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

STEREOLOGICAL AND HISTOLOGICAL EVALUATION OF DEXAMETHASONE, BETAMETHASONE, AND METHYLPREDNISOLONE IN PROMOTING PERIPHERAL NERVE REGENERATION; STUDY IN RAT MODEL

PhD Thesis

Mohammed Hamid Karrar ALSHARIF

SUPERVISOR
Doç.Dr. Mehmet Emin ÖNGER

SAMSUN
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ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled "Stereological and histological evaluation of Dexamethasone, Betamethasone, and methylprednisolone in promoting peripheral nerve regeneration; study in rat model" prepared by Mohammed Hamid Karrar ALSHARIF and supervised by Doç.Dr. Mehmet Emin ÖNGER 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 .../…/2021.

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ÖZET

DEKSAMETAZON, BETAMETAZON VE METİLPREDNİZOLONUN PERİFERİK SİNİR YENİLENMESİNİ DESTEKLEMEDE STEREOLOJİK VE HİSTOLOJİK DEĞERLENDİRİLMESİ; SIÇAN MODELİNDE ÇALIŞMA

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Histoloji ve Embriyoloji Anabilim Dalı
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Danışman: Doç.Dr. Mehmet Emin ÖNGER

Bu çalışmanın amacı, sıçan modelinde sağ siyatik sinir hasarından sonra Deksametazon (Dex), Betametazon (Bet) ve Metilprednizolon (Mps)’ un sinir rejenerasyonu ve fonksiyonel motor iyileşmeye muhtemel etkilerini incelemektir.

Altmış dört erkek *Wistar albino* sıçan (250 ± 30 g ağırlığında ve 12 - 14 haftalık), rastgele sekiz eşit (n=8) gruba ayrıldı. Kontrol (Cont) grubuna herhangi bir cerrahi işlem ve tedavi uygulaması yapılmazken, pozitif kontrol gruplarına, her grup için 2 mg/kg/gün dozunda Dex, Bet ve Meth verildi. Hasar (Inj) grubu ve Hasar+tedavi gruplarında siyatik sinir, 60 saniye boyunca 50 Newtonluk basınç uygulanarak ezilmeye maruz bırakıldı. Hasar+Dex grubunda, ratlara 10 gün boyunca 2 mg/kg/gün Dex verildi. Hasar+Bet grubunda, ratlara 14 gün boyunca 2mg/kg/gün Bet verildi. Hasar+Mps grubunda ratlara 14 gün boyunca 2 mg/kg/gün Mps verildi. Tüm madde uygulamaları cerrahi işlemden 24 saat sonra intraperitoneal yolla uygulandı. Her grupta siyatik sinir iyileşmesini değerlendirmek için stereolojik analizler, histolojik ve fonksiyonel incelemeler yapıldı.

Stereolojik analiz sonuçlarına göre; miyelinli akson sayısı açısından Hasar grubuna kıyasla Hasar+Dex grubunda azalan yönde bir istatistiksel fark ($p = 0,019$) bulundu. Ancak, Hasar+Bet ve Hasar+Mps grupları ile Hasar grubu arasında miyelinli akson sayısı, miyelin kılıf kalınlığı ve akson alanı parametreleri açısından istatistiksel bir fark bulunmadı ($p >0,05$). Siyatik fonksiyonel indeks (SFI) verilerine göre, Hasar+Dex ve Hasar+Mps gruplarında Hasar grubuna kıyasla artan yönde anlamlı istatistiksel fark (sırasıyla $p = 0,016$ ve $p = 0,005$) tespit edildi. Bununla birlikte, Hasar grubuyla karşılaştırıldığında, hiçbir tedavi grubunda EMG değerlendirmesi açısından anlamlı bir fark görülmedi ($p > 0,05$).

Sonuç olarak; Dex, miyelinli akson sayısı ve fonksiyonel iyileşme açısından olumlu etkiye sahipken, Mps sadece fonksiyonel iyileşmeye olumlu katkı sağlamıştır. Bununla birlikte, mikroskobik değerlendirmeler uygulanan tüm maddelerin siyatik sinir iyileşmesine olumlu etkileri olduğunu göstermektedir.

Anahtar kelimeler: Periferik sinir, Deksametazon, Betametazon, Metilprednizolon, Stereoloji.

ABSTRACT

STEREOLOGICAL AND HISTOLOGICAL EVALUATION OF DEXAMETHASONE, BETAMETHASONE, AND METHYLPREDNISOLONE IN PROMOTING PERIPHERAL NERVE REGENERATION; STUDY IN RAT MODEL

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The goal of this study was to examine the possible effect of administering Dexamethasone (Dex), Betamethasone (Bet), and Methylprednisolone (Mps) in promoting nerve regeneration and functional motor recovery after a crush injury to the right sciatic nerve in a rat model would.

Sixty-four male Wistar albino rats (weighing between 250 ± 30 g and age 12 to 14 weeks) were randomly divided into eight groups (n=8). The Control (Cont) group received no surgery or treatment, whereas the positive control groups received treatment with Dex, Bet, and Meth at a dose of 2 mg/kg/day for each group. The sciatic nerve was exposed to crush by applying 50 Newton for 60 seconds (sec) in the injury (Inj) or injury-treated groups. In the Inj+Dex group, the animals have received Dex for 10 days. In the Inj+Bet group, the rats were treated by Bet for 14 days. In the Inj+Mps group, the rats were given Mps for 14 days. All the treatments were performed intraperitoneally 24 hours after surgery. The stereological analysis, histological and functional examinations were done to assess the sciatic nerve in each group.

Stereological analysis revealed a significant difference in decreasing way in points of total numbers of myelinated axons in the Dex + Inj group compared to the Inj group ($p = 0.019$). However, the Bet + Inj and Mps + Inj groups revealed no significant differences in the total number of myelinated axons, myelin sheath thickness and axonal area compared to the Inj group ($P > 0.05$). According to sciatic functional index (SFI) evaluation, Dex+Inj and Mps+Inj groups demonstrated significant differences in increasing way ($p = 0.016$) and ($p = 0.005$) respectively. However, compared to the Inj group, there was no significant difference in the EMG assessment in any treated groups ($p > 0.05$).

In conclusion, Dex had a beneficial effect on myelinated axons number and functional improvement, whereas Mps only enhanced functional recovery. Furthermore, microscopic examinations revealed that all the proposed medications had beneficial impacts on the sciatic nerve sections.

Keywords: Peripheral nerve, Dexamethasone, Betamethasone, Methylprednisolone, Stereology

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Mohammed Hamid Karrar ALSHARIF

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LIST OF ABBREVIATIONS

°C: Degree centigrade

μm: Micrometer

ATP: Adenosine triphosphate

Bet: Betamethasone

CMAP: Compound muscle action potential

CNS: Central Nervous System

Cont: Control

Dex: Dexamethasone

ECM: Extracellular Matrix

Emg: Electromyography

Epo: Erythropoietin

ERK: Extracellular-signal-regulated kinase

IGF-1: Insulin-like growth factor-1

Inj: Injury

Mb: Methylcobalamin

Mps: Methylprednisolone

Mt: Melatonin

NAC: N-acetylcysteine

NGF: Nerve Growth Factor

PN: Peripheral Nerve

PNS: Peripheral Nervous System

TEM: Transmission electron microscopy

TP: Testosterone propionate

USA: United States of America.

WD: Wallerian Degeneration.

WRL: Withdrawal reflex latency

FIGURE LEGENDS

TABLES LEGENDS

1. INTRODUCTION

The human nervous system consists of two primary components: the Central Nervous System (CNS) and the Peripheral Nervous System (PNS) (Catala and Kubis, 2013). Regarding the CNS is composed of the brain and spinal cord (Laine and Smoker, 1998). Therefore, the CNS is directly related to PNS. On the other hand, the PNS is formed by 43 nervous divisions formed by 12 pairs of cranial and 31 pairs of spinal nerves which constitute a significant part of the PNS which take the functional responsibility of conducting the impulses to and from the CNS and the effector tissue or organ, which is known as afferent or efferent fibers (Splittgerber, 2018).

By the third week of development of the nervous system is achieved by thickening in the neural plate's ectoderm layer, which is eventually raised at the edges of the plate; which will give rise to the neural tube (T. W. Sadler, 2018); while the development of PNS takes place by several sources but chiefly by the strips of tissue called the neural crest prolonging longitudinally above the neural tube (Moore et al., 2018).

The nervous system's structural and functional unit is known as a neuron, which can be defined as the nervous system's fundamental unit responsible for processing sensory feedback from the outer environment or transmitting the motor impulses to the muscles effector. Neurons exhibit remarkable variations in size and shape. Consequently, neurons are classified into three function-based types, namely: sensory neurons, motor neurons, and interneurons (Pawlina and Ross, 2011).

The structural formation of neurons is formed by the neuronal body, and the processes, either to be axons or dendrites, that provide communication between the neighbouring neurons occurs by synaptic junctions. The neurons communicate through syntactic joints comprising a gap known as the synaptic spin, which transfers chemicals from the former neuron's axon or dendrites to the letter neuron's axon (Pawlina and Ross, 2011).

The communication between the CNS and the effector organ or tissue that occurs through the peripheral nerve is acknowledged by the peripheral nerve. The anatomical formation of the peripheral nerve is formed by the cell body, which can be found inside the CNS or exterior. The nerve fibers are the structural and functional unit of the peripheral nerve. The classification of nerve fiber depends on different criteria, such as conduction speed, functional role, and fiber diameter (Geuna et al., 2009).

Regarding the PNS, the nerve fibers are divided into two types; the myelinated nerve fibers are made of a single axon enveloped independently by the single Schwann cell. The unmyelinated nerve fibers are made of a group or collection of axons enveloped via a single Schwann cell (Gamble and Eames, 1964). The elements of the peripheral nerve surrounded and protected by connective tissue, separate sheaths play an essential role in giving adequate resistance to stretching factors to provide them with the force of compression used in the body's movement. The peripheral nerve material is classically arranged as separate fascicles bundles, surrounded by three distinct sheaths of connective tissue (epineurium, perineurium, endoneurium), directed from the outermost the innermost respectively; the epineurium is considered as a unifascicular or multifascicular connective tissue that encloses an external nerve trunk, while the perineurium defined as a tubular sheath, formed by specialized connective tissue wrapped around each nerve fascicle and the endoneurium is represented as loose connective tissue found in the innermost layer enveloping each single nerve fiber (Geuna et al., 2009; Parmantier et al., 1999).

Neural damage or injury may occur in both the CNS and the PNS. However, mainly after an injury, the difference is that the axon of PNS will regenerate rapidly; in comparison, CNS axons typically fail when regenerating. Peripheral nerve damage is accompanied by pathophysiological alterations in nervous disorders, including biochemical and morphological changes; this would be reflected as an interruption in axonal transport. Nerve injury can be classified into three categories according to pathophysiologic features: Neurapraxia, axonotmesis, and neurotmesis; each type can be briefly defined as follows; neurapraxia means a type of nerve injury is typified as disruption occurs in the myelin sheath, usually subsequent to compression, regarding the axonotmesis pathophysiological characteristics of this type are represented by a lack of continuity of the axon, followed by a variable degree of retention of connective tissue components, and the neurotmesis is defined as the worse damage in the nerve because it is supplemented with disruption of the whole nerve's physiological features (Pawlina and Ross, 2011; S. K. Lee and Wolfe, 2000).

The peripheral nerve changes after injury involve several neuropathological and morphological changes in proximal and distal segments. In the proximal segment, a lot of changes occur after nerve injury, including chromatolysis, which is characterized by swelling in the nucleus and accompanied by an eccentricity that the intern will modify the metabolic machinery from the propagation of nerve impulses to reconstruction of the wounded nerve. This may follow by the decomposition of polyribosomes to leave a thin "dust-like" powder, concerning neuropathological and morphological changes in the distal segment of peripheral nerve embrace a gradual process of degeneration (Moon, 2018). This degeneration mechanism is referred to as Wallerian degeneration; this process starts immediately after disturbance or damage to the myelin disintegration and propagation of the Schwann cell. In the injury site, Schwann cells and macrophages are activated and over time, the phagocytosis mechanism is formed for both myelin and cell debris (Geuna et al., 2009).

Regarding the mechanism of peripheral nerve regeneration, it is established when the effector organ or tissue is denervated, which takes place in two ways depending on the level of injury that involves the axon either to be exposed to damage or not exposed; the first way in the former type of injury the collateral branching considered as the primary process for regeneration; this process frequently initiates after four days subsequent to nerve injury, while in case of the latter type of injury the solitary way for recovery is the axonal regeneration (Lunn et al., 1990). Thus, the achieve complete healing or recovery, the nerve must undergo three major processes: Wallerian degeneration, regeneration of the axon, and re-innervation of the end or effector of the organ (Menorca et al., 2013).

Several scientific studies aimed to study the effect of pharmaceutical drugs conducted on animals to examine the role of medications to promote peripheral nerve regeneration based on the functional and histological criteria as an evaluation factor after inducing peripheral nerve injury (Bota and Fodor, 2019).

The following section aims to list the critical categories of drugs historically used in experimental studies of peripheral nerve regeneration and includes some examples for each category with an explanation of the action method.

Immunosuppressive medications have been prescribed for a long time due to widespread confidence in their beneficial role in encouraging peripheral nerve regeneration. These drugs working mechanisms can be summed up to reduce scar formation and facilitate regeneration by modifying immune and inflammatory reactions. An example of immunosuppressive medications that promote the functional recovery of the peripheral nerve is Tacrolimus (FK506), which has an affirmative effect in patients with upper limb transplantation, remarkably as peripheral nerve regeneration increased more rapidly (A. Y. Mekaj et al., 2014). Another example in the same drug family is Rapamycin which pharmacologically acts as an autophagy-inducer according to an experimental study; it showed a positive effect in reduced apoptotic cell count and encouraged histological and functional recuperation (Huang et al., 2016).

The neurological drugs are defined as the drugs that have a cellular function in the nervous system and act in the neural mechanisms, and the curative effect extends to give promoting peripheral nerve regeneration (Bota and Fodor, 2019), an example of a drug that falls under this family are Lithium, which is widely used to relieve effects of bipolar mood disorders and has recently shown its clinical effectiveness as a neuroprotective agent, also shown improvement in rats with sciatic nerve crush damage after providing a preoperative dose (Nouri et al., 2009). Gabapentin is also an example under this umbrella that acts as an anticonvulsant agent and also observed improvement in peripheral nerve response and enhancement in the characteristic feature of nerve morphology (Câmara et al., 2013). Also, Zonisamide, a medication prescribed to treat patients with epilepsy, is used in Parkinson's disease. It has also been proved that it promotes peripheral nerve regeneration by enhancing the neural growth factor and protecting from oxidative stress (Yagi et al., 2015).

The Nonsteroidal Anti-Inflammatory has been prescribed as a pain reliever to curb inflammation, decrease fever, and anticoagulant agent. The curative effect on peripheral nerve regeneration has also been proven. An example of this medication is Diclofenac, which showed particular enhancement in histological and functional nerve regeneration, which was justified by decreasing Wallerian degeneration severity (Tamaddonfard et al., 2014). In the same manner, Celecoxib has shown therapeutic efficacy in promoting peripheral nerve regeneration's functional regeneration after administration (Cámara-Lemarroy et al., 2008). Also, Ibuprofen, which has also confirmed its beneficial responsibility to promote peripheral nerve regeneration, can be explained by inhibiting the Ras homolog family member A (RhoA) cascade (Madura et al., 2011).

The Lipid-Lowering Medications (Statins) Pharmaceutical considered antagonists of 3-hydroxy-3- methylglutaryel coenzyme-A reductase, has been used to suppress cholesterol serum levels. The next mentioned medications are examples of statins whose therapeutic efficacy in promoting peripheral nerve regeneration has been proven experimentally. Simvastatin, along with the statins family, when this treatment is used at a high dosage, has been shown to leave a therapeutic benefit measured by an increase in histological recovery (Xavier et al., 2012). Another example worth mentioning is the Atorvastatin; the administration of this drug has a beneficial effect on functional regeneration in rats following damage to sciatic nerve crush injury (Cloutier et al., 2013).

Hormonal therapy is widely used to identify a collection of drugs where the type of treatment relies on the addition of hormones, blocks, or disabled hormones; it has also been shown to be used as medicinal agents that may facilitate the regeneration of peripheral nerves (Bota and Fodor, 2019). An example of hormonal therapy is Melatonin which is generated by the pineal gland responsible for the sleep-wake cycle. It has been investigated its role in the recovery of the peripheral nerves (Kaya et al., 2013). Also, Leptin is a hormone released from fat cells in the body tissue that showed a positive therapeutic effect by the occurrence of regeneration in the unmyelinated axons (Onger et al., 2017).

Another example of a pharmaceutical host that deserves to be mentioned is the Vitamins, where it can be defined as organic additives in food that are essential for growth and health preservation in minimal quantities. It shows a significant role in peripheral nerve regeneration. Methylcobalamin is known to be a cobalamin version of vitamin B12, which plays a substantial role in axon myelination (Okada et al., 2010). Another example is that Coenzyme Q10 is considered a vitamin-like substance with antioxidant effects. It plays an important factor in mitochondrial metabolism that is capable of promoting regeneration by improving the histological and functional features of the nerve after injury (Yildirim et al., 2015). Vitamin D is another example considered as fat-soluble secosteroids that play an important role in absorbing minerals in the digestive system, which had a potent effect in promoting structural and physiological nerve recovery (Chabas et al., 2013).

The last but not the least class of pharmaceutical family that will mention is the Corticosteroids are essential pharmaceutical drugs used to regulate allergic and inflammatory disorders or prevent excessive or inappropriate immune system responses. Clinically, the term corticosteroid is used to describe glucocorticoid action agents. In addition, it is worth mentioning that corticosteroids have pharmacological effects on the CNS and various endocrine functions (D. M. Williams, 2018). The current study focuses on synthetic steroids mimicking the action of naturally manufactured corticosteroids that could replace adrenal glands for corticosteroids in patients unable to develop sufficiently corticosteroids and, in particular, the following corticosteroids: Dex, Bet, and Mps; to evaluate their effectiveness in promoting the peripheral nerve regeneration followed the injury.

Dexamethasone: Considered to be a synthetic long-acting corticosteroid agent commonly used due to its analgesic and anti-inflammatory properties such as arthritis, blood/hormonal disturbances, allergic reactions, skin disorders, retinal problems, respiratory problems, in addition to these clinical indications, it is also recommended for postoperative nausea and vomiting (Ciobotaru et al., 2019). Dex is used for peripheral nerve injury therapy. Still, its modes of action are not well understood (Feng and Yuan, 2015). In the next chapter, we will mention a series of previous scientific studies that illustrated the therapeutic role of Dex in promoting peripheral nerve regeneration following injury.

Betamethasone: This medication is classified as a synthetic long-acting corticosteroid drug and treatments a broad range of diseases and disorders mainly due to the anti-inflammatory and immunosuppressive effects of glucocorticoids and their impact on circulatory and lymphatic system processes in palliatives for the treatment of a wide range of diseases such as skin disease (Drugs.com). Numerous studies concentrate on encouraging the effect of Bet on facilitating the functional rehabilitation of peripheral nerve regeneration (Dahlin et al., 1996), which will be discussed later.

Mps: Considered to be an intermediate-acting synthetic glucocorticoid, which belongs to the pregnane steroid hormone resulting from hydrocortisone and prednisolone primarily recommended for its anti-inflammatory and immunosuppressive function. It is used to treat conditions such as arthritis, blood disorders, severe allergic reactions to certain cancers, eye problems, skin diseases such as dermatitis that are often used to treat intestinal and pulmonary diseases other than immune system deficiencies; according to recent studies, Mps has been successfully used to treat COVID-19 (J. Liu et al., 2020; Timmermans et al., 2019). In addition, several studies focus on Mps's potential role in treating peripheral nerve injury (Dahlin et al., 1996).

2. GENERAL INFORMATION AND LITRETURE REVIEW

1.1. General Organization of the Nervous System

The Human nervous system is anatomically formed by two major components, which include the Central Nervous System (CNS) and the Peripheral Nervous System (PNS) (Catala and Kubis, 2013).

The CNS is composed of the brain and spinal cord, both of them lodged and protected in a bony structure skull and vertebral column, respectively. The brain, as described in most literature organized into the Forebrain (Cerebrum and Diencephalon), Midbrain, and Hindbrain (Medulla oblongata, Pons, and Cerebellum) (Laine and Smoker, 1998). The PNS is considered as nerves that arise from the brain (cranial nerves) and spinal cord (spinal nerves). Both types of nerves consisted of afferent sensory fibers and efferent motor fibers projected through the neuromuscular junction to innervate the skeletal muscles of the target tissues (Catala and Kubis, 2013). However, taken into consideration that some nerves are formed by purely afferent or efferent fibers (Splittgerber, 2018).

1.2. Development of the Nervous System

In men, the development of the nervous system takes place in the third week of embryonic development by the formation of thickening in the ectoderm layer in the neural plate, followed by an elevation occur in the edges of the plate; with further development, the edges fused to form the neural tube (T. W. Sadler, 2018).

The cephalic portion of the neural tube formed three dilatations named prosencephalon, mesencephalon, and hindbrain, while the caudal part will give rise to the spinal cord.

The prosencephalon in the fifth week of development will comprise two parts; the first part is called the telencephalon, which is situated between the outpocketings structures named the primitive cerebral hemispheres, and the second part is the diencephalon, which is marked by optic vesicle outgrowth (Moore et al., 2018).

In the same manner, the Rhombencephalon is also shaped by two segments; the first one is the metencephalon, which later constitutes pons and cerebellum; subsequently, the second part is named the myelencephalon, which will give rise in the future for the medulla oblongata (T. W. Sadler, 2018).

2.3. Development of the Peripheral Nervous System

The development of the PNS takes place from several sources, but primarily from the neural crest. All somatic and visceral sensory cells of the PNS are differentiated from neural crest cells. At the onset, all sensory periphery cells are bipolar. The processes are subsequently united in a single process with central and peripheral components that finally form a unipolar type of neuron. The termination of the peripheral process occurs in the sensory ending, while the central process recognizes the brain and spinal cord (T. Sadler, 2005).

Regarding the cell body of the afferent neuron its tightly incorporated by a capsule of modified satellite cells, which originate from the neural crest cells. However, this capsule is cumulative with the neurolemmal sheath of Schwann cells that wrap the afferent neurons. Furthermore, external to the satellite cells present a sheet of connective tissue that is continuous with the endoneural sheath of the nerve fibers. This connective tissue and endoneural sheath are derived from the mesenchymal origin (Moore et al., 2018).

2.4. Overview of the Neuron

The neuron is classified as the structural and functional unit of the Nervous tissue; the human body is estimated to contain about ten billion neurons. While neurons exhibit the greatest difference in size and shape compared to the other body cells, they can be classified into three general groups.

Sensory neurons**:** this type of neuron is functionally responsible for conveying the nerve impulses from the receptor to the CNS. The processes of this type of neuron involve the somatic and visceral afferent nerve fibers. The former type of afferent is responsible for conveying the general sensation from the surface of the body that includes the feelings of pain, touch, and temperature, as well as the proprioception sensation, which transmits the nonconscious sensation from tendons, joints, and muscles. The latter type is called the visceral afferent fibers; this type carries the sensation from the internal organ such as visceral organs, glands, blood vessels, and mucous membranes.

Motor neurons**:** unlike the previous type of neurons, this type conveys the impulses towards the opposite direction from the CNS (brain or spinal cord) to the effector cells. Like the former type of neurons, these neurons' process conveys the somatic and visceral efferent nerve fibers. The somatic efferent neurons conduct the voluntary impulses (motor impulses) to the skeletal muscles. The other type of efferent neurons is the visceral type, which sends involuntary (spontaneous) impulses to control the cardiac muscles' activity via Purkinje fibers, smooth muscles of the viscera, and the glands.

Interneurons: according to some literature, they are also called the intercalated neurons, which are neither sensory nor motor; instead, they are the integrating network bind between the motor and sensory neurons. This integration network is estimated to contain more than 99 % of all neurons (Pawlina and Ross, 2011).

