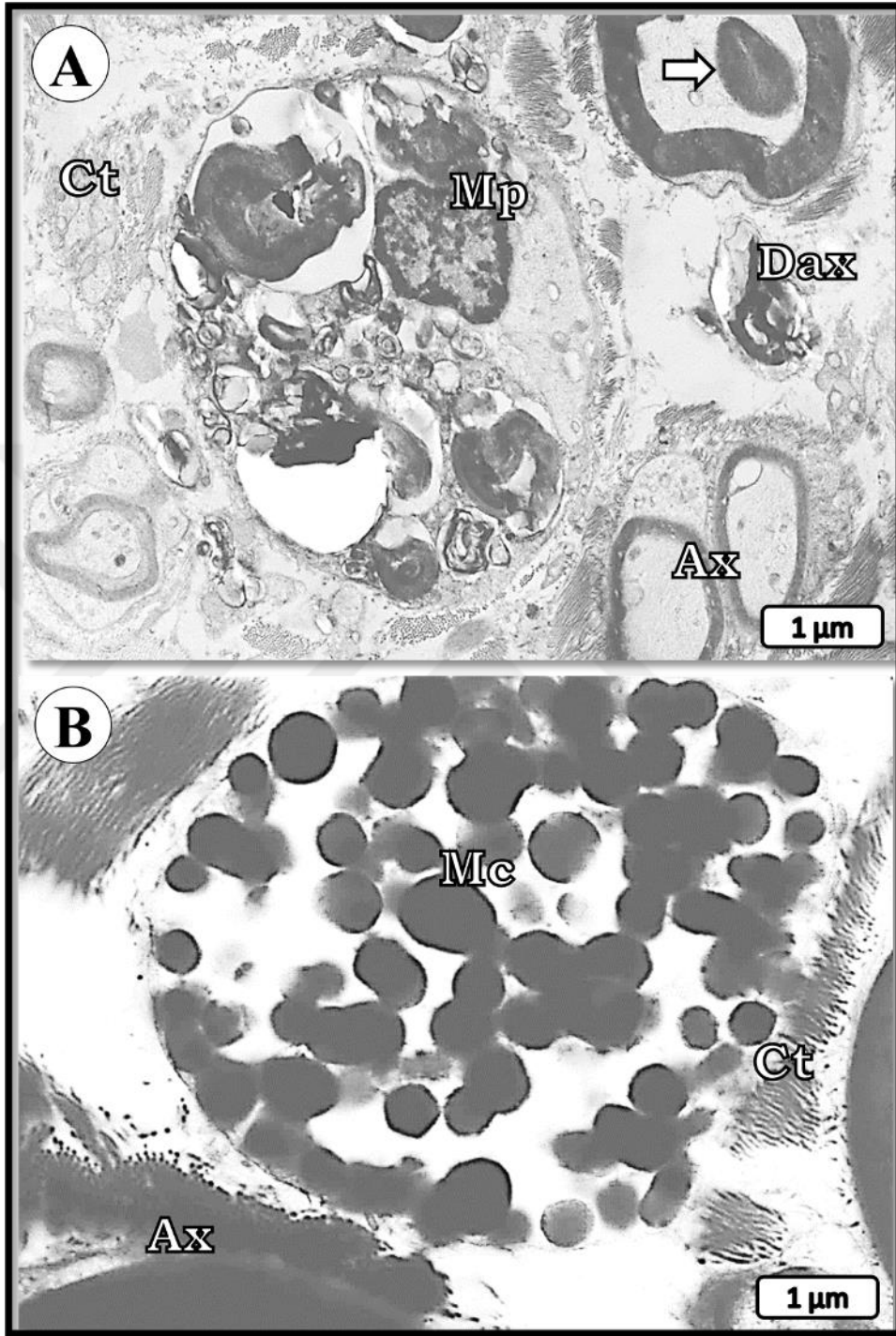


Figure 4.47. (A) and (B) are electron microscopic images of the sciatic nerve cross-sections taken from the Inj+EOOB group. The macrophages (Mp) that engulf the damaged axons are seen. The heterogeneity of axons size was observed. The protected nerve fibres were also observed (Ax), which proved the therapeutic effect of EOOB regarding axons number. The arrow indicates fragmentation of myelin sheath. The loose connective tissue between the nerve fibres is seen (Ct). The mast cell (Mc) was found in the loose connective tissue