2.5. The Structural Formation of Neuron

The body and neuron processes (axon and dendrites) form the neuron's structural formation, and the communication between the neighbouring neurons occurs by synaptic junctions (Pawlina and Ross, 2011).

The cell body of the neuron, also named the perikaryon in the typical type, is formed by a compact structure that contains the nucleus with a diameter that extends from 3 to 18 micrometers, which is responsible for maintaining all cell functions (Nadim and Bucher, 2014).

Concerning the processes of the neuron, as mentioned above, constitute the following: The axon, which is usually single and considered as the most extended type of neuronal process, plays a conductivity function by transmitting the received or the newly generated impulses to the synaptic junction (Nowakowski, 2006). The other type of neuronal process is termed the dendrites, which are characterized by its shorter length and more branched in comparison with the previous type that expedited its function to convey the impulses from the periphery (i.e., former neurons). The communication between the neurons occurs via synaptic junctions, which contain a gap called the synaptic cleft that transmits the excitatory or inhibitory neurotransmitter chemicals from the former neuron's axon, dendrites, or to the axon of the letter neuron (Pawlina and Ross, 2011).

2.6. Peripheral Nerves Structure

As mentioned earlier, the communication between the CNS and the effector organ or tissue occurs through the peripheral nerve.

The peripheral nerve (PN) can be defined simply as a bundle of nerve fibers that are gathered together via connective tissue in addition to the nutritive blood supply. As a mediator, the PN transmits sensory and motor signals from and to the targeting tissues (Kerns, 2008). Peripheral nerves are typically divided into three major groups, depending on fiber-type composition: motor, sensory, and mixed nerves (P. Williams et al., 1999).

The perikaryon or the cell bodies of the peripheral nerves can be found inside the CNS or exterior. In case the cell bodies located in the CNS present a cluster called nuclei, while if the perikaryon is placed in the PNS, it is termed the ganglia (Pawlina and Ross, 2011).

2.6.1. Parenchyma of the Peripheral Nerve

Understandably, the structural and functional unit of the PN is the nerve fibers. According to the literature, the nerve fibers classification depends on various parameters, such as the velocity of conduction, functional role, and fiber diameter. Moreover, anatomically the organization depends on the enclose of axons by Schwann cells. That enables us to distinguish between two fiber subgroups: myelinated and unmyelinated nerve fibers (Geuna et al., 2009).

The myelinated nerve fibers: are made of a single axon that is individually enveloped via a single Schwann cell. Within the PNS, the Schwann cell membrane is wrapped around the fiber to create a multi-laminated myelin sheath (Blaurock et al., 1986). The myelinated fibers lodge gaps or internodal space along the myelin-sheath called the Ranvier's node (P. Williams, 1999). As mentioned by *Matthews*, the myelination can be found either in CNS or PNS. However, it depends on axon diameter in the case of CNS, and it should be more than 1 micrometer, while in PNS, the axon diameter should exist 1.5 micrometers (Figure 1) (Matthews, 1968).

The unmyelinated nerve fibers: are made of a group or collection of axons enveloped via a single Schwann cell. Around 75% of mammalian axons of dorsal spinal roots and cutaneous nerves are unmyelinated, as well as the axons of autonomic ganglia are entirely unmyelinated. In comparison with the previous type, the diameter of the unmyelinated fibers is obviously smaller, arranged between 0.15 to 2.00 micrometers as a group in the sequential sequence of Schwann cells (Figure 1) (Gamble and Eames, 1964).

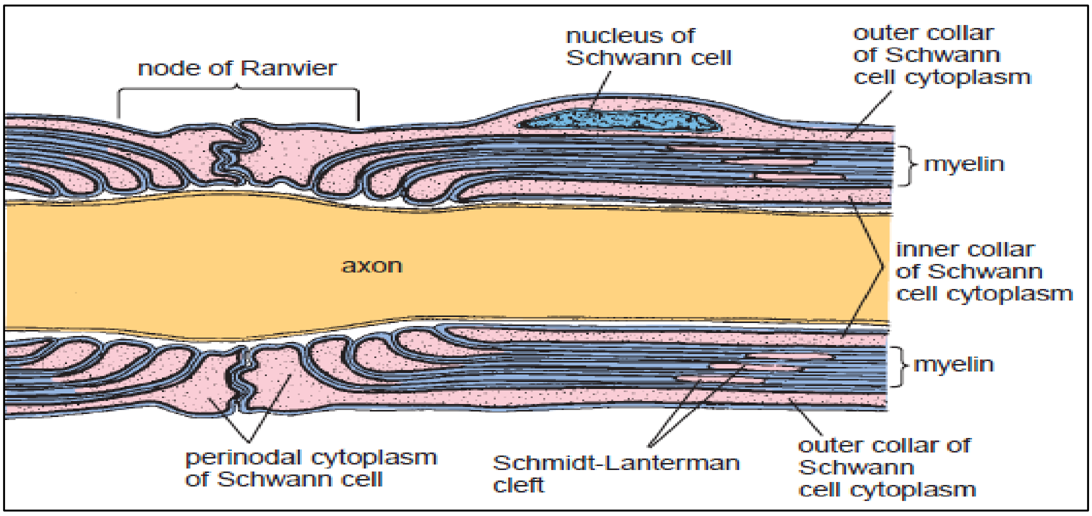


Figure 2.1. The diagram illustrates the axon's longitudinal section and its myelin relations (Pawlina and Ross, 2011)

2.6.2. The Stroma of Peripheral Nerve

One of the factors of difference in the CNS is that their nervous tissue elements (grey and white matter) are situated at the meningeal level, either in the brain or spinal cord. While the elements of PNS are surrounded and supported by a connective tissue distinct sheaths, which play an essential role in providing them with a sufficient resistance against the stretching factors, also supply them with compression forces applied during the body motions (Geuna et al., 2009).

The substance of a PN is classically organized as separate bundles acknowledged as fascicles, whether if the PN is unifascicular or multifascicular, encircled by three connective tissue distinct sheaths named: epineurium, perineurium, and endoneurium oriented from outermost to innermost respectively (Figure 2) (Seddighi et al., 2016a).

Epineurium: is a loose connective tissue that enveloped the nerve trunk from the exterior, either it is unifascicular or multifascicular; it also fills the spaces in between the fascicles; moreover, the epineurium is considered as nutritional and supportive constituent because it contains the intraneural vascular system, which contains the vasa nervorum that communicate and anastomosis with the arterioles and venules in the endoneurium sheaths. Developmentally the epineurium is derived from the mesodermal origin. Epineurium usually accounts for 30–70 % of the overall cross-sectional area of the nerve bundle in humans. It has been observed that there is a direct proportionality between the thickness of the epineurium and the number of fasciculi that are present in a peripheral nerve (Figure 2) (S Sunderland and Bradley, 1949).

Perineurium: can be defined as a tubular sheath formed by specialized connective tissue wrapped around each nerve fascicle (Jessen and Mirsky, 1999). Perineurium involves multiple layers of flattened polygonal (epithelial-like) cells and collagen fibers resting on a basal lamina; according to *Thomas and Olsson*, in mammalian nerves, these layers may reach up to 15 layers (Thomas and Olsson, 1984). One of the most crucial characteristic features for the cells that participate in the formation of perineurium; is the compact connection via gap junction that is termed as a blood-nerve barrier, which plays a vital role as a selectively permeable factor and that occur by preventing the unwanted substances from passing through. In turn, this leads to maintaining the endoneurium fluid pressure and osmotic balance (Parmantier et al., 1999).

Endoneurium: represents as loose connective tissue found in the innermost layer enveloping each single nerve fiber, it is composed of fibroblasts that synthesize the extracellular matrix, and the collagen fibers that will directly reflect on the gathering between protection and cushioning of fascicles, especially during the movement and against trauma may occur (Lundborg, 2004). We should also keep in mind that collagen fibrils are densely packed to form the nerve fiber is supporting walls (Figure 2) (Geuna et al., 2009).

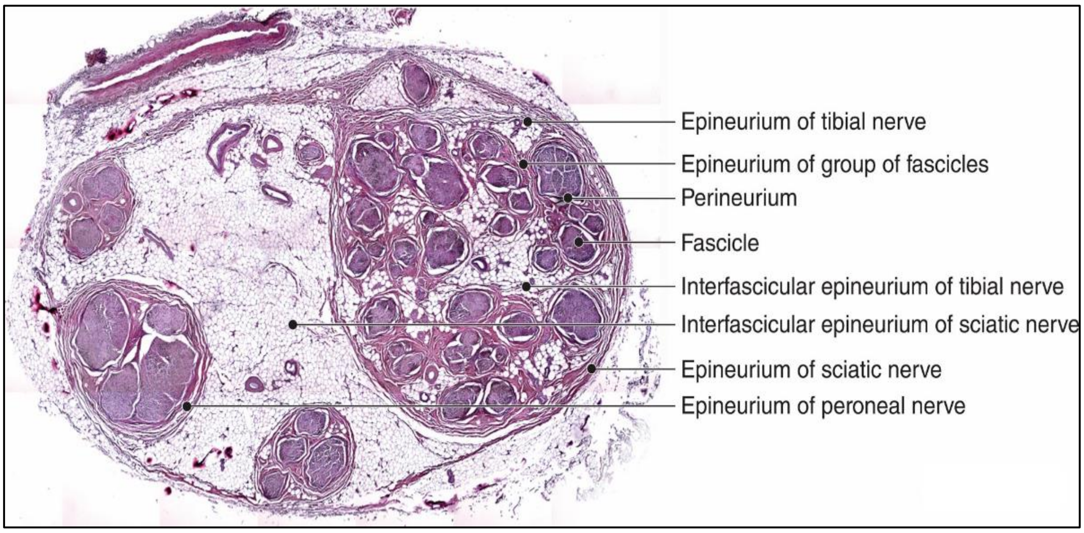


Figure 2.2 Sciatic nerve at the popliteal fossa level demonstrates the Peripheral Nerve's stroma (Reina et al., 2011)

2.7. Peripheral Nerve Injury

The neuronal damage or injury can occur in CNS and PNS. However, the variation mainly in the response after the injury that the axon of PNS has the ability to make rapid regeneration; in contrast, the axons of CNS usually fail in the regeneration. The justification is due to inattentive of the phagocytosing mechanism in the myelin fragments, which should occur by oligodendrocytes and microglial cells, on the other hand lacking a significant number of migrating macrophages due to the presence of the blood-brain barrier (Pawlina and Ross, 2011). This section will discuss the morphological and neuropathological features that follow the peripheral nerve injury in terms of degeneration and will talk about the possibility of regeneration and the factor of enhancement.

2.8. Peripheral Nerve Degeneration

Peripheral nerve injury is followed by pathophysiologic changes in the nerve's disruption location, inclusive of metabolic and morphological changes. That will reflect as an interruption in axonal transport. According to recent studies, these changes usually occur immediately after the crushing or transection injury of the nerve. It involves both proximal and distal portions disruption location (Geuna et al., 2009); each one of them will be discussed separately, taking into account changes in tissue organization.

Seddon mentioned that nerve injury could be classified into three categories according to pathophysiologic features: Neurapraxia, Axonotmesis, and Neurotmesis (S. K. Lee and Wolfe, 2000; Seddon and Wilkins, 1972).

Neurapraxia: this type of nerve injury is typified as the disruption that occurs in the myelin sheath, usually subsequent to compression. Continuity of the axon is maintained in this type of injury; subsequently, it is not associated with distal degeneration.

Axonotmesis: in comparison with the previous type of injury, the pathophysiologic features of this type are represented in lack of axon continuity, accompanied by a variable amount of connective tissue elements preservation.

Neurotmesis: is defined as the worse damage in the nerve because it is supplemented with disruption of the whole nerve's physiological features. In addition, it must be taken into account that it may or may not require the presence of complete nerve transection (S. K. Lee and Wolfe, 2000).

2.8.1. Changes in Proximal Segment of Peripheral Nerve After Injury

As mentioned previously, the nerve injury is followed by several neuropathological and morphological changes here; we will try to highlight the significant changes that may take place.

In the proximal segment of the peripheral nerve, the cell body response to this factor a few hours after the injury by making a morphological change called the chromatolysis, which is characterized by swelling in the nucleus and accompanied by an eccentricity that intern will lead to modification of the metabolic machinery from the propagation of nerve impulses to reconstruction the wounded nerve (Lieberman, 1971). According to *Moon,* chromatolysis can be defined as cytoarchitecture alterations, which fragmentation of rough endoplasmic reticulum leaving visible regions of Nissl-missing cytoplasm; in some situations, this can be followed by decomposition of polyribosomes to leave a thin "dust-like" powder (Moon, 2018). In other words, we can conclude that neurons turn to a "growing response mode" from a "signalling mode" (Fu and Gordon, 1997).

At the level of axon degenerate also occurs just back to the disruption or injury location, leaving an empty tubule or cylinder that originates from the basal lamina of Schwann cells; this process is known as retrograde degeneration. This retrenchment can extend to one or more internodal segments, depending on the lesion's nature (Cajal, 1928).

A few hours later, the proximal segment axons start to form a number of branches as a collateral structure called sprouts, which are heavily concentrated distally inside the basal lamina (Faweett and Keynes, 1990). These latest sprouts will be helpful as they can reconnect nerve signals along damaged paths and play a role in regeneration by restoring their function. However, according to experiments were done by *Witzel and Brushart*, the regeneration sciatic nerve has shown considerable behaviour variability (Witzel and Brushart, 2003). Although among the responses to nerve injury that occurs are the axoplasmic outgrowths, which can be defined as several biological processes, localized modifications in axonal cytoplasm composition have also been observed within hours after injury (Rishal et al., 2010).

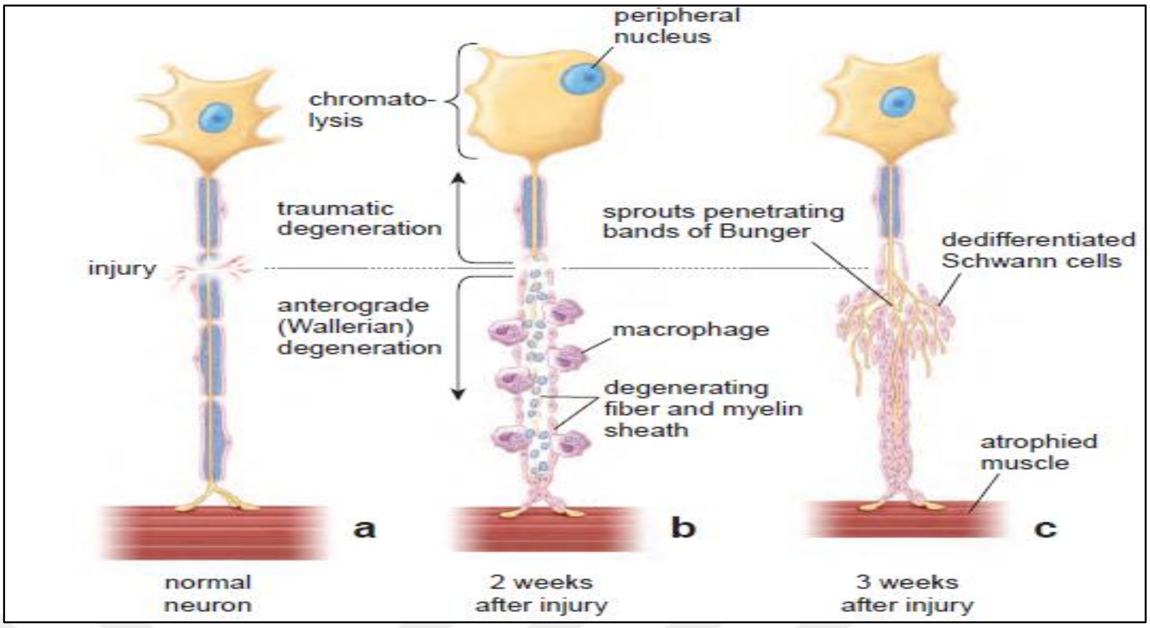


Figure 2.3. Degeneration sequences after nerve injury. (Pawlina and Ross, 2011)

2.8.2. Changes in Distal Segment of Peripheral Nerve After Injury

As a thread extension of peripheral nerve injury, we will focus in this section on the neuropathological and morphological changes that take place on the distal segment. After nerve injury, the distal segment is undergoing a gradual process of degeneration. This degeneration mechanism is referred to as Wallerian degeneration (Geuna et al., 2009). This phase begins instantly after disruption or injury with myelin disintegration and proliferation of the Schwann cell. In the injury site, Schwann cells and macrophages are stimulated, and during a period of time, they establish the phagocytosis process for both myelin and cell debris.

Among the events that occur in the early hours after injury and the axonal disintegration, within the first steps of this phase, the axoplasmic microtubules and neurofilament are granularly disintegrated by proteolysis (Lubińska, 1982; Schlaepfer, 1977; Vial, 1958).

According to experiments, the justification of Schwann cell proliferation is the loss of contact in the axon-Schwann cell as a preliminary signal (Thoenen et al., 1988). The proliferating Schwann cells are arranged into columns that call the bands of Büngner, and the regenerative axons are growing distally between its basal membranes. Factors stimulate the progress of regeneration, which occurs in the axon's distal segment termed the neurite outgrowth-promoting, including laminin and fibronectin (H. Liu, 1996).

Axon sprouts do not follow an exceptional route approaching a column of Schwann's or may spontaneously grow into the connective nerve tissue in the distal segment (Geuna et al., 2009, p.^pp.) was already discussed earlier. The original number of axons present may significantly increase in the distal nerve segment compared to the proximal segment of the same injured nerve (Povlsen and Hildebrand, 1993).

2.9. Peripheral Nerve Regeneration

The mechanism of peripheral nerve regeneration is established when the effector organ or tissue is denervated (Menorca et al., 2013).

Re-innervation takes place in two ways: the collateral branches of axons are not exposed to injury, or it may be occurring by the injured axon regeneration (Aguayo et al., 1973).

In 20-30% of nerve injury cases, the collateral branching is considered as the primary process for regeneration; this process frequently initiates after four days subsequent to nerve injury, and this will persist for around 3-6 months for rehabilitation or recovery occurrence (Aguayo et al., 1973). While in the case of nerve injuries that affect more than 90% of axons, the solitary way for recovery is axonal regeneration (Lunn et al., 1990).

Achievement of full rehabilitation or recovery, the nerve must encounter three significant processes: Wallerian degeneration, regeneration of the axon, and the end or effector organ reinnervation, as discussed earlier. The failed procedure of these processes may lead to a poor physical and clinical function that is typical in peripheral nerve injury patients (Menorca et al., 2013).

Regarding the intrinsic factors that promote the axonal regeneration influenced by Schwann cells, which are considered neurotrophic factors, which interact to modify the neuron gene expression pattern with tyrosine kinase receptors to encourage regeneration. The signal message is transmitted via retrograde transportation. Furthermore, we should consider the role of the Nerve Growth Factor (NGF), which has a limited function in healthy nerves. In contrast, in the case of nerve injury occurrence, the influence role emerges by upregulation in Schwann cells. NGF encourages the development and proliferation of the Schwann cells, as well as the trophism of the outgrowing axon, which is located on the receptors of the Bungner cell bands (Lunn et al., 1990). Many neurotrophic factors have been identified with wide functions ranging from enhancing cell survival through apoptosis prevention mechanisms to promoting neuronal and cell regeneration in Schwann cells. In addition, Schwann cells generate neuritis-promoting factors, including fibronectin and laminine, that are integrated into the extracellular matrix (ECM). These proteins are used by cones for adherence to the basal lamina of endoneurial tubes (Guenard et al., 1991).

After phagocytosing myelin, infiltrating macrophages also facilitate nervous regeneration via the secretion of interleukin-1, inducing NGF expression in Schwann cells and increasing the receptor of NGF density in Schwann cells. In addition, this feeding process results in the release of mitogens to further activate the proliferation of Schwann cells. However, macrophages also secrete interleukin-1 receptor antagonists, which reduces the regeneration of the myelinated and unmyelinated type of axons in the implantation tube in rats with sciatic nerves transaction (Battiston et al., 2009; Schröder and JM, 1972).

Once the growth cone approaches the endoneurial tube, that will increase the opportunity to reach the effector organ or tissue. Therefore, maturation should take place before the completion of the functional connection. The maturation mechanism involves the following sequences; starting with remyelination followed by axon enlargements to end with functional reinnervation. Axonal outgrowth generates ATP and acetylcholine, which facilitate a transition of Schwann cell's observable characteristics features from not myelinated to myelinated (Stoll et al., 1989).

2.10. Influence of Drugs on Peripheral Nerve Regeneration

According to recent studies, there is no proven peripheral nerve regeneration adjuvant drugs. However, many research types are conducted on animals to study medications' role to promote peripheral nerve regeneration based on the functional and histological criteria as an evaluation factor after inducing peripheral nerve injury. However, it is worth mentioning that the great majority of experiments have been preliminary in relatively few clinical trials (Bota and Fodor, 2019).

The goal of this section is to enumerate the main categories of drugs that have been previously used in the experimental studies of peripheral nerve regeneration and give some examples for each category with clarification of the method of action.

2.10.1. Immunosuppressants

Immunosuppressive drugs have been administrated for a long time due to the popular belief of their positive effect in promoting peripheral nerve regeneration. These medications' acting mechanism can be summarized in the decrease in scar formation and promote regeneration by altering immune and inflammatory responses.

Tacrolimus (FK506) (TA) is considered an immunosuppressive agent usually prescribed for patients with renal transplantation in case of renal failure to prevent organ rejection. It had been noted its affirmative effect in patients with upper limb transplantation especially in risen more rapidly the peripheral nerve regeneration, this, in turn, led to the conclusion that TA using in moderate doses can enhance the positive impact of peripheral nerve regeneration, this is precisely the opposite of that if high doses are used which proved that it lead to neurotoxicity. Tacrolimus act as an inhibitor for T-cell by linking FK-binding proteins and inhibiting other factors, leading to reducing the inflammatory cytokines (A. Y. Mekaj et al., 2014).

Rapamycin (RA): falls under the umbrella of the immunosuppressive agent, which pharmacologically acts as an autophagy-inducer. According to experimental studies, RA in sciatic nerve crush injury rats reduced apoptotic cell count and encouraged histological and functional recuperation, opposite to 3-methyladenine, which has similar categorization autophagy-inhibitor (Huang et al., 2016).

Due to the anti-inflammatory effect of corticoids, it is supposed to accelerate peripheral nerve regeneration; with variable outcomes, corticoids are expected to minimize scarring and improve the regeneration of the nerves (Bota and Fodor, 2019). *Morisaki* proved that low and high dose glucocorticoid hormone, which is administered to rats exposed to adrenal gland resection and a sciatic nerve crush injury, reveals improvement in histological recovery, proving its role glucocorticoid receptors in remyelination via Schwann cells (Morisaki et al., 2010). An example of a corticoid drug that has been used is Dex which proves it is a positive experimental result in promoting the histological and functional recovery in rats exposed to sciatic nerve crush injury, which expect occur due to immunosuppression acting and potential activity in neurotrophic (Feng and Yuan, 2015; Tanyeri et al., 2015). Another example worth mentioning is the administration of Mps in the same condition of sciatic nerve crush injury that showed enhancement in histological features recovery, in addition to reducing inflammation effect primarily when used

Mps in combined with Ozone as a mutual therapy (Ozturk et al., 2016; Yanilmaz et al., 2015; Liao et al., 2010).

2.10.2. Neurological Drugs

It is also named neuropharmacology drugs that are defined as drugs that have a cellular function in the nervous system and act in the neural mechanisms and extend to influence behavioural roles. Neuropharmacological drugs believe in their beneficial effect in promoting peripheral nerve regeneration (Bota and Fodor, 2019).

Lithium is commonly used to treat symptoms of bipolar mood disorders, and in recent times, it has proved its therapeutic efficacy as a neuroprotective agent, according to histological evaluation. In addition, Lithium also showed improvements in rats with a sciatic nerve crush injury after receiving a preoperative dose (Nouri et al., 2009).

Gabapentin: considered an anti-epileptic drug or anticonvulsant agent, has also proven its therapeutic efficacy in controlling the neuropathic pain that occurs by the herpes virus. Experimental studies were done in the rat model with sciatic nerve constriction in order to stimulate neuropathy verified that Gabapentin reduces the results of pain behaviours assessment which mean reduction of pain, also observed improvement the response of peripheral nerve and enhancement in the characteristic feature of nerve morphology (Câmara et al., 2013).

Pregabalin: classified as a neurological drug used to treat epilepsy, relieve neuropathic pain, and other medical uses. Regarding the effect in the peripheral nerve, it showed an advance in the structural and functional recovery, and that occurs by stimulating anti-inflammatory cytokine TGF-β1 in rat model study that received sciatic nerve crush injury (Celik et al., 2015).

Valproic acid: Like the previous one considered as anti-epileptical medication also acts as a mood stabilizer also reveal a positive impact on peripheral nerve regeneration depending on the criterion for improvement in structure and the function of the injured nerve that can be justified by activation of an extracellular signal-regulated kinase (ERK) (Rao et al., 2014).

Zonisamide: this drug used commonly to treat patients who have epilepsy also used in case of Parkinson disease; like the former medication is proven its therapeutic efficacy in promoting the structure and function regeneration in rat model study exposed to autograft sciatic nerve; these results are believed to have been obtained by enhancement neural growth factor and protection from oxidative stress (Yagi et al., 2015).

2.10.3. Nonsteroidal Anti-Inflammatory Drugs (NSAID)

Nonsteroidal anti-inflammatory drugs are associated with a drug class widely used as a pain reliever, curb inflammation, decrease fever, and act as an anticoagulant agent. The curative effect on peripheral nerve regeneration has also been proven, as mentioned by *Tamaddonfard*, that administration of Diclofenac, Celecoxib, and Vitamin B12 separately showed particular enhancement in histological and functional nerve regeneration. As well as the severity of Wallerian degeneration was further diminished when 0.1 mg/kg of vitamin B12 combined with 1 mg/kg of Diclofenac either with or without 0.2 mg/kg of Celecoxib (Tamaddonfard et al., 2014). In another study conducted, it was proved that Celecoxib has therapeutic efficacy in promoting the function regeneration after administration of the medication for seven days in a rat model study exposed to sciatic nerve crush injury (Cámara-Lemarroy et al., 2008). It falls under the same pharmacological classification, Ibuprofen, which also verified its positive effect in promoting the peripheral nerve regeneration, and that can justify by inhibiting the Ras homolog family member A (RhoA) cascade (Madura et al., 2011).

2.10.4. Lipid-Lowering Medications (Statins)

Statins: pharmaceutically have been used to suppress cholesterol serum levels by acting as inhibitors for the 3-hydroxy-3-methylglutaryel coenzyme A reductase; according to recent studies, it has proven the therapeutic effect as an anti-inflammatory agent as well as the neuroprotective properties (Pan et al., 2010). The next paragraph gives an example for some Statins that their therapeutic efficacy has been proven experimentally.

Simvastatin: along with statins family, the treatment performance of this drug was evaluated in rats exposed to sciatic nerve injury and found to be used in small doses 2 mg/kg/day prove lack of therapeutic effect in functional recovery (Daglioglu et al., 2010), while when the same medication used in high dose 20 mg/day found that it leaves a therapeutic effect which evaluated by improvement in histological recovery (Xavier et al., 2012), It is worth noting that the duration of treatment is having a significant impact as well in promoting the nerve regeneration.

Atorvastatin: also considered along with statins family according to experimental results, the administration of this medication has a positive effect in functional recovery in rats after exposure to sciatic nerve crush injury (Cloutier et al., 2013). Also, it has been proven that Atorvastatin's administration for seven days before the induce of sciatic nerve injury has demonstrated its protective effect, and that can be justified by the antioxidative and anti-inflammatory consequences of this medication (Pan et al., 2010).

2.10.5. Hormonal Therapy

The term hormonal therapy is commonly used to describe a group of medications that way of treatment action depends on adds hormones, blocks or disable hormones.

Hormones are administrated to control low hormone levels for certain conditions such as diabetes or menopause. In addition, synthetic hormones are used to adjust low hormone levels, which may occur in some pathological conditions (National Center Institute, 2020, November 30). It has also been found to be used as a medical substance that may promote the peripheral nerves' regeneration (Bota and Fodor, 2019).

Erythropoietin (EPO) is considered a hormone secreted by the kidney (renal cytokine), secreted by cells of the nervous system, and plays an essential role in hematopoiesis. According to literature, EPO has proven that EPO, due to antioxidative and anti-inflammatory consequences, has a positive effect on functional recovery in rats exposed to sciatic nerve crush injury (Yin et al., 2010).

Melatonin (MT): is a hormone produced by the pineal gland that is responsible for the regulation of the sleep-wake cycle, also known as the circadian rhythm, as well as playing roles in various physiological functions such as immunogenetic function, reproduction, and lipid metabolism. Several authors investigated the effect of MT in the recovery of the peripheral nerves after injury. As an example, the curative effect based on enhancing the histological and functional recovery of the rat sciatic nerve has been observed by an administration of MT in circadian rhythm (Kaya et al., 2013). In another comparative study conducted by performing a pinealectomy in a group of rats and the other group devoid of pinealectomy and treated by supplement MT in the injured sciatic nerve, the results showed improvement in the functional recovery (Atik et al., 2011). In another applied study was conducted to compare the therapeutic effect of a collection of drugs in the autograft buccal nerve, which is a branch of the facial nerve; the group of drugs includes Melatonin, Mps, Aminoguanidine to evaluate the regeneration in the target nerve, which shows the best result was documented by Aminoguanidine also the Melatonin revealed a significant therapeutic effect in enhancing the nerve regeneration.

Oxytocin: considered as a sex hormone that is secreted in both genders via the posterior pituitary gland, which proved through an experimental study that promotes the peripheral nerve regeneration and that can be explained by this hormone activate the insulin-like growth factor-1 (IGF-1) and nerve growth factor (NGF) also it plays an essential role in enriching the vascularization, in addition, to increase the number of axons consequently the nerve regeneration takes place (Gümüs et al., 2015).

Testosterone propionate (TP): this type of medication is classified as androgen, also considered as an anabolic steroid commonly used as testosterone substitution according to experimental study; it has been shown that TP has neurotrophic influence via increasing the brain-derived neurotrophic factor manifestation; however, it decreases the glial fibrillary acidic protein (Sharma et al., 2010), based on that; this leads to an improvement in neurotrophic factor expression (T. J. Brown et al., 2013). An example of therapeutic applications of TB to promote peripheral nerve regeneration is a research experiment conducted by *Sharma* in which a crush injury was induced in the recurrent laryngeal nerve of experimental rats, and the results confirmed the nerve functional recovery occurrence (Sharma et al., 2010).

2.10.6. Vitamins

According to the medical dictionary, vitamins can be defined as organic additives in food that are essential for growth and health preservation in minimal quantities. Vitamins D, E, B, and K, are included with the fat-soluble types and folates, among the examples of vitamins. For three factors, people are being treated with vitamins. The main explanation for the detection is to mitigate a vitamin deficit. Chemical methods are done and are ideal for the diagnosis of all vitamin shortcomings (Albahrani and Greaves, 2016). Also, visual tests such as a microscope blood cell analysis or an x-ray evaluation of the bones, or a visual exam of the eyes or the skin assist the diagnosis of vitamin deficiency; in the next paragraph, some examples of vitamins that attempted to be used for promoting the peripheral nerve regeneration (Albahrani and Greaves, 2016).

Methylcobalamin (MB) is considered the cobalamin equivalent of vitamin B12, which plays a significant role in axon myelination. It has been proved that MB effectively stimulated nerve regeneration by activation of protein kinases Erk 1/2 and Ark, which in turn promote neurite outgrowth and branching of neurites in the dorsal root ganglion (Okada et al., 2010). According to significant research has been done that the rats exposed to nerve injury recorded on the first day after injury increased in the level of vitamin B12. In contrast, seven days later occur a decrease in the level compared with the cont group, which can be justified by an increased local vitamin intake (Altun and Kurutaş, 2016). According to the literature, administration of MB cause a positive impact on the histological and functional restoration in the rat exposed to sciatic nerve injury (Okada et al., 2010). In comparative research has been compared MB with Pleiotrophin and Mps to assess the effect on rat ulnar nerve regeneration, which sutured to musculocutaneous nerve; the results reflect that MB has a therapeutic impact in promoting the morphological and physiological features. In contrast, the remaining drugs do not have a significant effect (Liao et al., 2010).

Coenzyme Q10: considered as an effective antioxidant, also it plays an important factor in mitochondrial metabolism; in an experimental study, it was administrated to rats that exposed to facial nerve injury, the results reveal Coenzyme Q10 promotes the regeneration by amelioration the histological and functional features (Yildirim et al., 2015).

Vitamin D: considered as fat-soluble secosteroids which play an essential role in mineral absorption in the digestive system, according to experimental results which examine two types of vitamin D: Ergocalciferol and Cholecalciferol were administrated to rats that exposure to an autographic reconstruction of the peroneal nerve; the results proved that Cholecalciferol had more potent effects in compare with Ergocalciferol on both structural and physiological nerve recovery (Chabas et al., 2013).

2.10.7. Other drugs

Pentoxifylline: also called Oxpentifylline, this type of medication is considered a vasodilator; it is classified under the umbrella of nonselective phosphodiesterase inhibitor commonly used as a painkiller also used to treat cramping and numbness.

It has been examined its effect in promoting the nerve regeneration in rat model study, the injury is induced by creating crush injury in the cavernous nerve, and it proved that Pentoxifylline enhances the histological and erectile function recovery, whereas it increases the synthase of nitric oxide (Albersen et al., 2011).

Metformin: this drug is the first-line medication for the treatment of type 2 diabetes, which reduces glucose production besides its other therapeutic functions as an antioxidant, neuroprotective influence, and anti-inflammatory effects; therefore, it deserves to be conducted laboratory experiments to assess its impact on peripheral nerve regeneration. The experimental study was conducted on diabetic rats also exposed to sciatic nerve crush injury, and the results proved that Metformin promotes functional and histological regenerations (Ma et al., 2016).

Sildenafil: Sold usually under the trade name Viagra, it has proven the therapeutic efficacy in treating males with erectile dysfunction, also it used to treat persons with pulmonary arterial hypertension. Sildenafil is considered as a selective phosphodiesterase-5 inhibitor. According to recent research, it has been shown positive effects in promoting angiogenesis, synaptogenesis, and neurogenesis. Based on the aforementioned, the curative effect in enhancing peripheral nerve regeneration has been tried in rats with sciatic nerve injury, and it has shown a therapeutic effect on the histologic and functional level but without statistical significance (Fang et al., 2013).

N-acetylcysteine (NAC): this medication, commonly used as a mucolytic agent, is also considered an antioxidant factor that can enable nerve regeneration, Although the precise processes of action were not disclosed. According to experiments were done in rabbits after transection of the facial nerve and subsequent repair and treatment with NAC and Mps, the results demonstrated that NAC promotes nerve regeneration specifically at the histological level. In contrast, in rabbits groups treated with Mps, despite the occurrence of degenerative signs of severe axonal detachment and a rise in myelin fragments, no substantial regeneration has been detected (Karlidag et al., 2012).

2.11. Corticosteroids

Corticosteroids are effective medicinal agents used to control allergic and inflammatory conditions or prevent excessive or inappropriate immune system reactions. Clinically the term corticosteroid is used to define the glucocorticoid action agents. Cortisol is considered as an endogenous glucocorticoid, noted for its influence on glucose metabolism furthermore maintains other corticosteroids immunological behaviour. Cortisol is generated by cholesterol metabolism in the adrenal gland. Regarding cortisol has a host of biochemical implications in humans as the main endogenous glucocorticoid. These symptoms are pedestrian and pleiotropic, which affect almost every organ and metabolism in the body (D. M. Williams, 2018). It is worth mentioning that corticosteroid has Pharmacologic effects on the central nervous system and various endocrine functions (Shaikh et al., 2012).

Concerning the steroid hormone originates from cholesterol metabolism, they share molecular characteristics of cortisol. Synthetic analogs are a 17-carbon androstane formation derived from cholesterol metabolism, which involves topical and systemic treatment. Three 6-carbon hexane rings, as well as five-carbon pentane rings, are accessible (Figure 2.4) (Moss, 1989).

The current study focuses on synthetic corticosteroid that mimics the behaviours of naturally produced corticosteroids which could be used to substitute adrenal glands corticosteroids in patients unable to manufacture sufficient quantities of corticosteroids, particularly the following corticosteroids: Dex, Bet and Mps are evaluate their action in promoting the peripheral nerve regeneration in a rat model study.

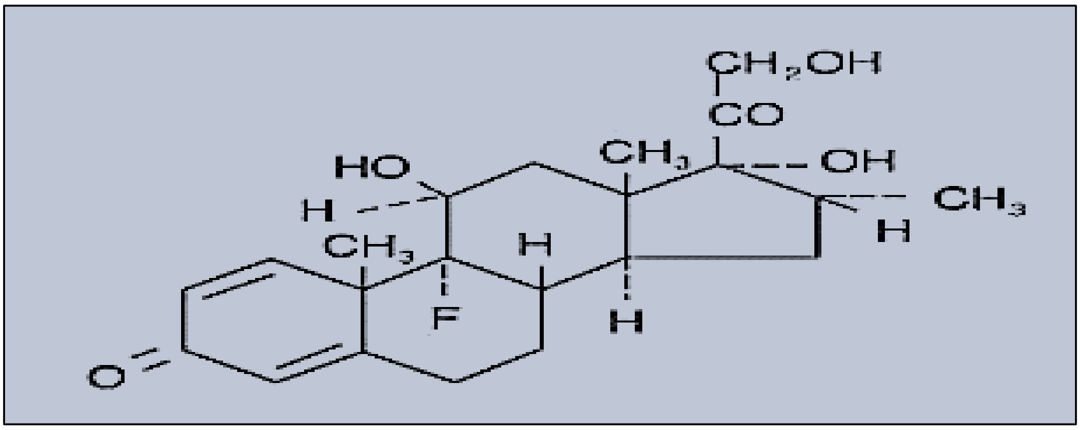


Figure2. 4. Structure of corticosteroid molecule

2.11.1. Dexamethasone

Considered as synthetic long-acting corticosteroid medication widely used due to its analgesic and anti-inflammatory effects such as arthritis, abnormalities of the blood/hormone, allergic responses, skin conditions, complications with the retina, respiratory problems, additionally to these clinical indications, it is also prescribed for postoperative nausea and vomiting (Ciobotaru et al., 2019; D. M. Williams, 2018).

Concerning the chemical composition of Dex is 9-fluoro-11 β, 17 dihydroxy 16α-methyl-21- (phosphonooxy) pregna-1, 4-diene-3, 20-dione disodium salt (Figure 2. 5) (Venkata Sairam et al., 2014).

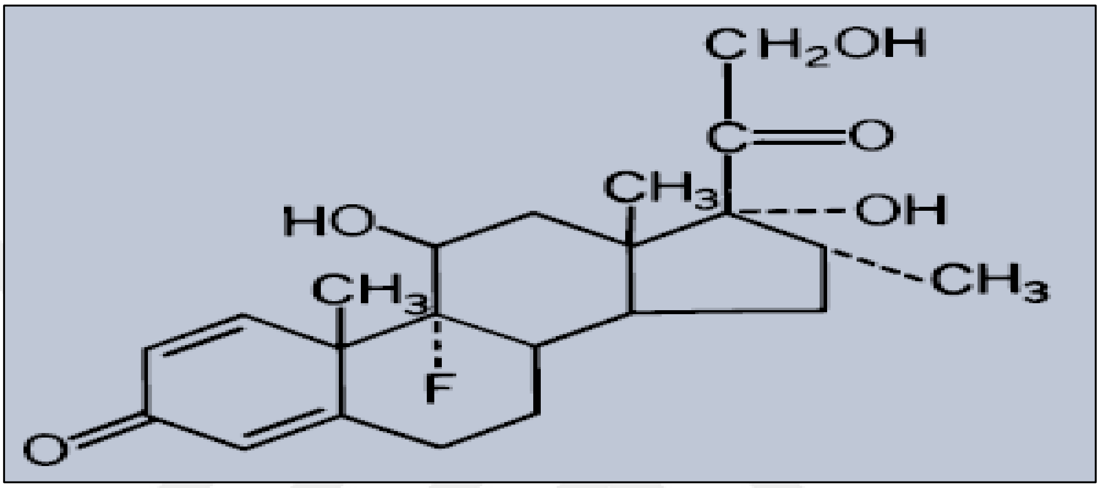


Figure 2.5. Structure of Dexamethasone

Dex is prescribed for the treatment of peripheral nerve damage, but its action mechanisms are not well known. In this section, we will highlight the studies conducted to assess the impact of Dex in promoting peripheral nerve regeneration after nerve injury; the studies included criteria that depend on the rat and rabbit model study (Feng and Yuan, 2015).

The first targeted study was conducted by *Feng,* in which he examined the effect of different doses of Dex in enhancing the functional recovery in rats sciatic nerve. Dex was injected into the muscle of site injury, and the evaluation depends on the following factors; motor activity assists by walking track analysis also by the mass ratio of the gastrocnemius muscle, as well as the histological evaluation, particularly the axon number, and immunohistochemical analysis. Regarding the results depending on doses first, the sciatic function index revealed the best result in the group of rats treated with Dex 2.0mg/kg; the beneficial impact had been noticed as the duration of therapy increased. The study also showed improvement in gastrocnemius ratio by using the medication with no significant difference in the result between the various doses. Also, the hypothesis supported by the immunohistochemistry result for CD3 expression depending on T-cell infiltration confirms that Dex therapy resulted in a decrease of CD3-positive cells, leading mainly to its immunosuppressive effect. Histological wise, the study also approves that an apparent increase in myelinated axon counts is directly proportional to the increase in Dex dose (Feng and Yuan, 2015).

In another comparative study, the Dex effect was compared with pyrrolidine dithiocarbamate effect in promoting regeneration of an inferior alveolar nerve after exposure to crush injury in a rat model study. Differential criterion depends on immunofluorescent result demonstrated that outcomes in the Dex population display are dramatically diminishing NF-κB expression. Western blot reveals the increased GAP-43 expression and the lesser NF-κB expression. In conclusion, all findings indicate that Dex causes a substantial increase in crushed inferior alveolar nerve regeneration due to inhibition of NF-κB (Gao et al., 2017).

In another comparative study, Dex has been targeting but with another administration route depending on the bridging technic for the injured sciatic nerve using the silicone tube. The tube is filled with the targeted medications in the study, namely 10 µL Dex and that by means of a specific dose equivalent (0.1 mg/kg) for the first rat's group, while the other group using the same silicone tube in the injured sciatic nerve but it filled with phosphate-buffered saline. The benchmark for the therapeutic effect is based on immunohistochemistry assessment by determining the site of reaction for the S-100; also, in the study takes into consideration the functional recovery of the sciatic nerve as a measurement factor, as well as the muscle mass by measuring the weight of gastrocnemius muscle. The results of the experiment showed significantly accelerated functional regeneration of the silicone conduit that was filled with Dex. Furthermore, regarding the muscle ratio, the yielded results proved that gastrocnemius muscle weight reduction had been increased by the administration of Dex. Thus, the results of immunohistochemistry and the histological morphometry were in line with its predecessor, which confirms that the group of rats treated with Dex exhibited enhancing the functional regeneration of the sciatic nerve. In conclusion, the study results demonstrate that topical use of Dex revealed a positive therapeutic effect in promoting the functional recovery of peripheral nerve regeneration (Mohammadi, Azad-Tirgan, et al., 2013).

Another study worthy of attention, based on a group comparison of medications, involves the following: Dex, Tacrolimus, Artesunate, and Petrolatum. The study was conducted by creating a crush injury in the sciatic nerve of the targeted groups and examining the effect of each medication separately, regarding the route of administration its depend on gelatine sponges which have been soaked and added to the damaged nerve regions with primed drug dilution for each single drug type. The findings of the experiment proved that all the used medication had beneficial effects in the regeneration of the nerves when the sciatic function index and electrophysiological tests were assessed; no significant variations between them were detected. After the histopathology test, the result showed that Artesunate lowered fibrosis and inflammation levels and increased myelinated axons diameter; fibroblast levels reduced while Dex decreased only the fibrosis rating Tacrolimus make lowering in fibroblast counts. Immunohistochemistry studies found that Dex and Artesunate gave more favourable immunoreactivity to the growth factor of the nerve (Uzun et al., 2019). In conclusion, like the previous one, this study reinforces the hypothesis that Dex exposed a positive therapeutic outcome in promoting the functional recovery of peripheral nerve regeneration.

In an exciting study, *Hongzhi Sun.* and his colleagues revealed that Dex might have a better therapeutic effect when combined with another prescription drug. The experimental rat's sciatic nerve was exposed to crush injury after dividing them into groups in the study. Rat groups that have undergone treatment are Dex (1 mg/kg) treated group; vitamin B12 (2 mg/kg) treated group the last group treated by the two medications together with the same previous doses. The medications were injected into the site of injury in order to examine the topical effect. The evaluation methodology targeted walking behaviour by tracing the footprint and assisting the electrophysiological and ultrastructural interpretation. The study's findings were impressive that the best therapeutic outcome was in the group of rats that received integrative therapy, including Dex and vitamin B12. The result can be justified as the two medications are acting synergistically to increase the quantity of Schwann cells

The occurrence of myelinated nerve fibers regeneration and the increase of Schwann cells demonstrates the positive effect that, however, leads to the positive impact reflected on afferent and efferent conduction and upgrading of the brain-derived neurotrophic factor countenance (Sun et al., 2012).

2.11.2. Betamethasone

This medication is also classified like the previous one as synthetic long-acting corticosteroid medication, which treats a broad spectrum of diseases and disorders primarily for the anti-inflammatory and immunosuppressive effects of glucocorticoids and their effects on circulatory and lymphatic system processes in palliatives treating a wide-ranging of diseases such as; skin disease (Dermatitis), allergic disorders including asthma, angioedema, and premature labour, to accelerate the growth of the infant's respiratory system. As a corticosteroid, it has the following derivatives: Bet sodium phosphate and Bet acetate. Bet has various administration routes depending on treatment condition to be orally or in injection form or topical form for dermal uses as cream and lotion (Drugs.com).

Concerning the chemical composition of Bet, it formed by $C_{22}H_{29}FO_5$ with a molecular weight 392.5g/mol, according to the International Union of Pure and Applied Chemistry (IUPAC) Bet has the following name: (8S,9R,10S,11S,13S,14S,16S,17R)-9-fluoro-11,17-dihydroxy-17-(2hydroxyacetyl)-10,13,16-trimethyl 6,7,8,11,12,14,15,16 octahydrocyclopenta[a]phenanthren-3-one (de Groot; "Explore Chemistry,").

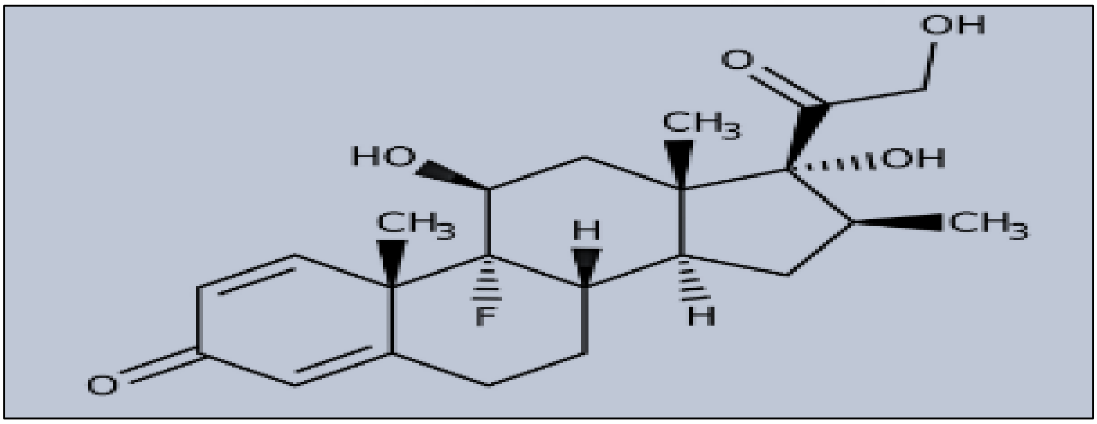


Figure 2.6. Structure of Betamethasone

The corticosteroid is prescribed for the treatment of peripheral nerve damage, but its action mechanisms are not well known (Dahlin et al., 1996). This section will highlight the studies conducted to assess the impact of Bet in promoting peripheral nerve regeneration after nerve injury; the studies included criteria that depend on the rat and rabbit model study.