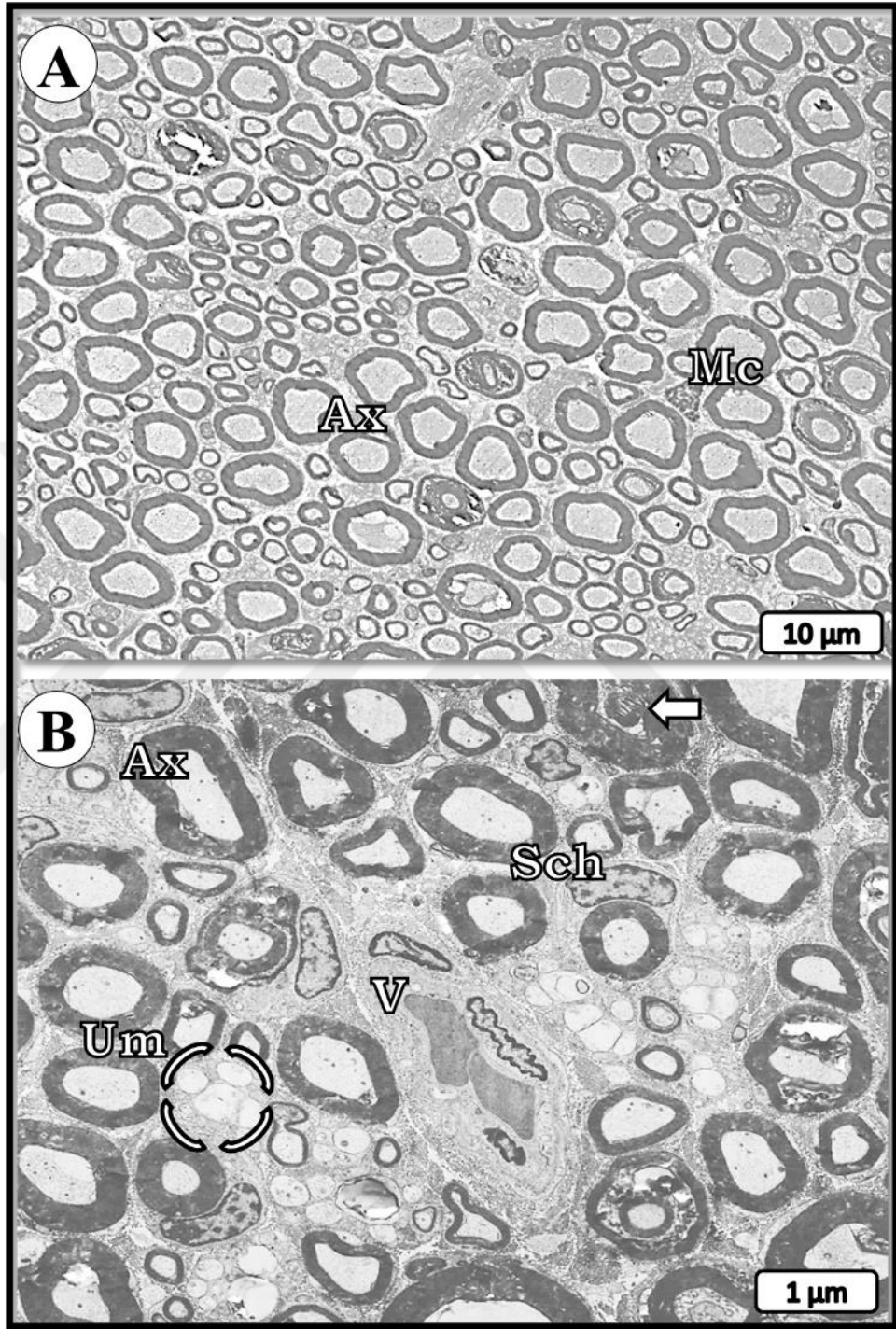


Figure 4.48. (A) and (B) are electron microscopic micrographs of sciatic nerve cross-section were taken from the AD group. The nerve elements look healthy. The nerve fibres were ensheathed by a thick and thin myelin sheath (Ax). The mast cell (Mc) was seen in the connective tissue of the nerve. Some physical impairments, such as fragmentation of myelin sheath was seen (arrow). The collection of unmyelinated axons was observed with well-defined borders (Um). Also, the Schwann cell (Sch) that coats the nerve fiber was observed

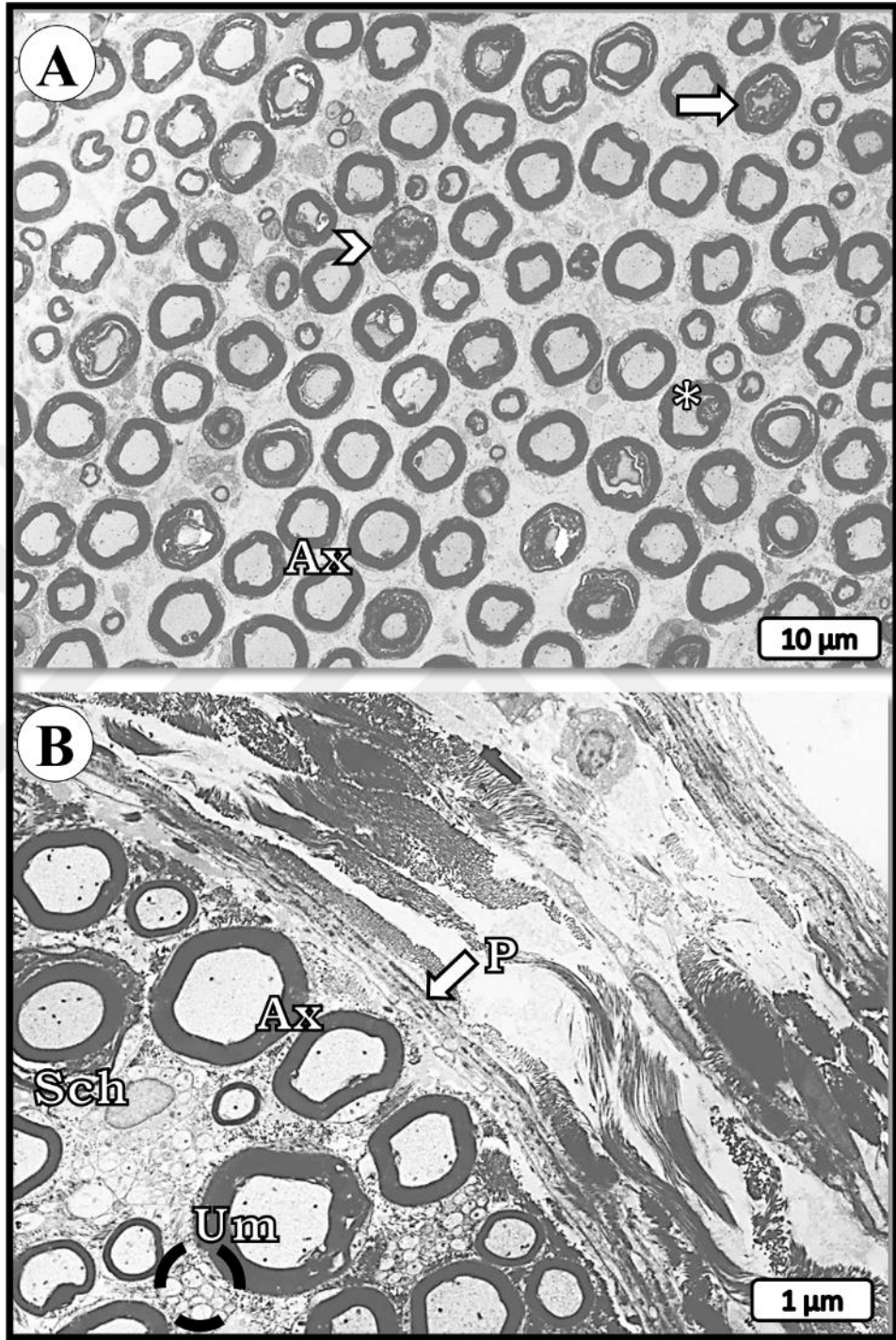


Figure 4.49. EM images of sciatic nerve cross-section belongs to the EVOO group. The morphology of the neuronal tissue looks healthy. The nerve fibres had uniform myelination distribution and structure (Ax). Some myelin sheath impairments such as cleft (arrow), fragmentation (*) and degenerated axon (arrow head) were observed. The connective tissue, perineurium (P) and its perineurial cells around the nerve bundle appeared intact. Sch is indicating to Schwann cell that coats axons. Um indicates a collection of unmyelinated fibres