Numbers of studies focus as mentioned above to assist Bet's impact on promoting the functional recovery of peripheral nerve regeneration; the first study analyzed the effect of Bet and amniotic membrane in repairing the injured sciatic nerve. The study depends on the following factors: measurement of withdrawal reflex latency, histological evaluation of the sciatic nerve as well as the electrophysiological results, and the functional assessment via the walking track analysis. The results of all these measurements demonstrated that the best result was in the group treated with Bet and the amniotic membrane together. In conclusion, this study proved that Bet and amniotic membrane are acting synergistically to enhance the peripheral nerve regeneration in rats model study (Sadraie et al., 2016).

Another study came in favour with the previous study to prove the beneficial impact of Bet in promoting sciatic nerve recovery. However, the effect of the medication evaluated depends on the immunohistochemistry factor by the usage of macrophage marker antibodies and p75. Based on that, the study results showed that the beneficial effect of a mild perioperative dosage of Bet on nerve regeneration is expressed in the recruitment of macrophages but only to a limited degree in the expression of p75 (Al-Bishri et al., 2008).

Besides the previous studies, *Rahim Mohammadi* conducted an experiment to assist the topical administration of Bet in promoting the peripheral nerve regeneration in Wistar rats by injecting the medication in vein graft which taken from doner rats external jugular vein and washed with a physiological solution and prepared to be used as a conduit for the transected nerve. The aim of the study is to compare the result of functional recovery, immunohistochemical outcomes of the groups of rats that treated topically by Bet injected into the conduit with an additional group of rats that considered as control which treated by phosphate-buffered saline, the result of the experiment showed that Gastrocnemius muscle mass in the group treated with Bet was found be substantially more significant than in the cont group; also the morphometric fiber indexes revealed that the myelinated fiber numbers and diameters of the group treated with Bet were significantly higher than those of the cont groups, as well as the location of the S-100 reactions in the group treated with Bet was slightly more optimistic than the cont group through the use of immunohistochemistry. In conclusion, Bet has increased the functional regeneration and quantitative morphometric indexes of the sciatic nerve when loaded on a vein graft. Furthermore, the implementation of this prepared agent provides the advantage of cost reduction and avoids systemic administration complications (Mohammadi, Amini, et al., 2013).

Another study favoured the previous studies in the beneficial therapeutic effect in enhancing peripheral nerve regeneration, which was conducted in rats. The results exhibited that the group of rats that received both chitosan membrane impregnated with Bet revealed the impact of sciatic function index and reflex response time of withdrawal, considerably reduced compared to the other groups. Furthermore, in contrast to the other groups, the electromyographical test latency and amplifying of the impulses in the group received chitosan membrane impregnated with Bet significantly increased; also, the histological evaluations conducted eight weeks after surgery found that the number of nerves fibers, nerve fiber size, and myelin sheath thickness in a group of rats received both chitosan membrane impregnated with Bet have improved dramatically relative to other groups. In conclusion, the study proved that Bet and Chitosan membrane have beneficial effects when acting synergistically on the transected sciatic nerve by accelerating the regeneration (Moattari et al., 2018). Another exciting study aimed to study the effect of Bet and nerve growth factor (NGF) in promoting nerve regeneration was more profound as it relied on studying the exact ultrastructural effects under an electron microscope in a rat model study. The study was initiated by exposing the rats to crush nerve injury, and they were distributed to groups based on the nature of the therapeutic. The experiment results proved that in the combined therapy group (Bet as well as the NGF), compared to experimental control and other treatment groups, the number of axons, axon diameters, and myelin thickness is substantially greater; thus, Bet, as well as the NGF, synergistically has a curative effect on promoting the peripheral nerve regeneration (Sencar et al., 2020).

2.11.3. Methylprednisolone

Considered as intermediate-acting synthetic glucocorticoid that belongs to pregnane steroid hormone consequential from hydrocortisone as well as prednisolone prescribed chiefly for its anti-inflammatory and immunosuppressive activity (Timmermans et al., 2019; Langhoff and Ladefoged, 1983).

Mps is used for treating symptoms including arthritis, blood abnormalities, extreme allergic responses to some tumours, eye problems, skin diseases such as dermatitis also used to treat intestinal and pulmonary diseases besides defects of the immune system; according to recent researches, Mps has been used successfully to treat serious COVID-19 (J. Liu et al., 2020).

Concerning the chemical composition of Mps, it formed by C22H30O5 with a molecular weight 374.5 g/mol, according to the International Union of Pure and Applied Chemistry (IUPAC) Mps has the following name:(6S,8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-6,10,13-trimethyl-7,8,9,11,12,14,15,16-octahydro-6Hcyclopenta[a] phenanthren -3-one ("compound/Methylprednisolone," 2004).

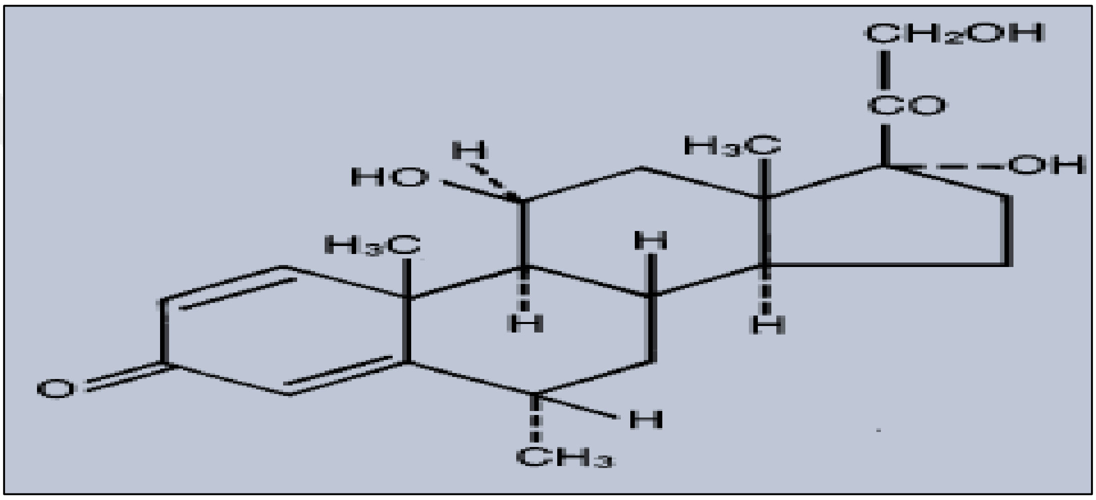


Figure 2.7. Structure of Methylprednisolone

As described above, corticosteroids are used to treat peripheral nerve injury, but their modes of action are not well understood (Dahlin et al., 1996). The study carried out to determine the effect of Mps on the development of peripheral nerve regeneration following nerve damage is illustrated in this section. The parameters used in the studies concentrate on the interpretation of the rats or rabbits model's findings.

Mps succinate (MPSS) is considered as a core of therapy after acute SCI synthetic glucocorticosteroid. However, the findings are published in the national studies on the spinal cord (NASCIS) II in the early 90's pattern trial (Bracken et al., 1990). Numbers of research focused on the effect of Mps on the promotion of the functional regeneration of the peripheral nerve; the first study aimed to histopathological evaluate of Mps and Ozone in promoting the functional recovery of the sciatic nerve after the crush injury. The experiment results showed significant differences in beneficial effect in nerve sheath atrophy, cellular intraneural inflammatory infiltration, and granulation of perineural tissue development, the proliferation of perineural vascular supply, inflammatory perineural neuronal invasion, peripheral tissue inflammation in the group of rats that received the combined medications (Mps and Ozone), also this group revealed remarkably low regeneration; while no change has been observed in nerve sheath cells in the group that treated with Mps. In conclusion, the Mps and Ozone act synergistically to give the best therapy than the remaining group (Ozturk et al., 2016).

The subsequent study also evaluates the effect of Mps in promoting the functional recovery of the rats sciatic nerve. The experiment results showed rapid recovery of regenerated axons in the Mps group supported by behavioural, functional, and electrophysiology findings compared with other groups. In addition, the total muscle weight ratio for gastrocnemius was calculated. The muscle weight ratios are statistically significantly different in the group also that treated with the Mps group. Also, the morphometric measures of regenerated fibers show a relatively higher number and diameter of myelinated fibers in the group that received Mps (Mehrshad et al., 2017).

The following study is also aimed at assessing the therapeutic impact. However, the standard was changed in it, as rabbits were used to fulfill the experiment as an alternative to rats, and the facial nerve was targeted as an alternative to the sciatic nerve. The study showed that N-acetylcystein and the Cont groups presented the best nerve regeneration, while the Mps group showed the weakest regeneration to be determined. In the group of N-acetylcystein, the rise in regeneration was slightly more generous than in the group of Mps due to Schwann cell and glial cell proliferation. On the other hand, no major regeneration was observed in the Mps group, despite degenerative signs of considerable axonal withdrawal as well as an increase in myelin debris. In conclusion, this study contradicts other studies that confirm the curative effect of Mps in promoting nerve regeneration and shown a lack of positive impact on nerve regeneration (Karlidag et al., 2012).

2.12. Stereological Background

Stereology considered as a subset of morphometric study, which defines as a series of techniques that are capable of obtaining three-dimensional data from nearly any form of structure, such as sections or images that originate from a two-dimensional source (Yurt et al., 2018; Altunkaynak et al., 2012). Stereology is dependent on the geometric principle of statistical sampling techniques. Stereological methods are structured to identify the biological tissue's quantitative features regardless of structure, size, or orientation (Deniz et al., 2018). Several stereological tools are currently used, including fractionator, disector, and nucleator (Altunkaynak et al., 2012).

2.12.1. Fractionator method

In this stereological approach, the targeted organ or structure to be analyzed is first systematically broken down into smaller fractions depending on a systematic basis, followed by random fraction items selected for counting. The only element that has to be identified is the particle ratio taken as a sample to the entire structure. The obtained specimens gathered are then categorized into histological sections (Altunkaynak et al., 2012; H.-J. G. Gundersen, 1986). The quantity of associated particles derived from these pairs of dissector sections is being used to approximate the specimens' numerical density in that particular segment of structure or organ. It is well known that, in advance of the proportions of the segment to the organ, the overall number of particles throughout the whole organ can be easily determined. There are two distinct forms of the histological fractionator system identified as the optical and physical fractionator (Deniz et al., 2018).

2.12.2. Physical fractionator

Considered as a type of fractionator technique that focused on the determination of the particle in a sampled pair of parts collected using a particular sampling method. In order to approximate the numeric density of the particle, it is necessary to achieve a volume through two neighbouring or adjacent sections. Concerning the volume can be measured using the certain two sections, and the space between them reflects the height; in contrast, height by area multiplication gives a volume called the disector volume (H. Gundersen, Bagger, et al., 1988).

2.12.3. Optical fractionator

The optical fractionator can be defined as a technique that is developed to allow the estimation of the cumulative number of particles or cells in dense sections. This technique involves a combination of a stereological optical disector to approach the quantified artifacts in a dense tissue field, a 3-dimensional metering sensor, and a systemic random sampling methodology. In this procedure, a small fraction reflecting the entire structure is sampled for study or analysis (H. Gundersen, Bendtsen, et al., 1988).

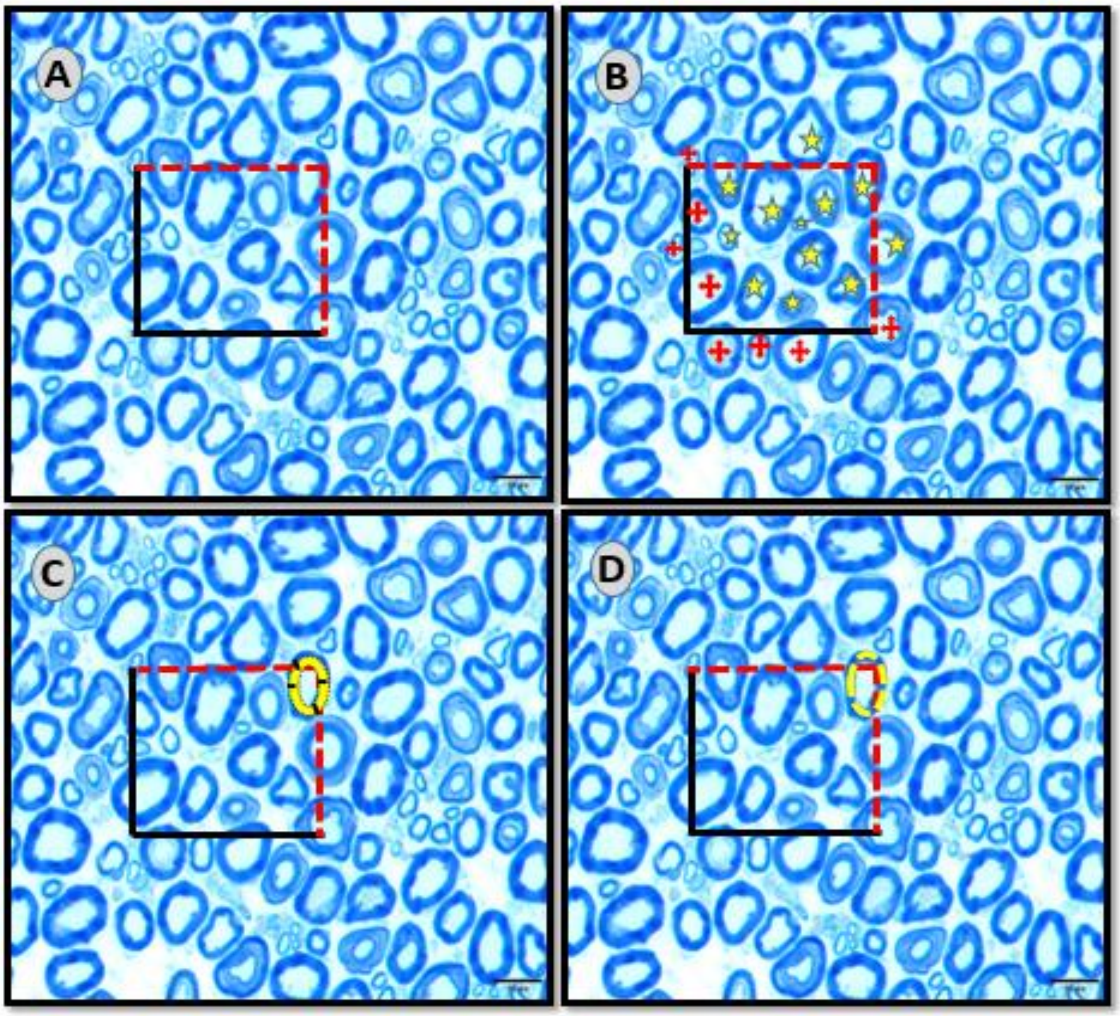


Figure 2.8. Image representation of the two-dimensional fractionator method (A) Showed the counting frame, (B) method of axons number counting, (C) myelin thickness measurement, (D) axonal area estimation method

2.12.4. The Nucleator

The nucleator technique can be defined as a tool used to measure an object volume using disector techniques and measures the sample volume using a particular sampling process based on the random central point` within the selected particle with a fixed nucleator configuration. This technique is considered an appropriate choice in the case of a computer-assisted stereological workstation, and the value obtained is the mean number-weighted (Altunkaynak et al., 2012). Consequently, the overall number of myelinated axons or the thickness of the axon as well as the myelin is very complicated to estimate or calculate. The stereology now offers applicable and impartial methods for counting and calculating the total number and volume of artifacts. The sampling technique is seen as a critical element in calculating the total number of myelinated axons (Kaplan et al., 2010).

3. MATERIAL AND METHODS

3.1. Experimental Animals

The study was carried out in the animal experimental research center of Ondokuz Mayis University (Samsun, Turkey). This research was accepted by the ethical committee and animal care of the institute of health science at the University of Ondokuz Mayis on 14. January. 2019 (meeting number 2018/45). The experimental method has been carried out cautiously in compliance with ethical committee rules and guidelines. The animal experimental research center of Ondokuz Mayis University provided experimental animals. In this study, all healthy adults, 64 Wistar albino rats, weighed 250 ± 30 g, with old age extend between 12 to 14 weeks, were used. The rats were kept in stainless steel cages in a temperature and humidity-controlled room with a 12-h dark/12-h light period at room temperature (22 ± 2° C) and humidity (50-60%). Animals were free to get standard rodent laboratory food and drink (Figure 3.1).

Figure 3.1 Experimental animals in this research (Wister albino rats)

3.2. Groups Design

Sixty-four male adult Wistar albino rats were divided randomly into eight equal groups; each group contains eight rats. The group was designed accordingly (Figure. 3.2).

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

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

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

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

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

Group 6: Dex group (n= 8)

Group 7: Bet group (n= 8)

Group 8: Mps group (n= 8)

Figure 3.2. Rats divided into eight groups

3.3. Surgical Procedure

The surgical procedure was performed under ideal circumstances. The rats were anesthetized by 5 mg/kg Ketamine (Ketalar®, Eczacibasi, Istanbul, Turkey) with 2 mg/kg Xylazine (Rompun®, Bayer, Istanbul, Turkey) by an intraperitoneal route of administration. Rats' hair was trimmed from the back of the right thigh immediately before the surgical procedure. The right sciatic nerve was exposed by creating a longitudinal incision between the musculature of gluteal and femoral regions then the nerve was released from the underlying tissue. The crushing damage was induced by standardized clamp forceps (Bahadır, Samsun, Turkey). The crush location was applied 5 mm from the sciatic notch, the crushing force was 50 Newtons, and the crush period was 60 seconds (Figure 3.3) (Torul et al., 2018; Bauder and Ferguson, 2012).

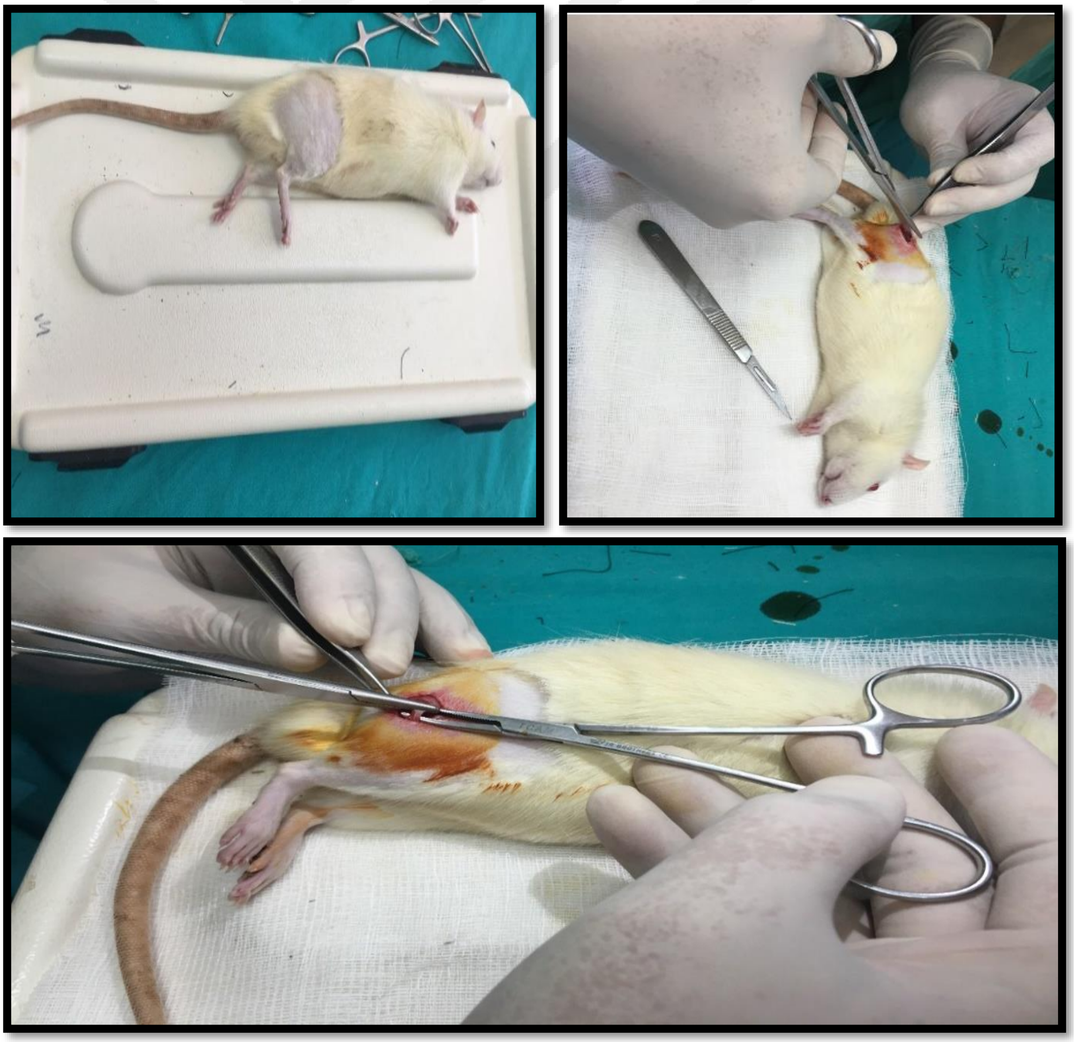


Figure 3.2. Steps of surgical procedure

3.4. Postoperative Care

Following exposure of the right sciatic nerve to crushing injury, the cut incision of skin was sutured back; then the experimental animals were subsequently received with antibacterial spray Pederipra (Hipra, Amer, Girona, Spain), which acted as an antibacterial agent that applied to incision place directly. Finally, in order to prevent infection, animals were administered (Sefazolin sodium Eqizolin 1g IM/IV) via an intramuscular route of administration for three days following surgery; this antibiotic was provided to rats in tapering doses (4, 2, and 1 mg/kg) respectively.

Figure 3.3. Antibacterial spray applied to incision place after skin suturing

3.5. Experimental animals groups

In the present study, experimental animals were grouped into eight; each group was housed in a special cage; these cages are designed to facilitate easy access to water and food. The specified groups were exposed to crush injury in the right sciatic nerve using standardized clamp forceps, where the nerve is exposed to 50 Newton for 60 seconds by the same surgeon. The experimental animals are summarized in (**Table 1**).

Group 1: Cont group

This group's experimental rats were not exposed to any particular protocol; they were left in the special cage for 28 days (experiment duration); the rats were free to access to get standard rodent laboratory food and water.

Group 2: Inj group

This group was received the right sciatic nerve crush operation only, where the crush location was applied 5 mm from the sciatic notch. The nerve is exposed to 50 Newton for 60 seconds, then the cut incision of skin was sutured back (Bauder and Ferguson, 2012; Torul et al., 2018).

Group 3: Inj + Dex

This group was received the right sciatic nerve crush operation, where the crush location was applied 5 mm from the sciatic notch. The nerve is exposed to 50 Newton for 60 seconds, then the cut incision of skin was sutured back; after that, Dex (2 mg/kg) was administered daily through the intraperitoneal route for ten days.

Group 4: Inj + Bet

This group was received the right sciatic nerve crush operation, where the crush location was applied 5 mm from the sciatic notch. The nerve is exposed to 50 Newton for 60 seconds, then the cut incision of skin was sutured back; after that, Bet (2 mg/kg) was administered daily through intraperitoneal route for 14 days.

Group 5: Inj + Mps

This group was received the right sciatic nerve crush operation, where the crush location was applied 5 mm from the sciatic notch. The nerve is exposed to 50 Newton for 60 seconds, then the cut incision of skin was sutured back; after that, Mps (2 mg/kg) was administered daily through intraperitoneal route for 14 days.

Group 6: Dexamethasone

This group was treated with Dex (2 mg/kg) was administered daily through the intraperitoneal route for ten days. No procedure of crush nerve injury was conducted on the animals of this group.

Group 7: Betamethasone

This group was treated with Bet (2 mg/kg) was administered daily through the intraperitoneal route for 14 days. Rats in this group were not received any crush nerve injury or any surgical intervention.

Group 8: Methylprednisolone

This group was treated with Mps (2 mg/kg) was administered daily through the intraperitoneal route for 14 days. Neither surgical intervention nor crush nerve injury was conducted on the animals of this group.

1.6. Drugs Doses and Route of Administration

Dex, considered synthetic long-acting corticosteroid medication, can be prescribed to treat peripheral nerve damage. Dex, in this experiment known by the brand name (Dekort 8mg/2ml I.M/I.V). The dosage used (2 mg/kg) will be administered daily through the intraperitoneal route for ten days (Earp et al., 2008).

Bet is a synthetic long-acting corticosteroid medication, which treats a broad spectrum of diseases and disorders primarily for the anti-inflammatory and immunosuppressive effects of glucocorticoids. Bet in this experiment is known by the brand name (Celestone Chronodose injection 3mg + 3mg/ 1ml. - Active ingredient Bet sodium phosphate/ Bet acetate). The dosage used (2 mg/kg) was administered daily through the intraperitoneal route for 14 days (Al-Bishri et al., 2008).

Mps is considered an intermediate-acting synthetic glucocorticoid that belongs to the pregnant steroid hormone consequential from hydrocortisone and prednisolone prescribed mostly for its anti-inflammatory and immunosuppressive activity. Thus, mps in the current experiment is known by the brand name (Prednol-l 40 mg liyofilize injectable ampul). The dosage used (2 mg/kg) was administered daily through the intraperitoneal route for 14 days (Figure 3.4) (Ozturk et al., 2016).

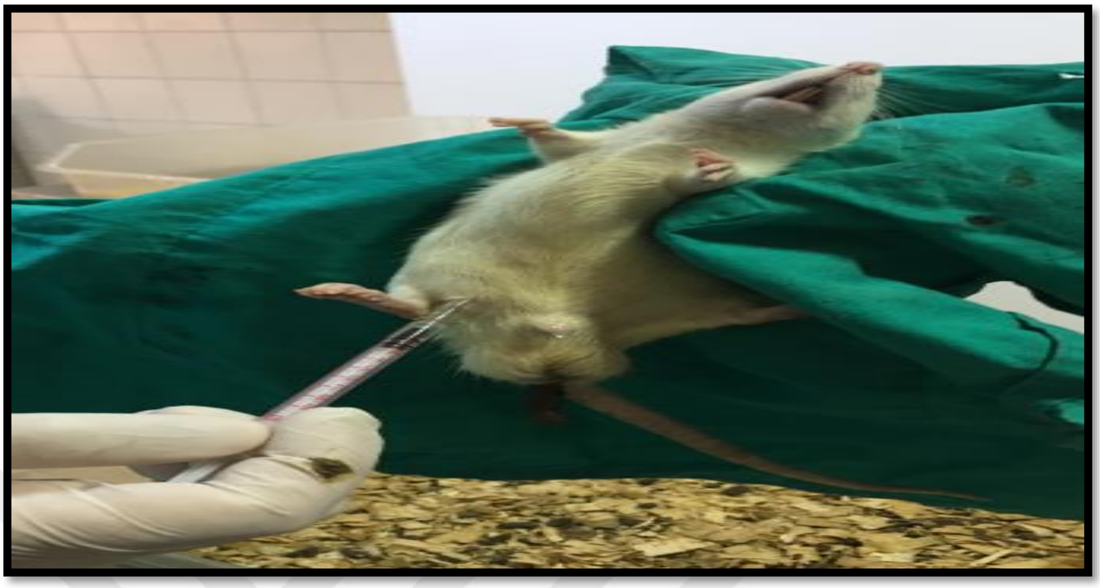


Figure 3.4. Rats received medication in the intraperitoneal route of administration

Table 3.1. Summary of experimental rats group

GROUP	DETAILS OF THE GROUP
Cont	Rats were not exposed to any particular protocol.
Inj	Rats received sciatic-only nerve crush operation.
Inj + Dex	Animals received sciatic nerve crush operations, with Dex (2 mg/kg) administered daily for 10 days.
Inj+Bet	received sciatic nerve crush operation, with Bet (2 mg/kg) administered daily for 14 days.
Inj + Mps	received sciatic nerve crush operation, with Mps (2 mg/kg) administered daily for 14 days.
Dex	Receive Dex (2 mg/kg) will be administered daily for 10 days without sciatic nerve crush.
Bet	Receive Bet (2 mg/kg) will be administered daily for 14 days without sciatic nerve crush.
Mps	Receive Mps (2 mg/kg) will be administered daily for 14 days without sciatic nerve crush.

3.6. Functional Evaluations

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

The evaluation of the SFI in rat groups was carried out at the end of the study by day 21 in order to examine the recovery of the motor activity of the hind limbs. The rats' posterior extremities were coated with ink; rats were permitted to walk down through a hallway, the hallway is fulfilling with paper. The paper is intended to capture the footprints. The distance between the toes of the footprint has been calculated, and the findings are assessed with a special formula.

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

Whereas:

IT: distance between the second toe to the fourth toe.

TS: distance from the first toe to the fifth toe.

PL: distance from the heel to the middle toe.

E: injured right foot.

N: normal left foot.

Walking-track evaluations and SFI are used to assess rat sciatic nerve damage and regeneration; zero is revealed as a normal or ordinary nerve motor function result, while -100 considered complete loss of nerve motor function (Figure 3.5) (Torul et al., 2018).

Figure 3.5. Walking track and footprint paper

3.6.2. Electrophysiological Evaluation

The electromyography (EMG) was considered another functional assessment used to evaluate the sciatic nerve's regeneration. At the end of the experiment, the rats were administered an intraperitoneal injection of 5 mg/kg ketamine (Ketalar®, Eczacibasi, Istanbul, Turkey) in addition to 2 mg/kg xylazine (Rompun®, Bayer, Istanbul, Turkey), which acting as anaesthesia. The right sciatic nerve of all rat groups was extracted from the sciatic notch to the bifurcation site and separated from the underlying connective tissue to conduct the EMG in order to demonstrate the skeletal's electrical ability in muscles and the motor regeneration of the sciatic nerve. For performing the EMG, the Power Lab4SP (ADInstruments, Sydney, Australia) was used, which is considered a system designed to correctly, continuously, and efficiently acquire data, and EMG Scope software (ver. 3.7.2, AD Instruments) is used to translate the results. The gastrocnemius muscle was assessed with a signal voltage of 0.01 mV to 10 mV. The bipolar stimulation electrode was located 10 mm proximal to the site of injury on the sciatic nerve. Gastrocnemius muscle was used for recording the potential for compound muscle action potential (CMAP). The recording electrode was positioned on the muscle's tissue; the procedure has been repeated three times, and the mean was measured and evaluated statistically. The amplitude can be defined as the frequency of the nerve impulse entering the muscle, while the latency is the time for the nerve impulse to reach the muscle via the nerve (Torul et al., 2018).

3.7. Preparation of Tissue Samples

Each animal was weighed on the day of the sacrifice, and their average weight was 260 ± 30 grams. Animals were first anesthetized with an intraperitoneal 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 to expose the skin; then, a vertical incision was made on the right thigh's back. The sciatic nerve was released from the sciatic notch to the bifurcation point, and the adjacent connective tissue was removed. The nerve segment comprising the crush site was withdrawn from the right sciatic nerve and injected with gluteraldehyde 0.5 % until the nerve was taken from the rat. A sharp blade was used to create a section in the nerve, nerve segment immediately immersion in a container containing the fixative solution, which is gluteraldehyde 0.5%, and it was replaced with a new solution with the same concentration three hours later the purpose of that is to remove the remnants of blood. The nerves tissue was located at 4°C in the refrigerator, left for two weeks in the fixative solution (Torul et al., 2018)

3.7.1. Tissue Processing

The nerve tissues were washed in four stages with 0.1-molar phosphate Buffered saline (PBS) (pH 7.4) in 15 minutes for each step, In the post-fixation stage, nerve tissues were placed in the solution of osmium tetroxide at 2% (Sigma-Aldrich Co. LLC., St. Louis, USA) and the tissues remained in the solution overnight until the tissues were becoming dark black in colour; the nerve tissues were kept away from the light in a dark field. The nerve tissues were then washed using PBS (pH 7.4) (0.1) molar in four steps, 15 minutes in each step, and PBS altered. Since cleaning the tissues, acetone was used at varying concentrations of 50% for 15 minutes, followed by 70% for 15 minutes, then 95% for 15 minutes, and eventually 100% for 20 minutes twice. After dehydration, the tissue was placed two times in propylene oxide for 20 minutes, and then the tissues were placed in propylene oxide solution for 50% and the resin for 50% for one hour. Then, beforehand blocking the tissues, it was placed in the resin for one hour; before the blocking step, the connective tissue around the nerve was carefully detached using fine forceps. At the final stage, the tissues were inserted in the resin at a temperature of 50°C, after that temperature was changed to intensify every 30 minutes before they eventually reached 62°C that will create the tissue blocks; then the tissue blocks were left in the oven for two days until the blocks are ready for sectioning (Figure 3.6) (Torul et al., 2018).

Figure 3.6. Sciatic nerve tissue blocks ready for sectioning

3.7.2. Sectioning Methods

Sections of nervous tissue resin blocks were taken with the ultra-microtome (Leica Ultra cut UCT, Leica Microsystems GmbH; Germany) and glass blade, semi-thin 0.5 μm thickness was taken for the light microscope investigation and ultra-thin sections 80 nm for the electron microscope examination. Parts of tissue (semi-thin) were inserted in a light microscope exam slide and kept dry (Figure 3.7) (Torul et al., 2018).

Figure 3.7. Ultra-microtome (Leica Ultra cut UCT, Leica Microsystems GmbH; Germany)

3.7.3. Staining Process

The thin sections obtained by the ultra-microtome were stained with blue toluidine. 1% toluidine blue stain solution was prepared after one-gram toluidine bleu was applied, and the solution was stirred to 100 ml deionized water by dissolving two grams of sodium borate. The solution was then filtered by filter paper (pore size: 11 microns) and stored at room temperature in an opaque container for two weeks. The nerve tissue was reinforced with a drop of toluidine blue using a pipette and left 20-30 seconds; then slide sank into the deionized jar of water and repeated the process was performed 3-4 times before the section was cleared. Regarding the ultra-thin sections were stained with uranyl acetate and lead citrate for the electron microscope analysis (Figure 3.8).

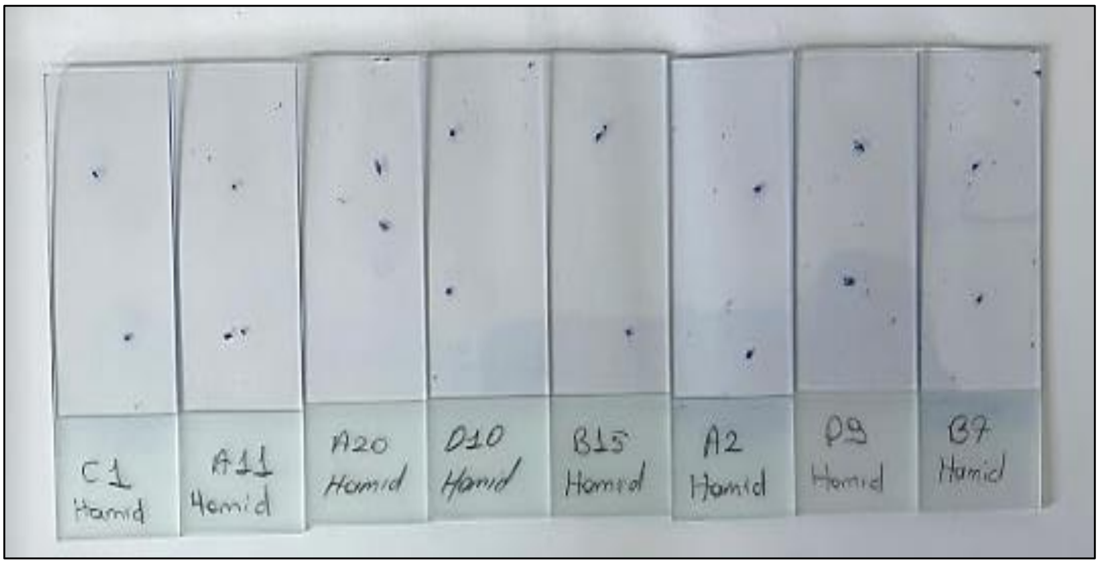


Figure 3.8. Sections sciatic nerve following stained using toluidine blue

3.8. Stereological Analysis

For stereological analysis, tissue sections of the sciatic nerve stained with toluidine blue were used. Stereological study was used to measure the total number of myelinated nerve axons by the light microscope depending on the fractionator method. Semi-thin sections (0.5 μm) have been stereologically examined by an appropriate magnification light microscope with a camera attachment (Olympus, BX43, Center Valley, PA, USA). The images taken are displayed on a computer provided with an entry microscope software package named CellSens. All of the above steps have been taken to aid the instruments and programs located at the Stereology Workstation of the Department of Histology and Embryology, Faculty of Medicine, University of Ondokuz Mayis (Figure 3.8). The current technique for calculating the total number of myelinated nerve axons was used in the study called the fractionator technique, while the thickness of myelin sheaths and the axonal area is measured through the nuclear probe. Counting of the myelinated axon number was performed using an X100 magnification. For counting myelinated nerve axons, an unbiased counting frame was used. The myelinated axons within the counting frame or the inclusion line were estimated, while the axon in the top right corner was used to measure the thickness and axonal area of the myelin's sheath. Regarding the unbiased counting frame is formed by a four-bound frame with two inclusion lines and two extension exclusion lines. In the calculation of the total number of axons of the nerve, the myelinated numbers were determined with the following formula:

The total number of the myelinated nerve axons

$$= \frac{\text{step size area (96486)}}{\text{counting frame area (1335)}} \times \text{number of axons counted}$$

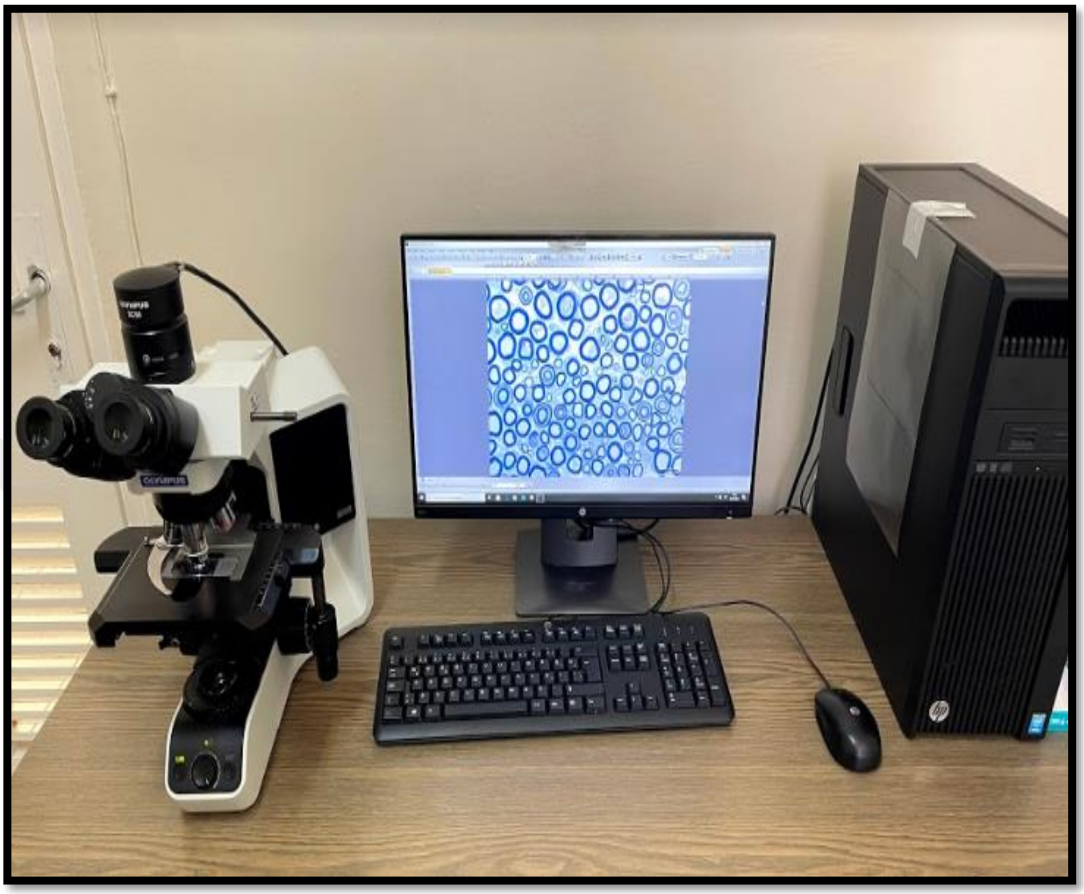


Figure 3.9. The stereological analysis system

3.9. Statistical Analysis

The software program SPSS (version 22.0) was implemented to analyze data obtained from recent research. Prior to the analysis, the normality test was conducted to select the appropriate statistical data for analyzing in the current study. A one-way ANOVA test (Post-Hoc Test) has been applied for the identification of significance. A P-value of less than 0.05 was considered significant.

4. RESULTS AND DISCUSSION

4.1. Results

This study aimed to investigate the therapeutic effects of Dex, Bet and Mps on promoting peripheral nerve regeneration in a study conducted in a rat model. The sciatic nerve was crushed by 50 Newton for 60 seconds. The presented study's data incorporates a number of parameters derived from stereological measurements. As a consequence of using stereological techniques, we were able to obtain quantitative data on the number of myelinated axons, axonal area, as well as myelin sheath thickness. Subsequently, the results were tabulated and analyzed to determine the occurrence of sciatic nerve protection or regeneration following crush injury. Furthermore, the findings from functional examinations, including the sciatic functional index and electromyographs, were used to determine muscle contraction ability besides the sciatic nerve motor function. Furthermore, the impact of Dex, Bet, and Mps on the crushed sciatic nerve was studied utilizing light and electron microscopic techniques through the use of sciatic nerve cross-section images. Throughout this study, both stereological and functional findings were represented in tables in addition to graphs.

4.1.1. Stereological Findings

The cumulative number of myelinated axons in the sciatic nerve was determined by taking advantage of the 2D fractionators technique. A nucleator technique was used to determine the axonal area as well as thickness of the myelin sheath in myelinated axons. Two criteria have been used in the sciatic nerves evaluation; in the Cont group, experimental rats were not exposed to injury or any particular protocol; on the other hand, the Inj group rats have received the right sciatic nerve crush operation only, where the crush location was applied 5 mm from the sciatic notch for 60 seconds. The Inj + Dex group received the right sciatic nerve crush operation. The nerve is exposed to crush injury for 60 seconds after that, and Dex was administered as a treatment. Right sciatic nerve crush surgery was performed on the Inj + Bet group. After that, the nerve was subjected to a 60-second crush injury, and Bet was used as a treatment. The Inj + Mps group received the right sciatic nerve crush operation. The nerve is exposed to crush injury for 60 seconds after that, and Mps were administered as a treatment. The Dex group was treated with

Dex with no crush nerve injury procedure on the animals of this group. As well, in the case of animals in the Bet group was given Bet with no inducing of crush nerve injury. Moreover, the last group, Mps, received therapeutic doses of Mps without undergoing any surgical intervention.

4.1.1.1. Estimation of Myelinated Axon Numbers

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

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

GROUPS	MEAN NUMBER OF THE MYELINATED AXONS ± SD
Cont	6354.119 ± 241.913
Inj	8056.932 ± 146.246
Inj+Dex	7133.349 ± 180.179
Inj+Bet	7672.457 ± 113.249
Inj+Mps	7803.022 ± 153.253
Dex	6391.374 ± 233.042
Bet	6350.660 ± 223.228
Mps	6417.347 ± 180.791

Concerning the mean number of the myelinated axons in the sciatic nerve, the standard deviation (SD) is demonstrated in Table 4.1 and Figure 4.1.

The mean number of myelinated axons in the Cont group was substantially lower than the mean number of myelinated nerve axons in the Inj group, according to statistical analysis of stereological data acquired by counting the numbers of myelinated axons in the rat sciatic nerve ($p = 0.00$). In terms of myelinated axons, mean numbers were observed; there was no statistically significant difference in Inj+Bet and Inj +Mps groups compared to the Inj group ($p > 0.05$). On the other hand, the statistical data reveals that significant differences appear when comparing the Inj group with the Inj+Dex group ($P = 0.019$)

Based on the initial statistical findings, it is possible to assume that Dex has a therapeutic impact in increasing the number of myelinated axons.

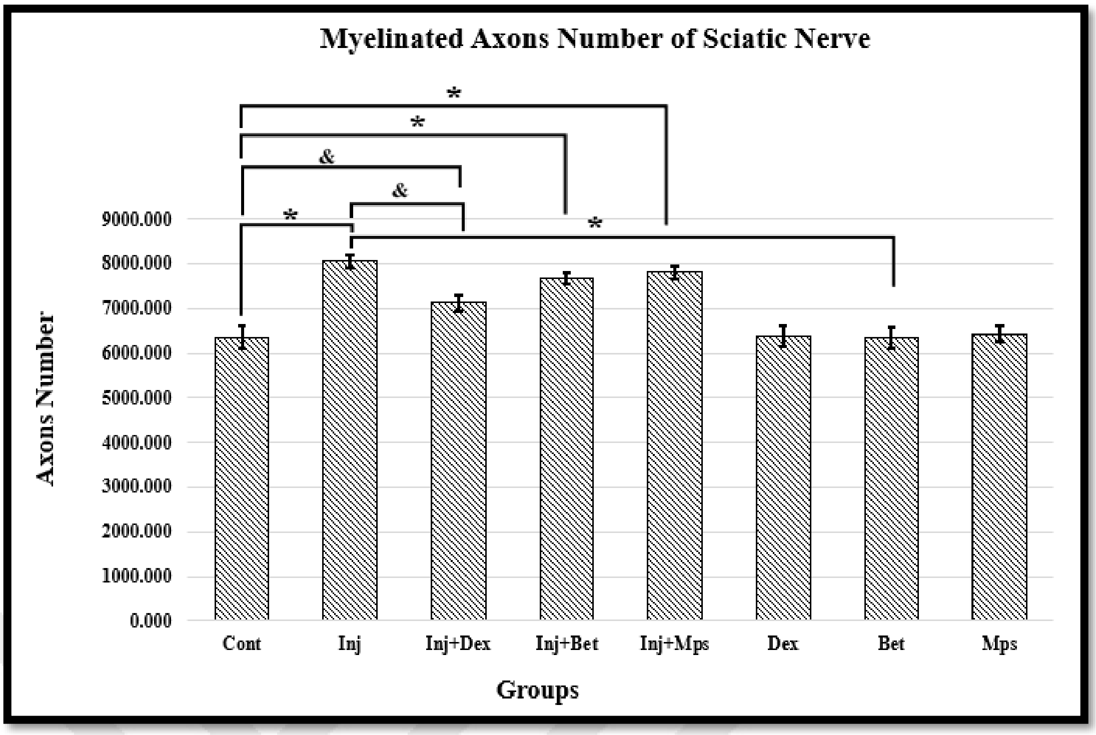


Figure 4.1. Exhibits the difference among the groups in terms of myelinated axons mean number of the sciatic nerve. The level of significance at p<0.01 is indicated by (*), while the significance level at p<0.05 is indicated by (&)

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

COMPARISON BETWEEN GROUPS	**DEGREE OF SIGNIFICANCE**
Inj with Cont	0.000
Inj with Inj+Dex	0.019
Inj with Inj+Bet	0.207
Inj with Inj+Mps	0.253
Cont with Dex	0.973
Cont with Bet	0.995
Cont with Mps	0.785
Cont with Inj+Dex	0.025
Cont with Inj +Bet	0.001
Cont with Inj +Mps	0.000
Bet with Beta + Inj	0.001
Bet with Mps + Inj	0.000
Bet with Inj	0.000

Dex with Dex + Inj	0.027
Dex with Beta + Inj	0.001
Dex with Mps + Inj	0.000
Dex with Inj	0.000

4.1.1.2. Measurement of Axonal Area

The mean axonal area in the sciatic nerve and the standard deviation (SD) are shown below in Table 4.3 and Figure 4.2.