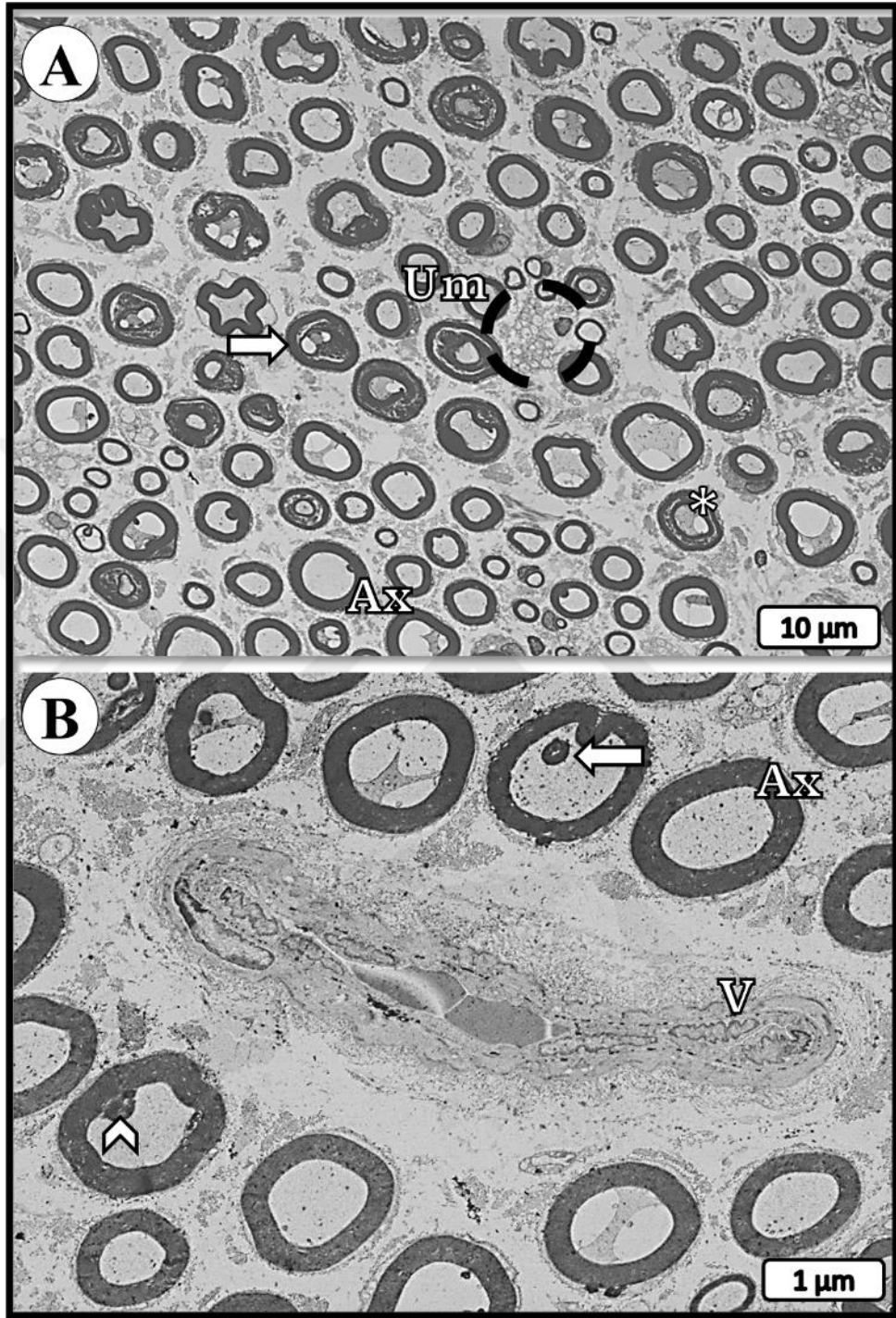


Figure 4.50. (A) and (B) EM images with different magnifications of the sciatic nerve cross-sections belong to the EOOB group. The general structure view of the nerve looks normal. The nerve fibres had uniform myelene distribution and structure (Ax). Arrow and indicating impaired axons. The aggregation of unmyelinated fibres was seen (Um). Arrowhead indicates fragmentation. The blood vessel with mature endothelial cells was observed normal (V)

4.2. Discussion

The current study was carried out to explore the regenerative effect of natural herbs such as AD, EVOO and EOOB as potent antioxidant substances on the rat's sciatic nerve after inducing peripheral neuropathy through implementing the crush nerve injury using a clumb with a pressure force of 50 Newton for 60 sec.

We used the stereological technique to estimate the number of myelinated axons and measure the myelin sheath's thickness besides the axonal area of the sciatic nerve. For analysis purposes, The 2D fractionator method was used to estimate the number of myelinated nerve fibres, while to measure the myelin thickness and axonal area, the nucleator was employed.

Based on the stereological findings, it was revealed that Inj+AD appreciated a significant decrease regarding the mean number of myelinated fibres and a significant increase in terms of mean myelin sheath thickness compared to the Inj group. In contrast, no significant difference was found in the mean axonal area between Inj+AD and Inj groups. These results indicate a beneficial impact of AD substance in the functional restoration of the injured nerve. Regarding the EVOO results concerning the mean number of myelinated fibres, a significant difference was detected among Inj+EVOO and Inj groups. On the other hand, no significant increases were observed between Inj+EVOO and Inj groups in terms of mean axonal area and the mean of the myelin sheath thickness, demonstrating that EVOO improved repairing an injured sciatic nerve. Furthermore, a significant alteration was found between Inj+EOOB and Inj groups regarding the mean number of myelinated fibres. While regarding the mean axonal area and mean myelin sheath thickness, there was no significant increase among Inj+EOOB and Inj groups; these findings reflect that EOOB showed a positive neuroprotective impact on nerve regeneration.

It is worth noting that the results revealed a significant difference concerning the mean number of myelinated fibres, myelin thickness, and the axonal area of the Cont group compared to the Inj group.