Table 4.3. Represents the mean the axonal area and the standard deviation (SD)

GROUPS	AXONAL AREA MEAN (μm^2) ± SD
Cont	65.611 ± 1.280
Inj	16.927 ± 1.465
Inj+Dex	18.289 ± 1.563
Inj+Bet	17.031 ± 2.868
Inj+Mps	15.944 ± 1.323
Dex	66.230 ± 3.329
Bet	68.010 ± 3.165
Mps	68.432 ± 3.699

According to the results of a statistical analysis of the stereological data acquired from the measurement of the myelinated axons of the right sciatic nerve, the Inj group mean the axonal area was significantly lower than that of the Cont group ($p = 0.000$). Similarly, when comparing the Inj group to the Inj+Dex, Inj+Bet, and Inj+Mps groups, no significant differences were seen in the mean axonal area ($p > 0.05$). However, significant differences were seen when comparing the Cont group to Inj+Dex, Inj+Bet, and Inj+Mps groups in terms of the mean axonal area ($P = 0.019$, $P = 0.003$ and $P = 0.000$) respectively.

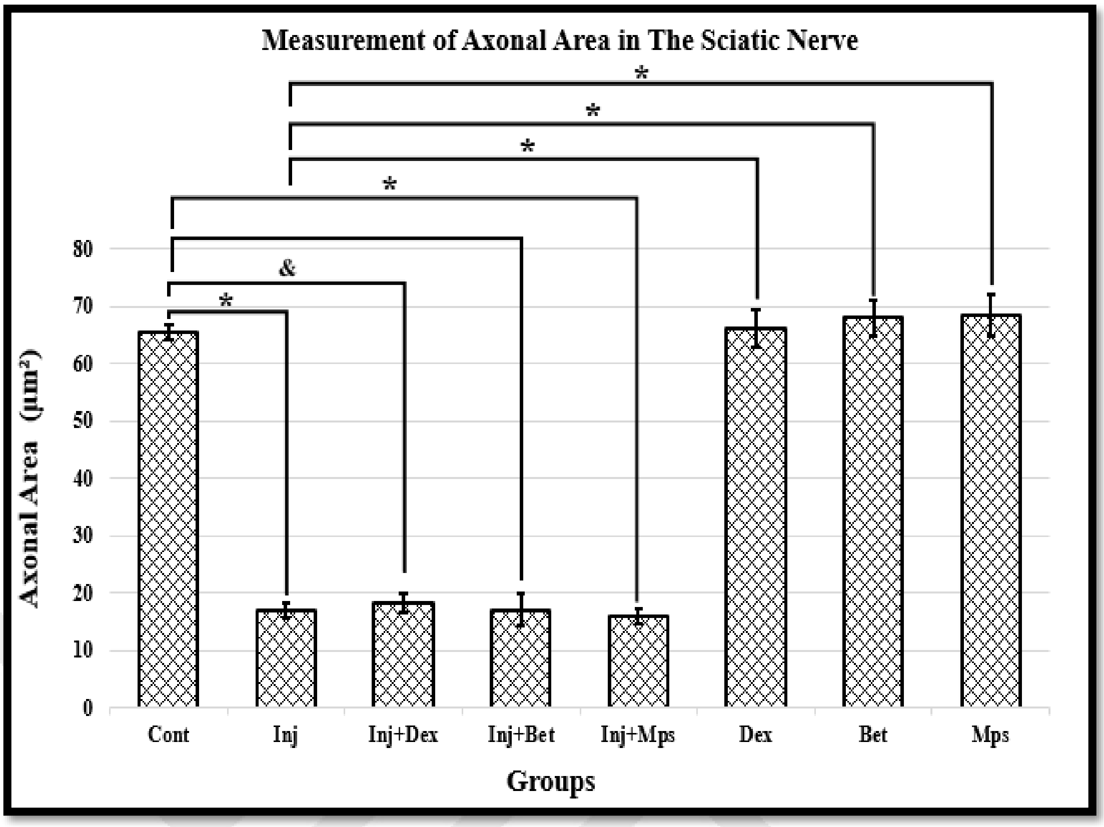


Figure 4.2. Display the differences of the axonal area mean (µm2) across groups. The level of significance at $p<0.05$ is indicated by (&), The level of significance at $p<0.01$ is indicated by (*)

Table 4. 4. Shows degree of significance between groups in terms of myelinated axonal area

COMPARISON BETWEEN GROUPS	DEGREE OF SIGNIFICANCE
Inj with Cont	0.002
Inj with Inj+Dex	0.435
Inj with Inj+Bet	0.861
Inj with Inj+Mps	0.666
Cont with Dex	0.817
Cont with Bet	0.437
Cont with Mps	0.495
Cont with Inj+Dex	0.019
Cont with Inj+Bet	0.003
Cont with Inj+Mps	0.000
Dex with Mps+Inj	0.000
Mps with Mps+Inj	0.000

Beta with Mps+Inj	0.000
Beta with Inj-Dex	0.001
Beta with Inj-Mps	0.000
Beta with Inj-Beta	0.000
Dex with Inj-Dex	0.010
Dex with Inj-Mps	0.002
Dex with Inj-Beta	0.002

4.1.1.3. Measurement of Myelin Thickness

The mean of myelin sheath thickness in the sciatic nerve, as well as the standard deviation (SD), are shown below in Table 4.5 and Figure 4.3.

Table 4.5. Represents the mean and standard deviation of myelin thickness (SD)

GROUPS	THE MYELINE THICKNESS MEANS VALUE (µm) ± SD
Cont	1.638 ± 0.018
Inj	0.475 ± 0.028
Inj+Dex	0.492 ± 0.051
Inj+Bet	0.506 ± 0.081
Inj+Mps	0.508 ± 0.042
Dex	1.551 ± 0.327
Bet	1.663 ± 0.045
Mps	1.644 ± 0.030

According to the statistical study performed on the stereological evidence derived from the numbering of the myelin thickness in the right sciatic nerve, the myelin thickness of myelinated axons in the Inj group was significantly lower than the corresponding number of myelinated axons in the Cont group ($p < 0.01$). In the same way, myelin sheath thickness was also shown to be highly significantly different between the Inj group associated to Dex, Bet, and Mps groups ($p = 0.000$). However, significant differences were seen when comparing the Cont group to Inj+Dex, Inj+Bet, and Inj+Mps groups in terms of the myelin thickness ($p < 0.05$). Correspondingly, when comparing the Inj group to the Inj+Dex, Inj+Bet, and Inj+Mps groups, no significant differences were seen in the mean myelin thickness ($p > 0.05$).

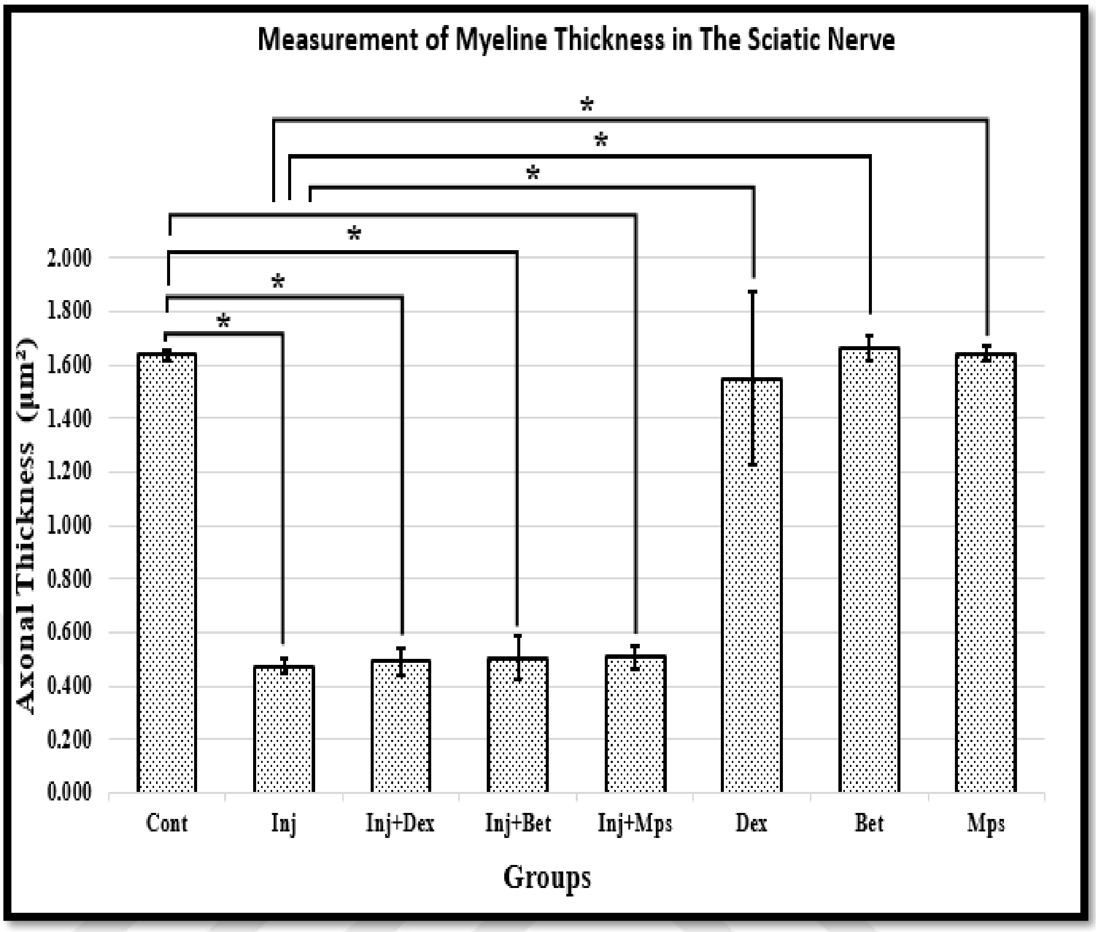


Figure 4.3. Shows the difference of myelin thickness mean in the sciatic nerve among the groups. The level of significance at $p<0.05$ is indicated by (&), The level of significance at $p<0.01$ is indicated by (*)

Table 4.6. Shows degree of significance between groups in terms of myelin thickness

COMPARISON BETWEEN GROUPS	DEGREE OF SIGNIFICANCE
Inj with Cont	0.001
Inj with Inj +Dex	0.594
Inj with Inj +Bet	0.49
Inj with Inj +Mps	0.558
Cont with Dex	0.548
Cont with Bet	0.562
Cont with Mps	0.811
Cont with Inj+Dex	0.003
Cont with Inj+Bet	0.004
Cont with Inj+Mps	0.003

Inj with Mps	0.000
Inj with Beta	0.000
Inj with Dex	0.000
Dex with Inj-Dex	0.000
Dex with Inj-Mps	0.001
Dex with Inj-Beta	0.000
Mps with Inj-Dex	0.001
Mps with Inj-Mps	0.000
Mps with Inj-Beta	0.000
Beta with Inj-Dex	0.000
Beta with Inj-Mps	0.001
Beta with Inj-Beta	0.001

4.1.2. Functional Examinations of the Sciatic Nerve

4.1.2.1. Sciatic Functional Index Findings

The mean of the sciatic functional index (SFI) and standard deviation (SD) are shown below in Table 4.7 and Figure 4.4.

Table 4.7. Presents the mean value and the standard deviation (SD) of SFI

GROUPS	MEAN VALUE OF SFI ± SD
Cont	-0.625 ± 0.122
Inj	-21.786 ± 3.419
Inj+Dex	-16.459 ± 3.388
Inj+Bet	-17.439 ± 3.481
Inj+Mps	-15.913 ± 3.670
Dex	-6.348 ± 1.615
Bet	-6.763 ± 2.608
Mps	-7.468 ± 2.105

Findings on SFI indicated that there was a significant difference between the Inj and Cont groups ($p = 0.000$) based on statistical analysis. No significant difference was observed between the Inj group and the Inj+Bet group ($p > 0.05$);

however, significant differences were identified between Inj+Dex and Inj+Mps groups (P = 0.016 and P = 0.005), respectively. However, highly significant differences were seen when comparing the Cont group to all injury treated groups, including Inj+Dex, Inj+Bet, and Inj+Mps in terms of the SFI (p = 0.000). We also obtained the same high significant results when comparing the injury treated groups with other groups that received the medications without injury, namely Dex, Bet, and Mps, in terms of SFI (p = 0.000).

Consequently, the findings of a statistical study of the SFI that was performed revealed that Dex and Mps medications promoted recovery of motor nerve fibers.

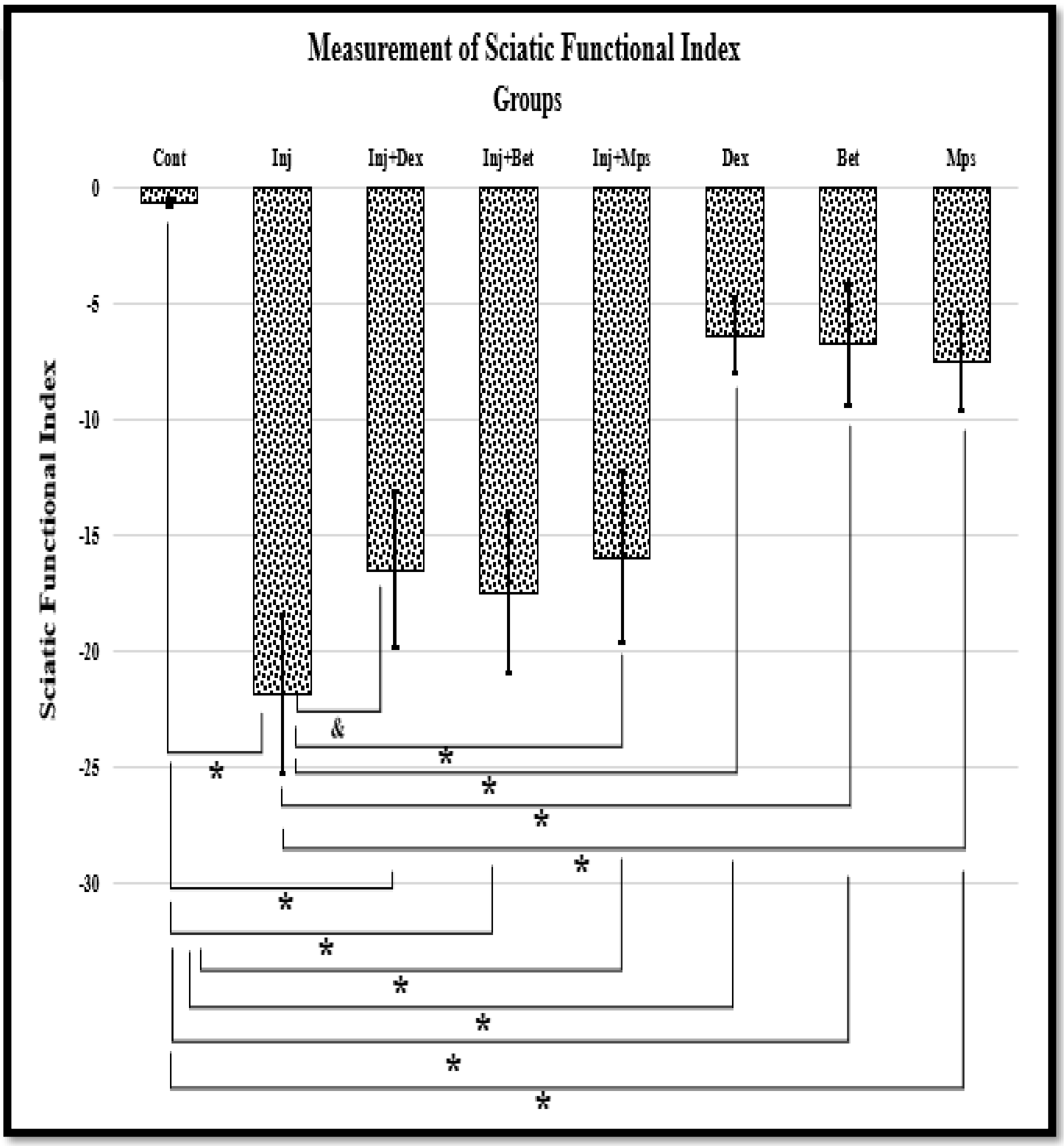


Figure 4.2. Display the differences of mean SFI across groups. The level of significance at p<0.05 is indicated by (&), while the significance level at p<0.01 is indicated by (*)

Table 4. 8. Shows degree of significance between groups in terms of functional sciatic index

COMPARISON BETWEEN GROUPS	DEGREE OF SIGNIFICANCE
Inj with Cont	0.000
Inj with Inj +Dex	0.016
Inj with Inj +Bet	0.091
Inj with Inj +Mps	0.005
Cont with Dex	0.007
Cont with Bet	0.003
Cont with Mps	0.001
Cont with Inj+Dex	0.000
Cont with Inj+Bet	0.000
Cont with Inj+Mps	0.000
Dex with Inj-Dex	0.000
Dex with Inj-Mps	0.000
Dex with Inj-Beta	0.000
Mps with Inj-Dex	0.000
Mps with Inj-Mps	0.000
Mps with Inj-Beta	0.000
Beta with Inj-Dex	0.000
Beta with Inj-Mps	0.000
Beta with Inj-Beta	0.000

4.1.3. Electromyography Findings

4.1.3.1. Amplitudes

The mean amplitudes and the standard deviation (SD) are shown in Table (4.9) and Figure (4.5).

Table 4.9. Demonstrates the mean amplitude and standard deviation (SD) across the groups

GROUPS	**MEAN VALUES OF AMPLITUDE (MV) ± SD**
Cont	47.965 ± 4.360
Inj	26.699 ± 0.148
Inj+Dex	25.686 ± 5.001
Inj+Bet	25.642 ± 6.671
Inj+Mps	26.453 ± 3.628
Dex	46.090 ± 4.416
Bet	49.954 ± 6.277
Mps	66.906 ± 17.825

The mean amplitude values in the Inj group were significantly higher than in the Cont group ($p = 0.000$), based on a statistical analysis of the acquired data. When comparing the Inj group to the Inj+ Dex group ($p > 0.05$), Inj+Bet group ($p > 0.05$), and Inj+Mps group ($p > 0.05$), no significant differences in mean amplitude values were observed. In addition, when the Cont group was compared to the Dex, Bet and Mps groups in terms of mean amplitude values, no significant differences were noted ($p > 0.05$) (Table 4.10).

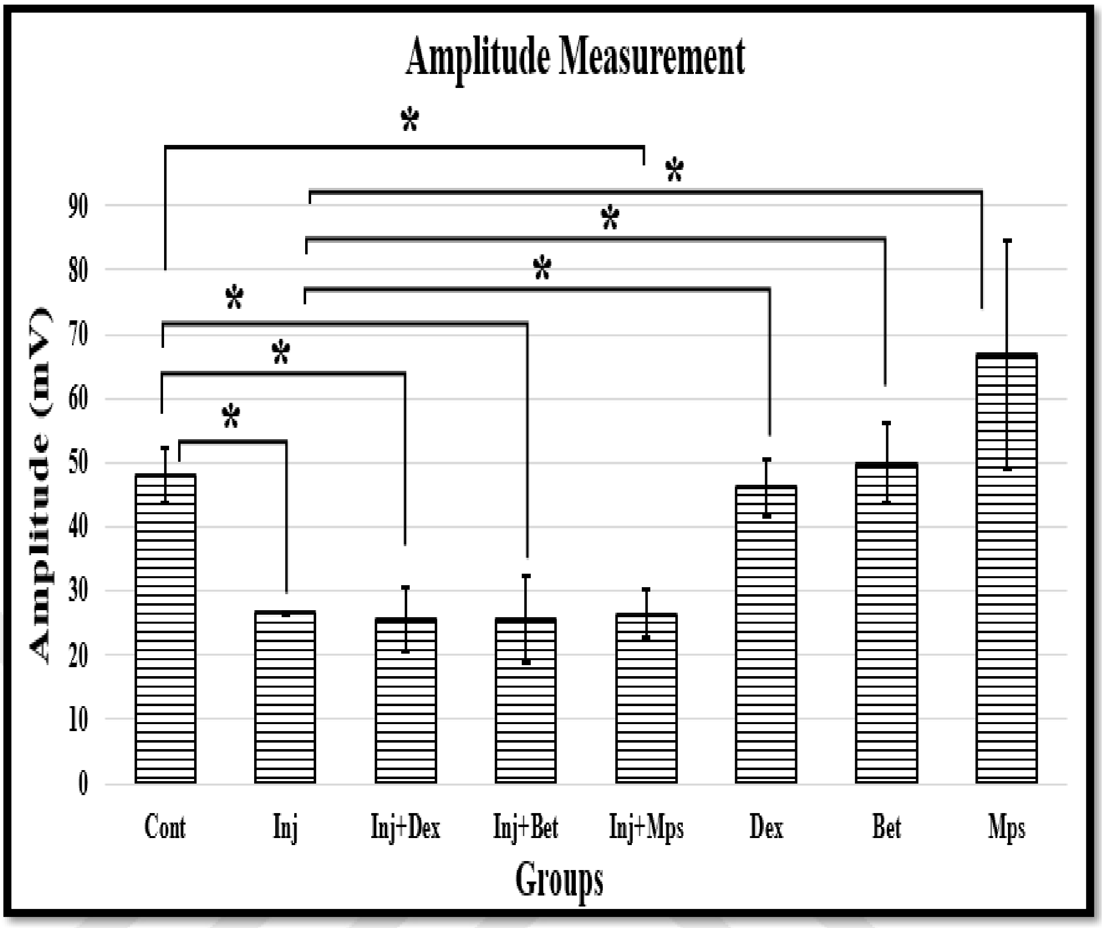


Figure 4.3. Shows the differences among the groups in terms of the mean value of amplitude. The significance level at p<0.01 is indicated by (*)

Table 4.10. Shows degree of significance between groups in terms of amplitude

COMPARISON BETWEEN GROUPS	DEGREE OF SIGNIFICANCE
Inj with Cont	0.000
Inj with Inj+Dex	1.000
Inj with Inj+Bet	1.000
Inj with Inj+Mps	1.000
Cont with Dex	1.000
Cont with Bet	0.528
Cont with Mps	0.528
Cont with Inj+Dex	0.000
Cont with Inj+Bet	0.000
Cont with Inj+Mps	0.000
Dex with Inj-Dex	0.001
Dex with Inj-Mps	0.000

Dex with Inj-Beta	0.001
Mps with Inj-Dex	0.009
Mps with Inj-Mps	0.013
Mps with Inj-Beta	0.008
Beta with Inj-Dex	0.002
Beta with Inj-Mps	0.003
Beta with Inj-Beta	0.001

4.1.3.2. Latencies

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

Table 4.11. Shows the groups' mean latency and standard deviation (SD).

GROUPS	MEAN VALUES OF LATENCY (SEC) ± SD
Cont	0.231 ± 0.023
Inj	0.173 ± 0.022
Inj+Dex	0.164 ± 0.019
Inj+Bet	0.172 ± 0.035
Inj+Mps	0.168 ± 0.020
Dex	0.228 ± 0.029
Bet	0.239 ± 0.021
Mps	0.232 ± 0.020

The mean latency values in the Inj group were significantly higher than the Cont group ($p = 0.006$), according to statistical analysis of the acquired data. Once comparing the Inj group to the Inj+ Dex group ($p > 0.05$), Inj+Bet group ($p > 0.05$), and Inj+Mps group ($p > 0.05$), no significant differences in mean latency values were observed. In addition, when the Cont group was compared to the Dex, Bet and Mps groups in terms of mean latency values, no significant differences were noted ($p > 0.05$) (Table 4.12).