Furthermore, two functional evaluations were applied to investigate the motor recovery of the sciatic nerve. These examinations are SFI using walking track analysis and EMG by recording the latency and amplitude from the muscle action potential curve. In general, the findings of SFI measurement showed differences among the groups; therefore, AD, EVOO and EOOB exhibited a promising influence as significant differences were found between Inj+AD and Inj, Inj+EVOO and Inj, Inj+EOOB and Inj groups, respectively. These findings are in line with the stereological results. Furthermore, the innervation of the gastrocnemius muscle was evaluated to measure the amplitude value of CMAP. The nerve conductivity was examined by testing the latency, time delay between the muscle response's peak voltage and nerve stimulation. The EMG results revealed that EVOO and AD could restore the motor function of the injured nerve.

Moreover, microscopic examinations were applied to recognize the sciatic nerve features' changes from the histological view; that might occur in different study groups. Due to the excellent regenerative effect of AD, EVOO, and EOOB, we observed a significant improvement in the crushed sciatic nerve structure.

4.2.1. Peripheral Neuropathy (PN) and Peripheral Nerve Injuries (PNIs)

PN referred to any damage that occurs in peripheral nerve components. Several causes led to PN, such as direct trauma or prolonged compression (Fuller, 2004). However, PNIs attracted much of the scientific community's attention as they are considered a global severe public health problem resulting in many illnesses. PNIs spent more than 150 billion dollars annually in the USA (Taylor et al., 2008).

The peripheral nerve fibres can usually be quickly damaged as they are the most fragile body structure. The causes of PNIs include traumatic and non-traumatic injuries. These injuries are linked to socioeconomic disabilities, in addition to the functional disability of innervated structures with sensation and motor impairment (Sasso et al., 2020). The crush, the Gunshot wounds, chronic compression, lacerations, major surgery, radiotherapy, trauma, or the full transaction are risk factors of PNIs (Burt et al., 2017; Secer et al., 2009). When nerve injury was developed, the connection abnormalities

between the brain and another organs in the body will be detectable (Prakash and Bidkar, 2016).

For the classification of PN and neurosurgery, World War I and II have given useful insights, unreliable parallel repair methods, and a new point in the history of neuropathy treatment. In 1850, Sir Augustus Waller described "Wallerian degeneration", and the loss of the distal nerve portion achieved a landmark in understanding nerve injury. The study of nerve healing has dramatically focused on these findings (Waller, 1850). Furthermore, the scientists have agreed that the nerve repair approach at any time may change forever with an important knowledge-based discovery (Terzis et al., 1997).

According to the seriousness of the nerve injury, nerve composition might be affected totally or partially; the most small structure (the axon) will be impacted first when the damage is less severe. When the highly intense injury, the whole nerve structure is usually affected (Torul et al., 2018). The PNIs have been classified into main categories based on the severity of the damage by two pioneer scientists, Sir Seddon and Sir Sunderland. Seddon's classification was first, and his classification types are: neuropraxia that defined as impulses blockage due to compression with fast recovery, axonotmesis "continuity lesion" is severe axon damage although the surrounding tissue remains healthy, thence; the last class is neurotmesis (cutting) is a complete detachment of a nerve into two parts (Seddon, 1942). Accordingly, Sir Sunderland was added his input as reclassified the Axonotmesis into different grades as; Grade II is a condition in which the damage will be in the nerve fibre and its myelin sheath while the connective tissue is survived (Hussain et al., 2020; Zuniga and Radwan, 2013), whereas, Grade III in that grade the damage will extend to endoneurial layer, whereas the other layers remain intact. Finally, grade IV is the most severe grade in which the epineurium only survives, whereas the axonal sheath and all different layers are damaged (Hussain et al., 2020; Flores et al., 2000). Nowadays, a combination of histopathological features of the four mentioned grades has been indicated as the sixth grade of injury (Sullivan et al., 2016). Although, in the sixth degree, the impacted fascicles are not all; therefore, few fascicles would be included in the recovery, the recovery rate implementation is damage severity based (Boyd et al., 2011).

If the injury is caused by compression, several complications will occur on the nerve, such as the functional and impulses conductivity impairments due to the mechanical force. Accordingly, it is worth noting that the pressure force and duration of the crush compression are directly related to the injury severity degree; the signals conductivity is decreased when the pressure force and duration were increased (Wong et al., 2019). The previous study reported that the nerve structure and function is affected by the injury. Sensory and motor abnormalities (partial or total) will follow the damage based on many factors such as injury application technique, duration and intensity (Türedi et al., 2018). In addition, some ischemic alterations will take place on the nerve after short-term injury, such as fluid leakage due to compression of the nerve's blood vessels that will lead to an inflammatory response such as oedema (Beer et al., 2001).

On the other hand, the microvasculature of the nerve will be disturbed in long-term injury. Regarding nerve degeneration and regeneration; the peripheral vessels that provide the nerve with nutrients and oxygen supply play a vital role, myelin protein production depends primarily on the continuous blood supply, oxidative stress caused by the free radicals accumulation that results in damage, thus, the continuing blood supply is crucial for the nerve healing process. The crushing type of the injuries is consistent with the axonotmesis explained by Seddon; the approach used is broadly implemented (Beer et al., 2001).

Following injury, many pathophysiological changes occur in the damaged neuron, injury site, and neuron's cell body show degenerative features. The degenerative and regenerative alterations occur in the proximal fragment, while the distal part of the injured nerve undergoes WD. In 24 to 48 hours after injury, WD occurs as the destruction of the SCs mitotic division and the myelin sheath. The calcium influx is induced by SCs, which results in protease production, which destroyed the myelin sheath and interrupted nerve impulse conduction. At the injury site, the portions enlarge immediately after an injury. Close to WD's termination, axonal ends move away from the target organ, the structure of nerve shrinks, inflammatory response like oedema, and fibrosis will ultimately occur. In contrast, the proximal segment changes, such as the cell body swelling and Nissl body disappearance. Furthermore, the chromatolysis incidence indicates degeneration. Simultaneously, myelination interrupted, the release of cytokines

and recruitment of macrophages by SCs induce the myelin debris phagocytosis; subsequently, the regeneration process begins (Hussain et al., 2020).

The PNS has a more remarkable ability to restore the function and regenerate, unlike the CNS, which does not have the same capacity; hence, the CNS recovery is minimal (Huebner and Strittmatter, 2009). Following the nerve injury, and for nerve regeneration purposes, the scavenging of myelin and axonal debris is the primary step accomplished quickly by SCs and a huge number of the macrophages; this explains why the axonal regrowth is achievable in the PNS. On the other hand, the microglia assisted by a few numbers of the macrophages recruited from the blood circulation are scavenging debris very slowly; that is why the axonal repair is restricted in the CNS (Tallon and Farah, 2017). The peripheral nerves' capability to recover is attributed to the surrounding circumstances appropriate to axon development. The growth factors appear to be a key factor in the growth of axons, and a huge body of evidence suggested that SCs produce growth factors and proteins of extracellular matrix (Yuan et al., 2010). Likewise, mesenchymal cells could be an essential contributor to the regeneration process of axons in the peripheral nerve (Toma et al., 2020).

The literature reported that regeneration relies on critical factors, especially cellular and tissue levels, such as renewing endoneurial blood vessels that are considered indispensable for axonal regrowth (Fang et al., 2020).

Many authors studied the degeneration mechanism. They conclude that oxidative stress is the leading cause of the neurodegenerative disorder, as reactive oxygen species (ROS) targeting mitochondria led to cell death and conclusively nerve conduction impermeant (Ullah et al., 2018). Furthermore, oxidative stress plays a fundamental role in peripheral nerve injury-induced degeneration; oxidative stress is associated with nerve recovery suspension (Lu et al., 2019; Qiu et al., 2019). The literature showed that many approaches had been employed to enhance the regenerative capacity of the injured nerves, such as neurotrophic substances, artificial tubes and scaffolds, etc. (Arino et al., 2008; Johnson and Soucacos, 2008). However, many factors related to these approaches, such as side-effects and efficiency, are still unproven (Sagir et al., 2019; Hausner et al., 2012).

PNI management through antioxidants could be a novel method to avoid the accumulation of oxidative stress, which preserves the normal activity of SCs and promotes remyelination (Dong et al., 2019).

4.2.2. Aqueous *Adansonia digitata* (AD)

The literature has elegantly shown that AD is recognized as a potent antioxidant as it contains an extraordinary quantity of polyphenolic compounds and vitamins (Sokeng et al., 2019; Braca et al., 2018; Ghoneim et al., 2016; Fernandes de Oliveira et al., 2012; Lamien-Meda et al., 2008; McDougall et al., 2005) especially fruit pulp which possesses very high in vitamin C component more ten times than in the orange (Sabina et al., 2020). It is reported that the free radicals are the central component in degenerative neuropathy (Muthuraman and Sood, 2010; Namikawa et al., 2006). Therefore, the capacity of AD as an antioxidant was examined in terms of ameliorative regenerative impact following sciatic nerve crush in the existing study; based on the stereological results; it was observed that the mean of myelinated nerve axons number and the mean of the myelin sheath thickness in Inj+AD has significantly differed than the Inj group. On the other hand, there was no significant increase in the mean axonal area of Inj+AD compared to the Inj group. It would be suggested that AD protects injured nerve fibres from deterioration and helps axons maturity following injury. It is proposed that AD could be used as neuroprotective substance due to its high polyphenolic compounds and vitamins content.

The regenerative effect of *Achyranthes bidentata* polypeptides (ABPP) aqueous extract from *Achyranthes bidentata* Blume (one of the Chinese medicinal plants) on nerve regeneration was investigated; the nerve crush injury model was applied twice for 30 seconds with an interval of 10 seconds by smooth-jawed micro-forceps on the sciatic nerve of the mice model, the treatments were 1, 4, and 16 mg/kg through venous injection in the tail for 21 days with an interval of 3 days; the finding evidenced by physiological, histological assessments and suggested that ABPP are alternative for neurotrophic factors which enhanced the regeneration at the molecular level. Hence, ABPP is considered a neuroprotective agent to crush injury-induced peripheral neuropathy (Yuan et al., 2010). Another study was conducted to examine the potential

effect of Crocin which is a constituent of saffron herb, on peripheral nerve regeneration in the Wistar rat model using functional and histopathological evaluations; the crush injury duration was 60 sec with a pressure of 0.5-1 kg/mm² by a small haemostatic forceps. The rats were treated with doses (5, 20, 80 mg/kg) following the injury through intraperitoneal injection for ten days. They revealed that Crocin accelerated the regeneration and recovery of the sciatic nerve function through decreases the serum malondialdehyde (MDA) level, which is a biomarker of oxidative stress (Tamaddonfard et al., 2013). The anti-nociception action of the aqueous bark extract of AD with different doses was studied recently on the sciatic nerve of Wistar rats; using constrictive ligatures by tying 4.0 silk at four places on the nerve without affecting blood flow to the nerve structures; the finding was that aqueous AD minimized a systemic prostaglandin E2 degree and level of lipid peroxidation production which cure the oxidative stress and induce neuropathic pain relief (Owoyele and Bakare, 2018). The aqueous bark extract of AD has also been investigated to obtain antioxidant activity on degenerative diseases of CNS; it has been concluded that it has an antidepressant role in enhancing the mice model's behaviour (Shehu et al., 2019). It was documented that baobab fruit pulp extract could be considered a cardioprotective agent against isoproterenol which induces oxidative stress in a rat model (Ghoneim et al., 2016). Due to the findings mentioned above, these results seem to be remarkably constant with our stereological outcomes. Accordingly, AD improved nerve recovery and re-myelination of the sciatic nerve following injury by protecting the nerve fibres from oxidative action.

4.2.3. Extra-Virgin Olive Oil (EVOO)

Due to the extreme level of naturally occurring phenolic contents, EVOO is considered unique among all vegetable oils. The scientific knowledge indicates that EVOO possesses strong antioxidant activity due to its components like vitamins B, E and phenolic bioactive constituents (such as HT and oleuropein) (Amel et al., 2016; Owen et al., 2000). There is a substantial body of evidence that show the potential favourable properties of EVOO and its phenolic components in the prevention of damage by oxidative stress in cardiovascular, nervous and other body systems as an anti-toxicity agent due to it possesses antioxidant, anti-inflammatory, anti-cancer properties

(Robles-Almazan et al., 2018; Khayyat, 2016; Cicerale et al., 2012; Tasset et al., 2011; Carrasco-Pancorbo et al., 2005). The previous works documented very few studies investigating the regenerative effects of EVOO and its components on the peripheral nerve. In the current study, we assessed the ameliorative impact of EVOO on the sciatic nerve following the crush injury model. We utilized EVOO as a neuroprotective herb to promote nerve recovery. According to the stereological findings, it was observed that the mean number of the myelinated nerve axons in Inj+EVOO was high significantly fewer than in the Inj group ($P=0.000$). Contrarily, the mean of the myelin sheath thickness and mean axonal area (diameter) in Inj+EVOO did not significantly differ from the Inj group. Therefore, it would be suggested that EVOO treats injured nerve fibres due to its phenolic components. Many authors have reported the antioxidant activity of EVOO on CNS degenerative diseases; in this regard, this study is the first to assess the regenerative potential of EVOO on sciatic nerve recovery following the crush injury model inducing peripheral neuropathy.