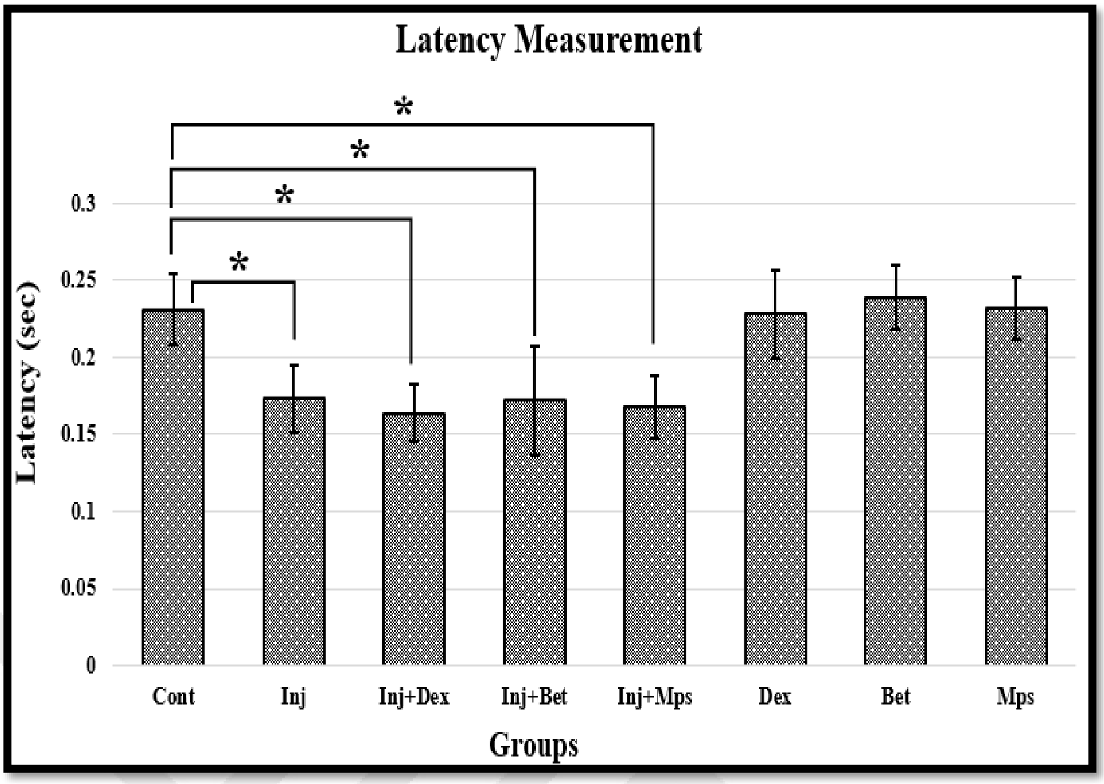


Figure4.4. Shows the differences between the groups regarding the mean of latency. The significance level at p<0.01 is indicated by (*)

Table 4. 13. Shows degree of significance between groups in terms of Latency

COMPARISON BETWEEN GROUPS	DEGREE OF SIGNIFICANCE
Inj with Cont	0.006
Inj with Inj+Dex	0.622
Inj with Inj+ Bet	0.527
Inj with Inj+ Mps	0.909
Cont with Dex	0.865
Cont with Bet	0.777
Cont with Mps	0.978
Cont with Inj+Dex	0.002
Cont with Inj+Bet	0.001
Cont with Inj+Mps	0.003
Dex with Inj-Dex	0.003
Dex with Inj-Mps	0.001
Dex with Inj-Beta	0.001

Mps with Inj-Dex	0.005
Mps with Inj-Mps	0.002
Mps with Inj-Beta	0.002
Beta with Inj-Dex	0.001
Beta with Inj-Mps	0.000
Beta with Inj-Beta	0.000

Table 4.4. The table displays the mean values of the data from higher to lower among the three corticosteroids in terms of assessed parameters. Despite the fact that certain drugs appear to have a favourable impact by raising (axonal area, myelin thickness, SFI and amplitude) or decreasing (number of axon and latency) the parameters which underlined, they were unable to achieve the statistical values as shown in the third column

PARAMETER	MEAN VALUE	SIGNIFICAT DIFFERENCES (P VALUE) AMONG SUBSTANCES	STATISTICAL GRAPH NUMBER
Axon Number	Mps > Bet > Dex	NO	Figure 4.1.
Area	Dex > Bet > Mps	NO	Figure 4.2.
Thickness	Mps > Bet > Dex	NO	Figure 4.3.
SFI	Mps > Dex > Bet	NO	Figure 4.4.
Amplitude	Mps > Bet > Dex	NO	Figure 4.5.
Latency	Bet > Mps > Dex	NO	Figure 4.6.

4.1.4. Light Microscopic Findings

Semi-thin sections taken from the sciatic nerve were stained with toluidine blue and examined by a light microscope (LM). The obtained results are shown below.

4.1.4.1. Light Microscopic Findings in the Cont Group

Semi-thin sections taken from the sciatic nerve of the Cont group were examined by LM; the general histological structural view appeared normal.

Epineurium that surrounds the whole nerve looks normal. The nerve fascicles are surrounded by thin perineurium that contains blood vessels normally. The heterogeneity of myelinated axons was observed normal and enclosed by a thick myelin sheath. The nerve fibres coherence is deemed normal. The myelin sheath of some axons showed a physical impairment appearance (vacuolization/tubulisation) due to improper fixation, poor tissue processing, or unknown reason.

Moreover, a few physiological impairments of the myelin sheath were also observed, such as Schmidt-Lanterman clefts. The unmyelinated axons were also found. Blood cells inside the blood vessel were also seen. Likewise, the Schwann cell's nucleus was appeared very clear in some sections. (Figures 4.7- 11).

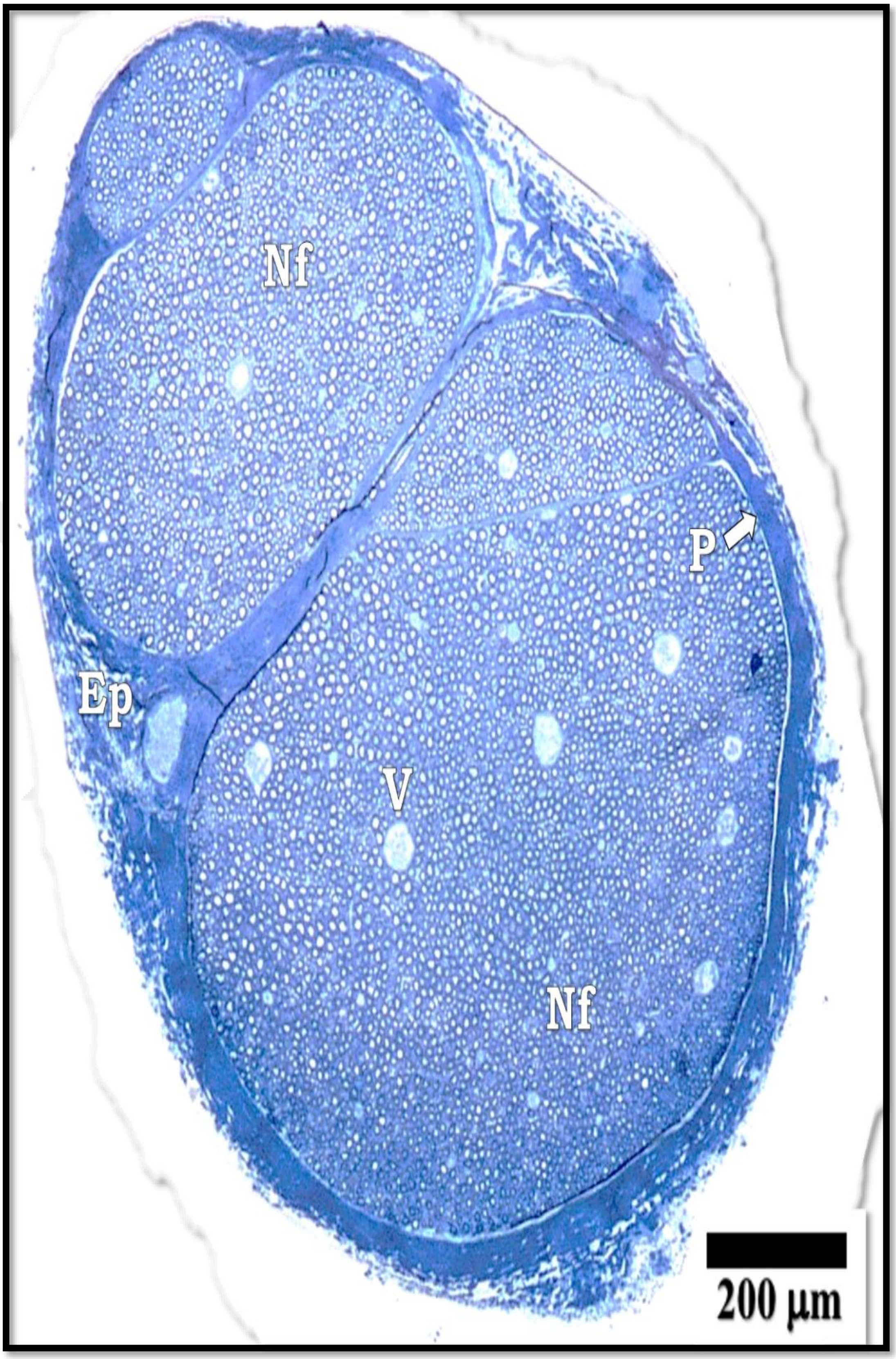


Figure 4.7. Image taken from the sciatic nerve of the Cont group is seen. Healthy nerve fascicles (Nf) surrounded by intact perineurium (P) are seen (arrow). Intact epineurium (Ep) around the entire nerve structure is observed. A blood vessel (V) in the middle of the nerve fascicle is seen. The general structure of the nerve looks healthy

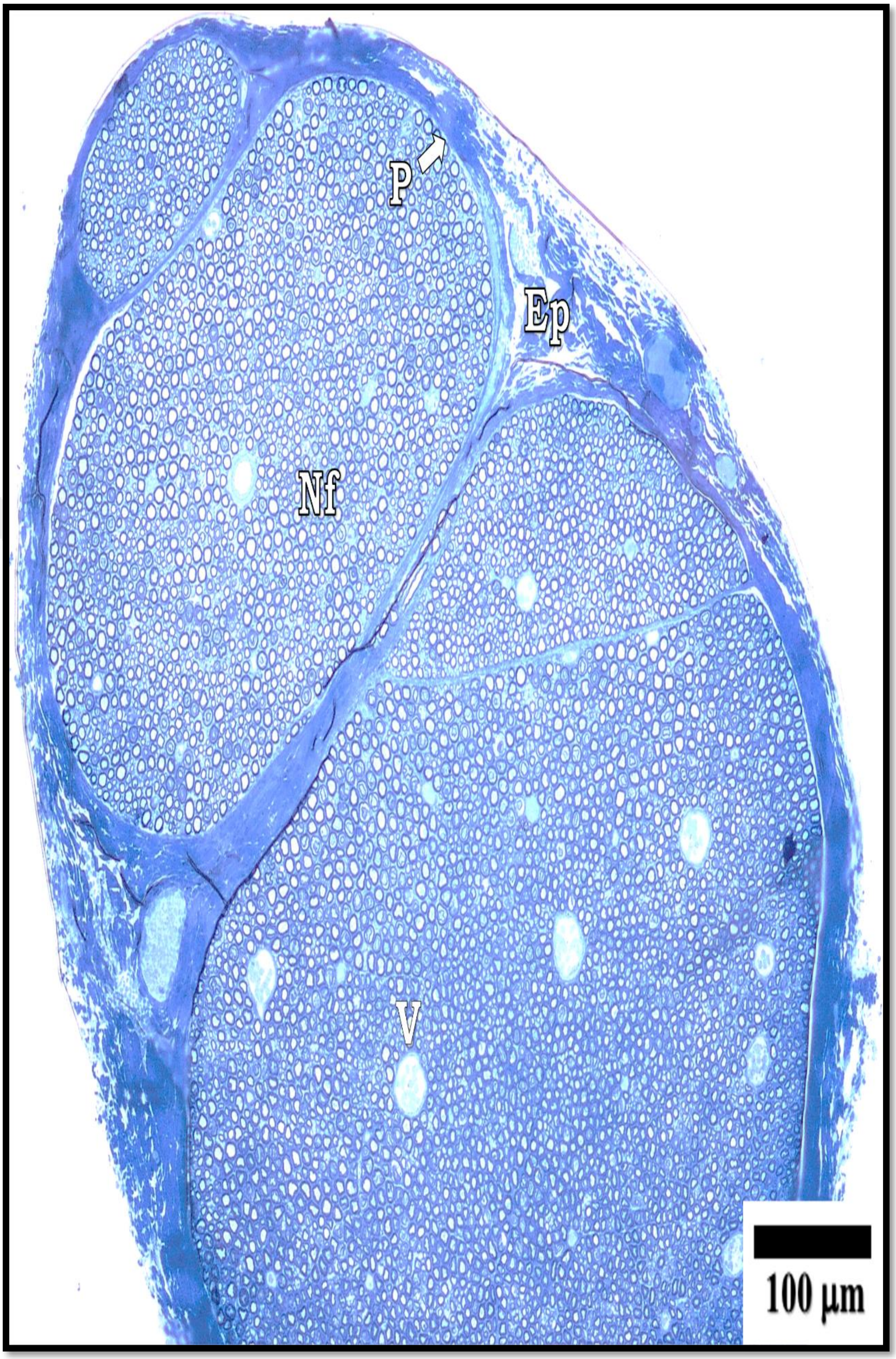


Figure 4.8. A Photomicrograph taken from the sciatic nerve of the Cont group is seen. A very healthy nerve fascicle surrounded by intact perineurium (P) is observed. The myelinated nerve fibres in the nerve are seen as normal. A blood vessel (V) with a continuous wall can be seen in this section. Intact epineurium (Ep) around the whole nerve is clearly observed

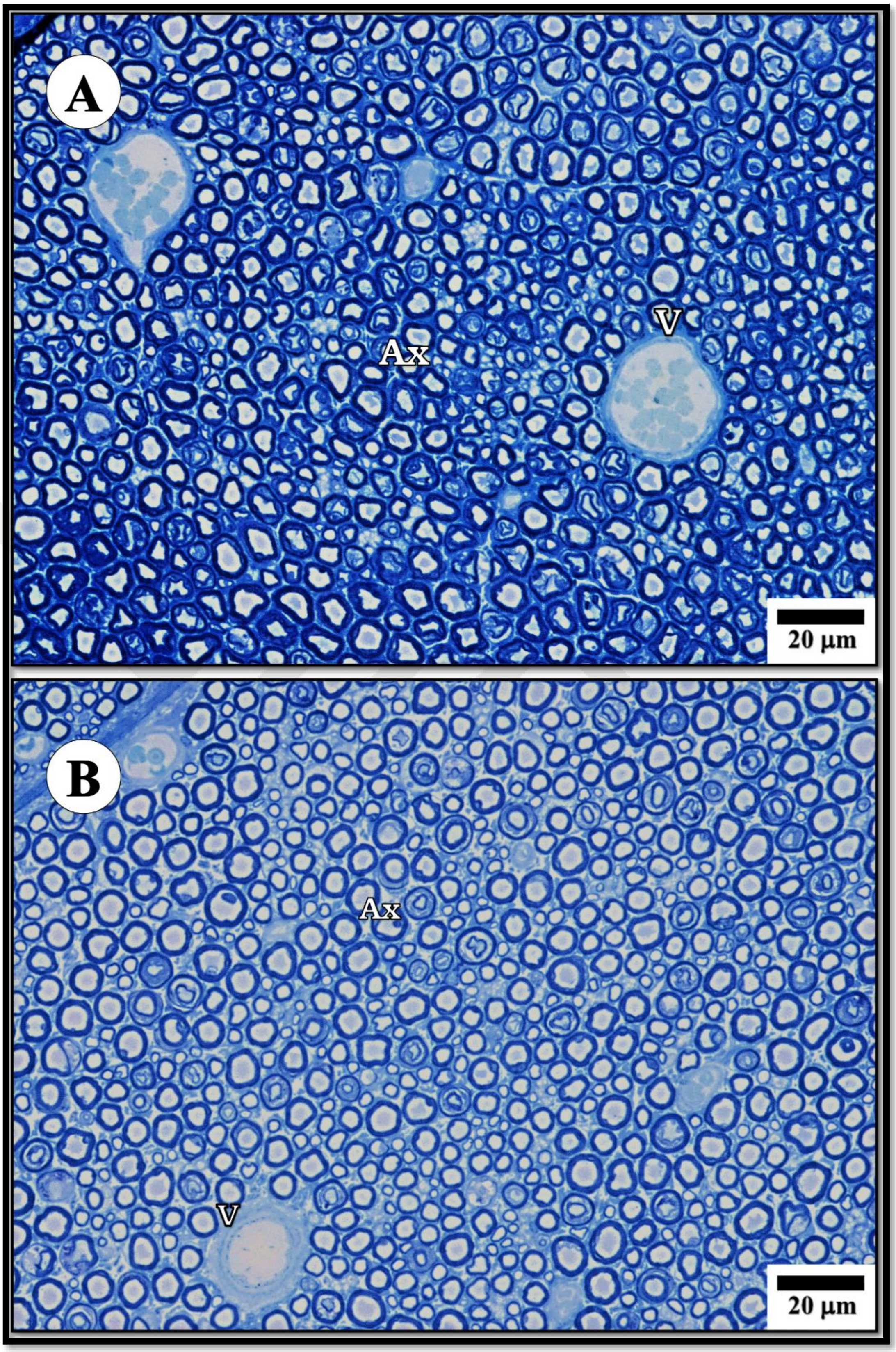


Figure 4.9. (A, B) Photomicrographs taken from the sciatic nerve of the Cont group are seen. The general structure of the nerve looks normal. A healthy blood vessel (V) with blood cells is observed. The nerve fascicle and myelinated nerve axons are appeared normal. Group of healthy myelinated nerve axons (Ax) is seen

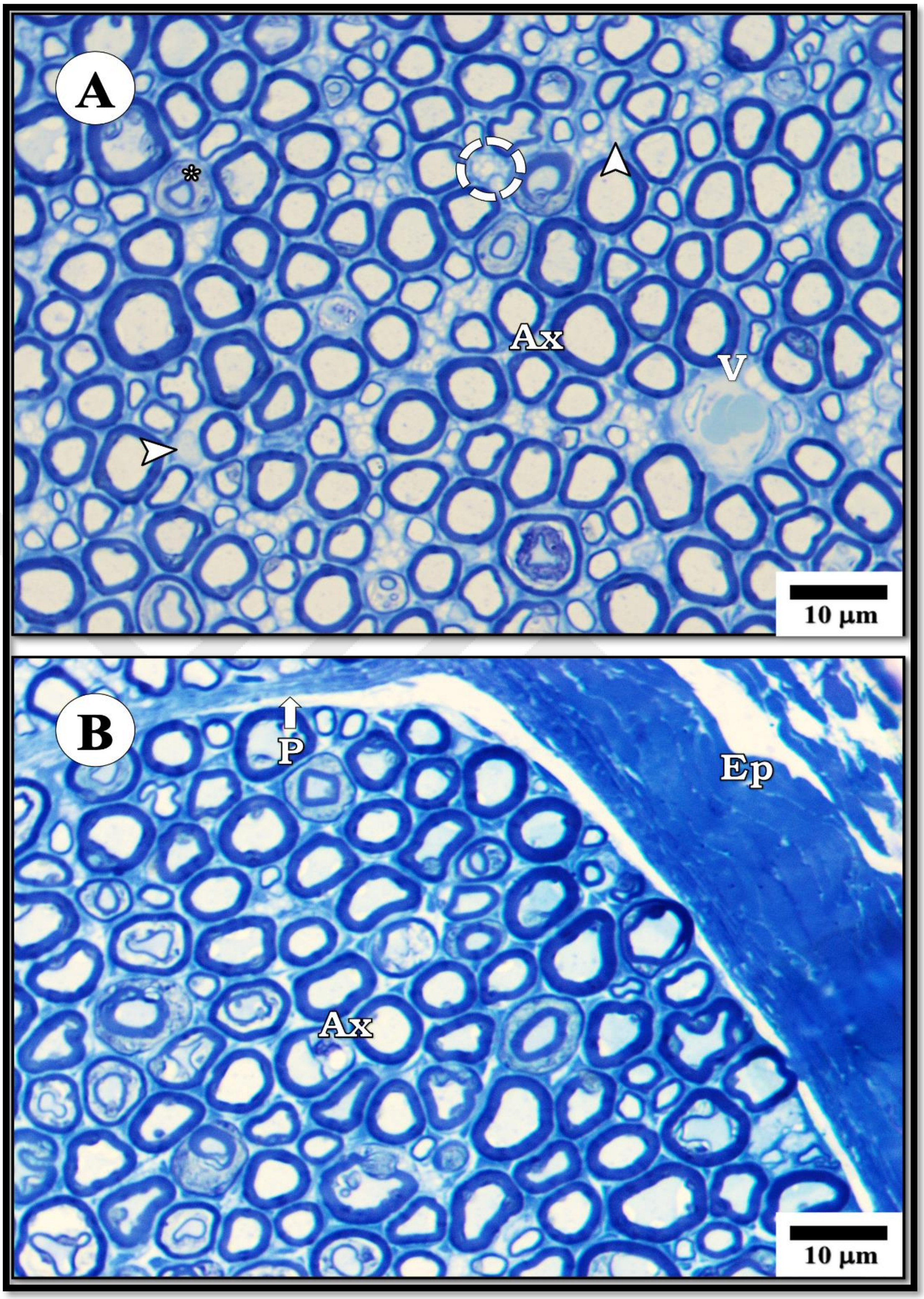


Figure 4.10. (A, B) Photomicrographs taken from the sciatic nerve of the Cont group are seen. Most of myelinated nerve axons (Ax) and myelin sheath thickness looks normal. A thick blood vessel wall (V) is seen with many blood cells inside the lumen. The Schwann cells nuclei were seen closed to myelinated axons (arrowhead). Some parts of the myelin sheath were impaired by the Schmidt-Lanterman cleft that is pointed by (star). Intact perineurium (P) and epineurium (Ep) are clearly observed. The unmyelinated axons were also indicated by (white circle)

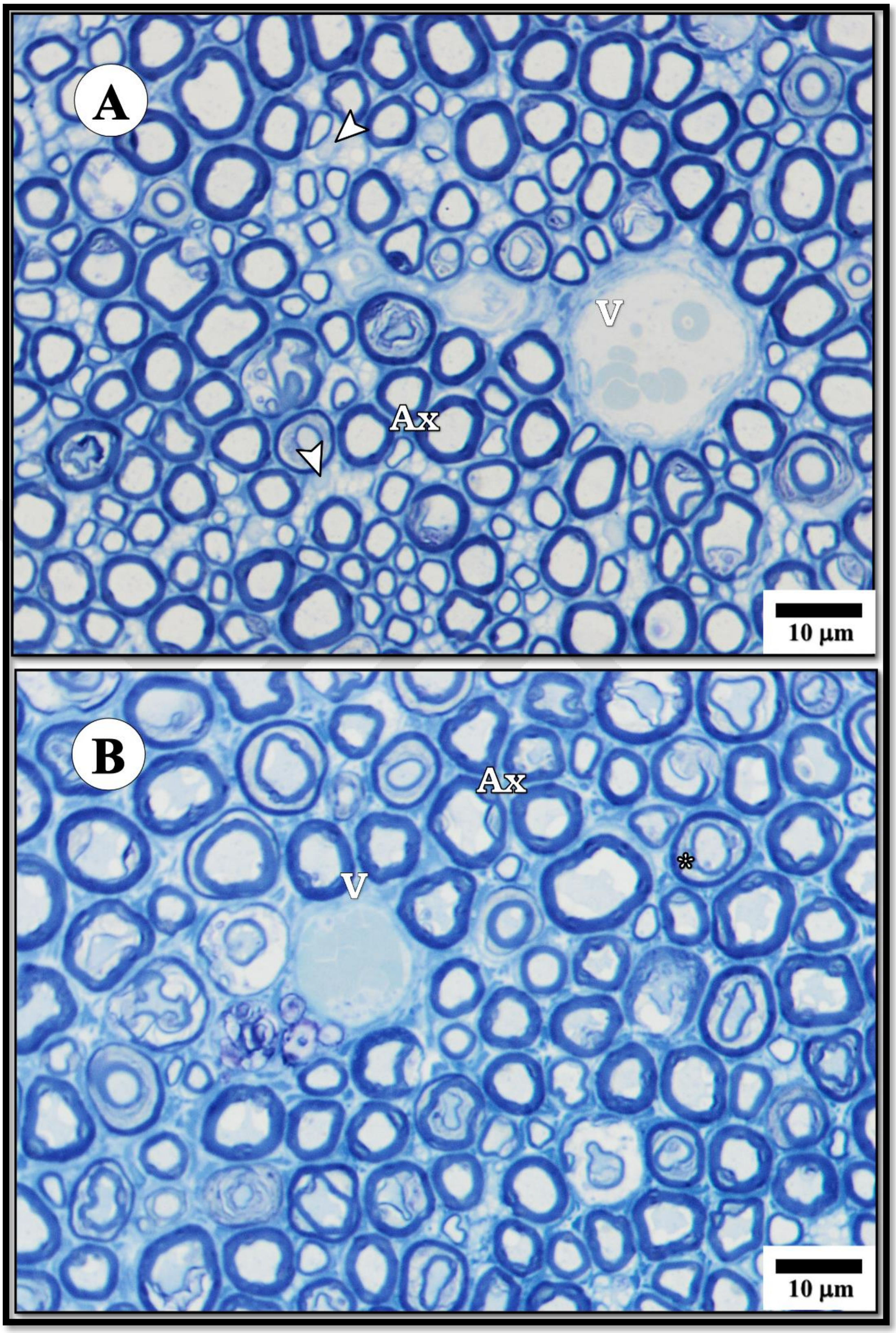


Figure 4.11. (A, B) Photomicrographs taken from the sciatic nerve of the Cont group are seen. Most of myelinated nerve axons (Ax) and myelin sheath thickness looks normal. A thick blood vessel wall (V) is seen with many blood cells inside the lumen. Some parts of the myelin sheath were impaired that are pointed by (star). The Schwann cells nuclei were seen closed to myelinated axons (arrowhead)

4.1.4.2. Light Microscopic Findings in the Dex Group

Semi-thin sections taken from the sciatic nerve of the Dex Group are seen. All nerve structures were normal. The nerve bundles are surrounded by intact epineurium in addition to healthy perineurium, which encloses each bundle. Most myelinated axons were ensheathed by thick myelin sheath were observed. Some myelinated fibres defects were seen. The blood cells within the lumen of blood vessels were seen as normal. The Schwann cells could be identified by their nuclei localized close to myelinated fibers (Figures 4.12- 15).