A previous study explored the potential impact of evening primrose oil (EPO) on nerve regeneration using morphometric and microscopic evaluations; the crush injury applied was 15 sec with consistent pressure in Sprague-Dawley rats which treated with 6000mg/day by oral gavage, the authors summarized that EPO promoted nerve recovery due to its ability to improve the SCs proliferation (Ramli et al., 2017). The therapeutic competency of curcumin and propolis was used in the regenerative medicine field to manage crush injury using physiological and microscopic assessments; the doses were 100-mg/kg of curcumin and 200-mg/kg of propolis orally for 21 days. They reported that curcumin and propolis have remarkable regenerative capacities due to their antioxidants contents as they minimized nitric oxide and malondialdehyde levels, besides their free radicals scavenging activity (Yüce et al., 2015). Recently, the neuroprotective impact of EVOO lignans ((+)-Pinoresinol and (+)-1-acetoxypinoresinol) was studied against Penitrem. A deterioration induced neurotoxicity on human SCs (hSCs) *in vitro* and on mice sciatic nerve as *in vivo* studies. The authors conclude that EVOO lignans have scavenger activity as long as they protect the PA-induced neurotoxicity of PNS *in vitro* and *in vivo* (Qusa et al., 2020).

Hydroxytyrosol (HT) is the most antioxidant component, has also been investigated in vitro study to explore its capacity to increase the hSCs proliferation; the authors pointed out that HT improved the generation of hSCs, as the acceleration of SCs proliferation is closely related to activation of the phosphoinositide 3-kinase (PI3K / Akt) pathway. This result was demonstrated by an increase in markers used in protein expression of the proliferation of hSCs (GFAP and p75 NGFR) along with an increase in the proliferation index of hSCs (Kamil et al., 2020). Furthermore, much evidence exists to examine the effect of Oleuropein (an EVOO-derived polyphenolic) treatment on cognitive neurodegenerative disease (Alzheimer's disease); the therapeutical action is indicated by several cellular processes (such as activation of autophagy gene expression) (Cordero et al., 2018). Furthermore, the antioxidant influence of HT compound has also been studied on diabetic peripheral neuropathy in male Sprague–Dawley rat model; early stage of diabetic neuropathy was initiated by streptozotocin with a single dose of 60 mg/kg through intraperitoneal injection; then the animals were treated with different doses of 10, 100 mg/kg/day for six weeks. They concluded that HT has an antioxidant effect by lowering ROS levels and cure diabetic peripheral neuropathy (Ristagno et al., 2012). According to the results stated above, these findings have also shown approval with our stereological assumptions. Consequently, EVOO enhanced the regrowth of the sciatic nerve following crush injury by protecting the nerve axons from oxidative stress-based degeneration.

4.2.4. Essential Oil of *Ocimum basilicum* (EOOB)

There is a growing body of in vivo and in vitro reports indicated that EOOB has radical scavenging and antioxidant activities in treat oxidative damage associated with some inflammatory cases; its protective properties might refer to essential oil ingredients, such as methyl chavicol, methyl cinnamate, eugenol, camphor, and linalool (Kavoosi and Amirghofran, 2017; H. Li et al., 2017; Rodrigues et al., 2016; Tshilanda et al., 2016). Hence, we examined the regenerative ability of EOOB as a therapeutic agent to combat the peripheral neuropathy caused by a crush injury. The stereological findings revealed that the number of the myelinated nerve axons in the Inj+EOOB group was significantly decreased than the Inj group ($p=0.014$). On the other hand, no significant

differences were observed between the Inj+EOOB and Inj groups regarding the myelin thickness and axonal area (diameter). Therefore, we suggested that EOOB promotes regenerating the damaged sciatic nerve in terms of myelinated fibres number due to its phenolic components, especially methyl chavicol.

To the best of our knowledge, a previous study about the antioxidant efficacy of flower extract of *Azadirachta indica* herb on peripheral neuropathy following crush surgery of 30 sec in the sciatic nerve of diabetes mellites (DM) rat model has been conducted to evaluate the functional restoration, the rats treated with different doses throughout study duration which is 21 days. They conclude that *Azadirachta indica* flower extracted the sensory and motor functions of the sciatic nerve and cope with nerve degeneration of DM rats after crush surgery (Sriraksa et al., 2019). Another *Ocimum* species is *Ocimum sanctum* has been investigated as methanolic extract with a study duration of 14 days to assess its regenerating capability on the sciatic nerve after the complete transaction (axotomy); however, the nerve was tightly ligated with silk in two different sites, then the nerve was transacted between those points (about 5 mm), which induces PN in the rat model. Nevertheless, the authors indicated that the *Ocimum sanctum* promoted regeneration and restored neural function (Muthuraman et al., 2008). Moreover, it was reported that EOOB inhibits the neuronal excitability of rat sciatic nerve after trauma because it contains (-)-Linalool as a major constituent (Medeiros Venancio et al., 2016). Many authors also documented the efficient characteristics of EOOB on neurodegenerative events of CNS to explore the possibility of counteracting the adverse effects of oxidative stress; it is reported that EOOB attenuated multiple sclerosis and cognitive defects induced by ethidium bromide, which is caused by mitochondrial dysfunction (Garabadu and Singh, 2020), and cure Alzheimer disease which caused by chronic unpredictable stress (Ayuob et al., 2018). Based on the literature findings mentioned above, these results align with our stereological outcomes. Accordingly, EOOB ameliorates the functional and structural neuronal damage of the peripheral nerve induced by the crush injury.

4.2.5. Functional Evaluation

Functional assessments are extensively used in peripheral nerve regeneration studies; however, EMG and SFI tests are good examples of functional evaluation. In this context, to ensure more accurate results, the authors used to combine both tests (Casal et al., 2020). Moreover, the correlation between histomorphometry and functional tests are widely documented (Wang et al., 2018). Therefore, besides stereological analysis and light and electron microscopic examinations in the current study, we applied EMG and SFI tests as functional evaluations.

The information obtained from the SFI test answered the degree of functional recovery following crush application. Accordingly, in our study, we employed walking tract analysis as the SFI test approach; the hind limbs footprints of the rats were collected and analysed by comparing the footprint of the left and right hind limb for each animal. According to statistical analysis, significant differences were found when comparing Inj+AD with Inj, Inj+EVOO with Inj, Inj+EOOB with Inj groups. Unfortunately, there was no enough reports are investigating AD, EVOO, and EOOB on peripheral nerve regeneration using SFI. In this regard, a recent study in Sprague-Dawley rats was conducted to examine the role of Qian-Zheng-San; Chinese medicinal herbs, in nerve function recovery following the application of crush injury. Based on the SFI results postoperatively, *Qian-Zheng-San* was shown to enhance the injured hind limb (Wang et al., 2019). Furthermore, another study assessed the repair value of the Epimedium extract in sciatic nerve crush injury of rats. It was revealed that the SFI test showed significant improvement after crush surgery (Kou et al., 2013). Moreover, it is reported that the SFI test is a useful tool to reflect the neuronal function recovery after applying injury, as concluded when examined the therapeutic value of frankincense extract on rat injured nerve model (Jiang et al., 2016). In this study, AD, EVOO and EOOB showed positive influence in terms of the SFI test as they restored the motor function of injured nerves.

Additionally, EMG was employed in the current study. EMG is deemed a standard functional test that reflects the neuronal conductivity, muscle status, site and severity of the injury, and the degree of muscle atrophy (Kamble et al., 2019). In our study, EMG

was used for gastrocnemius muscle, and CMAP was recorded and analysed as amplitude and latency values. Regarding the latency and amplitude values in the current study, a significant difference in sciatic nerve regeneration was seen when comparing injured treated groups with the Inj group. Furthermore, according to the EMG test results, the amplitude findings showed fast muscle contraction in the Inj+EVOO due to strong signals transmitted through the nerve to the muscle as the significant alteration was appreciated between Inj+EVOO and Inj groups. In contrast, no significant difference was found among Inj+EVOO and Cont groups.

On the other hand, the latency results showed a very short duration of nerve conduction velocity to reach the muscle in the Inj+AD and Inj+EVOO as significant differences were also detected among Inj+AD and Inj groups, as well Inj+EVOO and Inj groups. When comparing stereological results to functional (EMG) test findings, the obtained data do not agree. The myelin sheath thickness is related to latency value, and the axonal area is related to amplitude. However, EVOO enhanced amplitude and latency values without improving the axonal area and myelination thickness. The existing stereological finding pointed out that EVOO enhanced the axonal area as the difference among Inj+EVOO and Inj groups intended to differ significantly ($p=0.074$); this finding might explain why axonal area and amplitude was inconsistent in the Inj+EVOO group.

Moreover, it is worth mentioning that the full nerve regeneration rely on the study duration; therefore, the best findings of the study examinations can be obtained in a long-term study. However, we carried out this study in only three weeks. Alternatively, these results may also be related to the dosage of the EVOO, which could not manifest the statistical difference in terms of stereological parameters.

In summary, we observed beneficial findings regarding EVOO in terms of amplitude and latency values. In comparison, AD showed a positive effect in terms of latency result on damaged sciatic nerve regrowth.

4.2.6. Microscopic Examination

The histological examination is used as a standard tool to investigate the changes which occurred at the tissue and cellular level of neuronal tissue after injury. The light

and electron microscopic evaluations are offer accurate information about the histological observations of the regenerated and degenerated peripheral nerves. Several histological alterations occurred following PNIs indicated by the myelin breakdown and increased number of macrophages, besides some degenerative changes of SCs, including vacuoles of myelinated nerve axons and swelling in both connective tissue non-myelinated nerve axon (Helvacioğlu et al., 2018). The distinctive signs of degeneration caused by WD after PNIs are observed in axons and myelin sheath, such as reducing myelin sheath thickness and increased axon number due to nerve sprouts formation from existing axons. However, some of them reconnect their destination. Moreover, the tissue debris was removed by macrophages and SCs; therefore, the macrophage presence is evidence of nerve degeneration. Furthermore, some structural changes in the neuronal cell body following the injury include chromatolysis and cellular swelling, the peripheral position of the nucleus, dendrites shrinking, and size increase of nucleus and nucleolus (Allodi et al., 2012).

Based on the histological structures of the peripheral nerve, which can be observed and analysed, the light and electron microscopes are useful to examine the nerve tissue. However, the diameter of the myelinated axon, myeline coat integrity and cells (like mast cells, SCs and macrophage) are the essential structures identified in nerve cross-sections during the microscopic evaluation. Moreover, the blood vessels and blood cells should be considered, and the connective tissue, particularly the epineurium, has an considrable value that can decide the nerve state. In our study, the rat sciatic nerve was assessed by light microscopy to investigate the structural changes of the regenerated and degenerated fibres. Accordingly, the general appearance of the nerve structure in the Cont group looks healthy. The myelinated nerves fibres and the myelin covering were also seen as normal. Likewise, the blood vessels wall and blood cells and epineurium, which is deemed the connective tissue of nerve, have a normal appearance.

On the contrary, the general structure of the rat sciatic nerve in the Inj group showed recognizable destruction. As a result of injury, pronounced changes were observed when comparing with the Cont group. However, micromorphological alterations appeared abnormal such as the myelin thickness reduction, epineurium

destruction and myelin sheath dissociation. Moreover, a quantity increase of the connective tissue and adipose cells was detected; besides, the macrophages with distended cytoplasm (foamy appearance) due to myelin debris phagocytosing and aggregated at the injury site the increased blood capillaries with premature endothelial cells.

The previously mentioned features and the increased number of SCs deemed to be evident for nerve degeneration. To the best of our knowledge, the degenerative changes were attributed to ischemia due to obstruction of the microvasculature of nerve tissue. The stereological findings of Inj + AD show that AD enhanced the nerve damage repair in terms of the number of myelinated axons and myelin coat thickness. Likewise, AD beneficial impact is apparent in terms of light and electron microscopic evaluation. In this respect, most of the cross-sections of this group were observed integrated. However, the myelinated axons and myelin sheath thickness are seen protected due to AD effect with some small-sized axons covered a thin myelin sheath in regenerated nerve; in addition to an increased amount of SCs nuclei and well-preserved epineurium appearance around myelinated fibre, all of these observations reflect remyelination and repair process in this portion. On the other hand, the myelin sheath impairment and myelinated axons degeneration were also seen in some sections.

According to morphometric analysis results of the Inj+EVOO group, it was found that EVOO showed regenerative value in terms of myelinated axons number. EVOO recovery effect is observed clearly in light and electron microscopic evaluation. In this regard, most of the nerve cross-sections of this group were kept integrated, but a few of them were seen with impaired sheath. However, the myelinated axons are observed healthy due to EVOO impact. The small-sized axons that ensheathed by a thin myelin sheath in regenerated nerve were dominant. The increased SCs nuclei, intact epineurium appearance, and macrophages presence indicate the nerve recovery process.

The findings of stereological analysis showed that treatment with EOOB had improved the nerve regrowth, as observed in the myelinated axons number of the injured sciatic nerve. Correspondingly, the microscopic evaluation showed that the recovery of the nerve was observed. In this context, the epineurium was seen as normal. The

myelinated nerve fibres were protected entirely. The heterogeneity of axons size was marked as a considerable number of axons were small and enclosed by a thin myelin sheath. The physical and physiological myeline sheath impairments were found in some nerve fibres. The blood capillaries with their premature endothelial cells were also observed. Moreover, the macrophages which engulf myeline waste and a huge number of SCs nuclei were detected. Therefore, all of the mentioned findings reflect the nerve restoration process.

Bearing the previous findings in mind, we can state briefly that most of the nerve tissue structures appeared healthy in the Cont group. In contrast, in the Inj group, most of the nerve components include axonal area and myelin thickness, showed intense structural damage. The injured groups exposed to AD, EVOO or EOOB treatment showed protected nerve components mainly with some parts deterioration of the nerve tissue. On the other hand, in the groups exposed to AD, EVOO or EOOB, the nerve structures mostly in these groups were observed healthy with the presence of some devastated structures of the nerve in some cross-sections. A recent study revealed that sciatic nerve structural deterioration and WD of myelin sheath in rat model occurred following administration of aflatoxin, which induced neurotoxicity. These findings were indicated by using light and electron microscopic examination (Makhlouf, 2020). Also, it was reported that creatine showed a positive effect in coping with degenerative events of the sciatic nerve, which occurred after the crush model. This finding was confirmed by electron microscopic evaluation (Helvacioğlu et al., 2018). The histological examination findings showed the regenerative efficacy of leptin and acetyl-L-carnitine administration after expose to the resection injury model (Onger et al., 2017).

Our study suggests that AD, EVOO and EOOB showed promising ameliorative results on sciatic nerve repair after the crush injury model. This outcome is supported by functional assessment, microscopic evaluation and stereological analysis.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The current work points out that Cont and Inj groups' stereological evaluation findings showed that the crush injury of the sciatic nerve significantly decreased the mean value of myelin sheath thickness and the axonal area and increased the mean number of axons.

- AD, EVOO and EOOB mitigated the peripheral neuropathy induced by the crush injury model.
- AD afforded regrowth improvement of damaged nerve in terms of axons mean number and myelin sheath thickness. Moreover, it restored the motor function of the injured nerve in terms of the SFI and latency.
- EVOO showed ameliorative ability and the best results in all functional tests SFI and EMG (amplitude and latency values of CMAP) in addition to the mean number of axons.
- Furthermore, the EOOB showed promising results in terms of the mean number of axons and SFI.
- EM and LM examinations were proved these positive outcomes.
- Based on the mean values, AD is the best substance in terms of myelin thickness, axonal area and latency. EVOO was showed the best results regarding axon number, SFI and amplitude (Table 4.13).

5.2. Recommendation

- We recommend further quantitative studies to investigate the AD, EVOO and EOOB effect in injury-induced PN with different study duration, different dosages and other examinations such as immunohistochemistry and sensory recovery.
- Consumption of herbs and medicinal plants extraction rich with antioxidants components like AD, EVOO, and EOOB are recommended as alternative options for injury-induced PN management.

- EVOO application is highly recommended in peripheral nerve repair since it rapidly accelerates the functional nerve recovery, as this proved by SFI, amplitude and latency of CMAP findings in short term duration.



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APPENDICES

Ethical committee approval certificate

	<p>T.C. ONDOKUZ MAYIS ÜNİVERSİTESİ Hayvan Deneyleri Yerel Etik Kurulu</p>
Sayı : 68489742-604-E.4775 Konu : Hadyek İzin Onayı Hk	26/02/2019
<p>DOÇ.DR.MEHMET EMİN ÖNGER PROJE YÜRÜTÜCÜSÜ</p>	
<p>Laboratuvar Hayvanlar üzerinde Araştırma amaçlı çalışma yapmak üzere başvuran Doç. Dr. Mehmet Emin ÖNGER'in 2019/01 Kabul nolu "Siyatik sinir hasarına bağlı nöropatik sıçan modeli üzerine Ocimum sanctum esansiyel yağının potansiyel etkileri: Stereolojik ve histolojik bir çalışma" başlıklı projesi 14.01.2019 tarihli Kurul toplantısında OMU- HADYEK 'in yönergesi kapsamında değerlendirilmiş ve Hayvan Hakları ve Deney Etik İlkelerine Uygun bulunmuştur. Karar onayı ekte sunulmuştur. Gereğini bilgilerinize rica ederim.</p>	
<p>e-imzalıdır Prof. Dr. Mustafa AYYILDIZ HADYEK</p>	
<p>Ek: ETİK KURUL KARARI 2019/01 DOÇ. DR. MEHMET EMİN ÖNGER</p>	

CURRICULUM VITAE

Nagi Mahmoud Ahemd Bakhit started his education at Human Anatomy, Omdurman Islamic University, Faculty of Basic Medical Sciences 2002-2006 Khartoum, Sudan. He graduated from the Human Anatomy, University of Gezira, College of Medicine-Deanship of Graduate Studies and Scientific Research in 2011 with his master's degree. He started his Ph.D. education in Ondokuz Mayıs University Faculty of Medicine, Department of Histology and Embryology in 2015.

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Publications:

- Sagir, D., Bereket, C., Onger, M. E., **Bakhit, N.**, Keskin, M., & Ozkan, E. (2019). Efficacy of Extracorporeal Shockwaves Therapy on Peripheral Nerve Regeneration. *Journal of Craniofacial Surgery*.
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