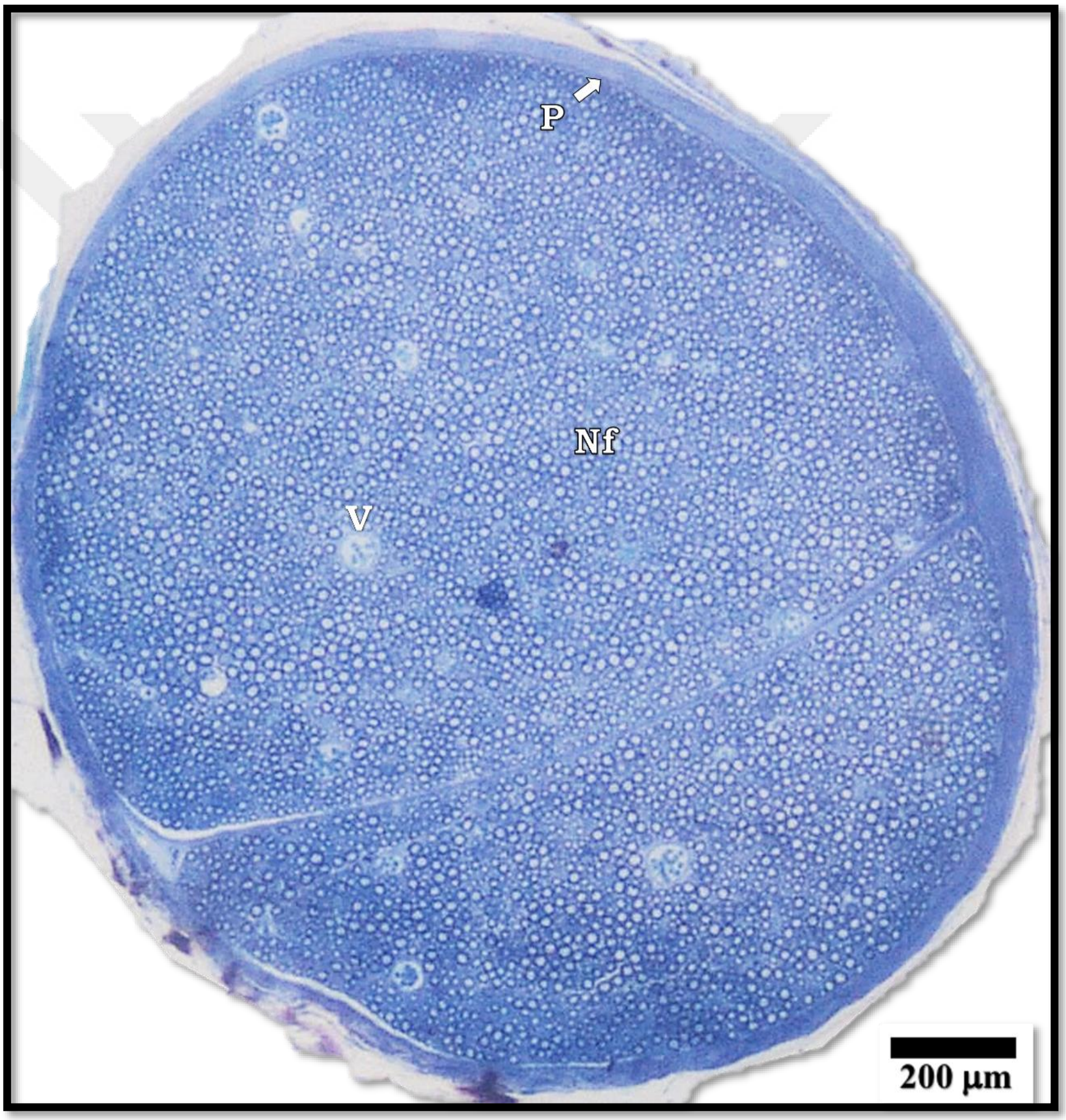


Figure 4.12. A Photomicrograph taken from the sciatic nerve of the Dex group is seen. A very healthy nerve fascicle surrounded by intact perineurium (P) is observed. Nerve fascicle (Nf) is seen and encircled by perineurium (P). The Myelinated nerve fibers in the nerve are seen as normal. A blood vessel (V) with a continuous wall can be seen in this section

Figure 4.13. Image taken from the sciatic nerve of the Dex group is observed. Normal axons (Ax) is seen. Blood vessel (V) is also seen. Schwann cell is also seen (head arrow)

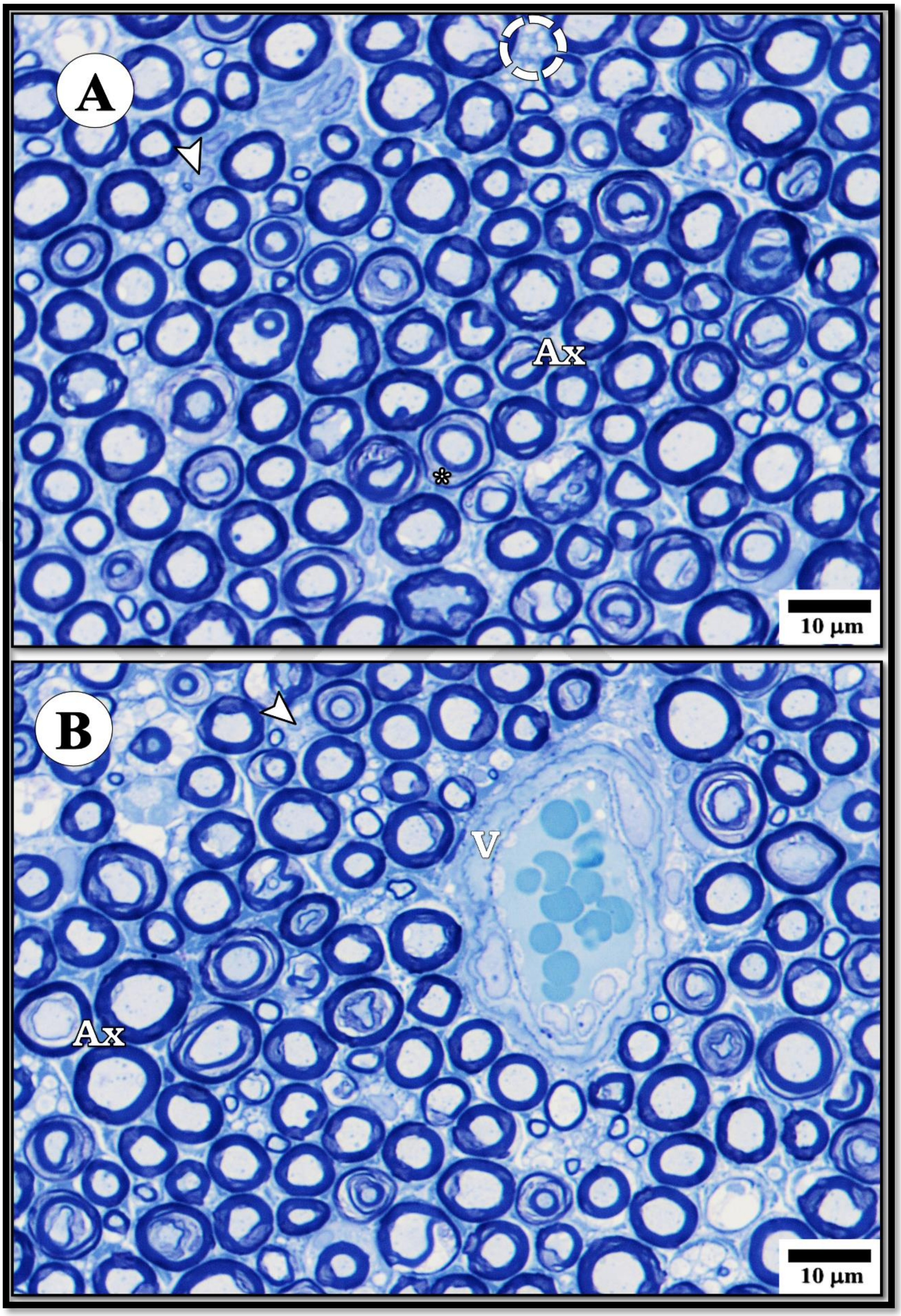


Figure 4.14. (A, B) Photomicrographs taken from the sciatic nerve of the Dex group are seen. Myelinated nerve axons (Ax) and myelin sheath thickness look normal. A thick blood vessel wall (V) is seen with many blood cells inside the lumen. Some parts of the myelin sheath were impaired that are pointed by (star). Schwann cell is also seen (head arrow). A group of unmyelinated axons around the Schwann cell is also seen (interrupted white circle)

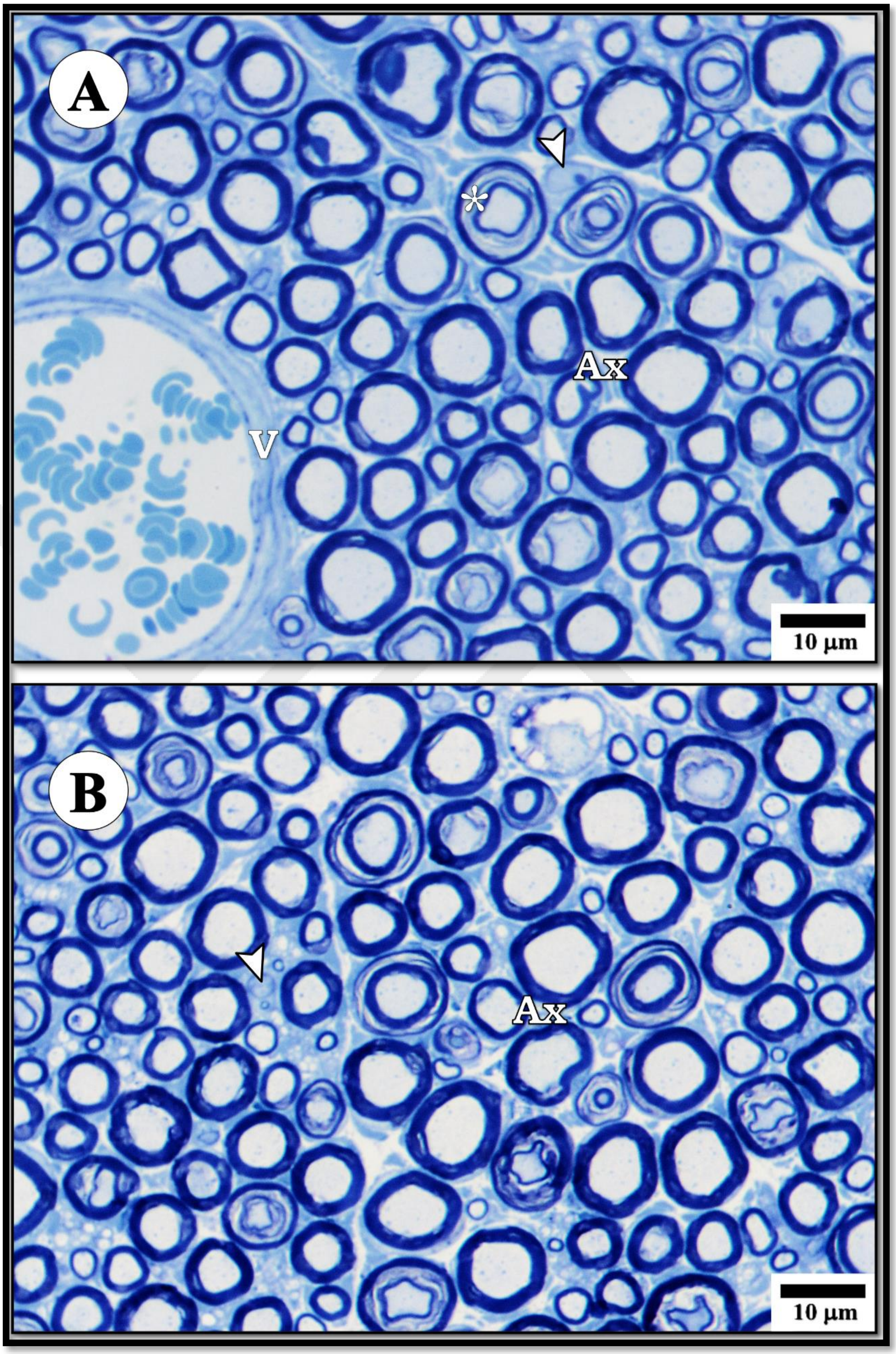


Figure 4.15. (A, B) Photomicrographs taken from the sciatic nerve of the Dex group are seen. Myelinated nerve axons (Ax) and myelin sheath thickness look normal. A thick blood vessel wall (V) is seen with many blood cells inside the lumen. Some parts of the myelin sheath were impaired that are pointed by stars. Schwann cell is also seen (arrow). Schmidt-Lanterman cleft was also found pointed by (star)

4.1.4.3. Light Microscopic Findings in the Bet Group

The cross-sectioned of semi-thin sections taken from the sciatic nerve of the Bet group were evaluated using LM. The general histological architecture appeared healthy. The connective tissue of the epineurium and perineurium was observed intact. The Schwann cells nuclei were demonstrated closely to myelinated fibers. The blood cells normally appeared within the blood vessels lumen. Most of the myelinated axons were ensheathed by a thick myelin coat, and some unmyelinated fibers and myelin sheath impairments were observed. (Figures 4.16- 20).

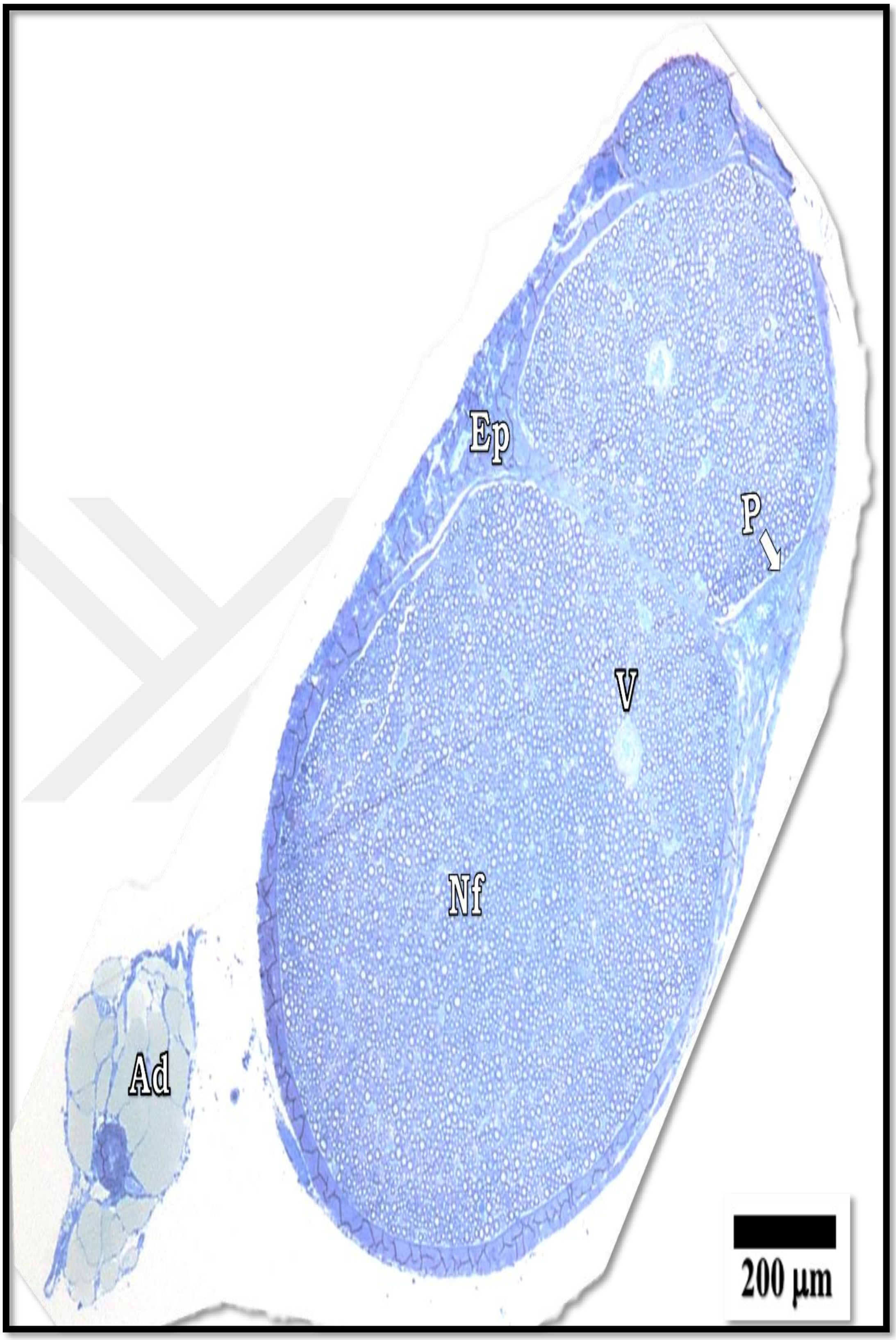


Figure 4.16. Image taken from the sciatic nerve of the Bet group is seen. Healthy nerve fascicles (Nf) surrounded by intact perineurium (P) are seen (arrow). Intact epineurium (Ep) around the entire nerve structure is observed. A blood vessel (V) in the middle of the nerve fascicle is seen. Adipose tissue (Ad) close by the nerve is also seen. The general structure of the nerve looks healthy

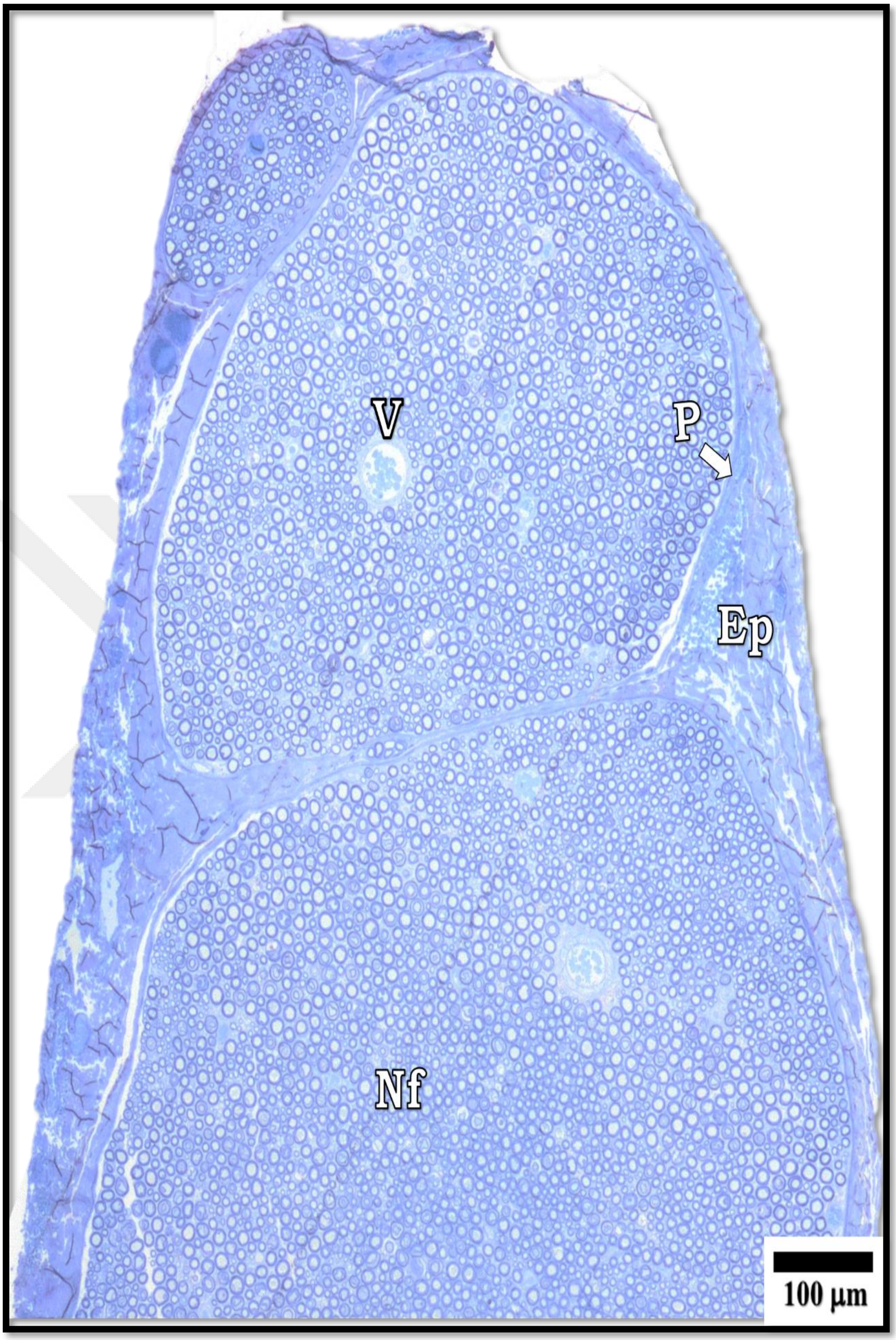


Figure 4.17. A Photomicrograph taken from the sciatic nerve of the Bet group is seen. A very healthy nerve fascicle surrounded by intact perineurium (P) is observed. Nerve fascicle (Nf) is seen and encircled by perineurium (P). The Myelinated nerve fibers in the nerve are seen as normal. A blood vessel (V) with a continuous wall can be seen in this section. Intact epineurium (Ep) around the whole nerve is clearly observed

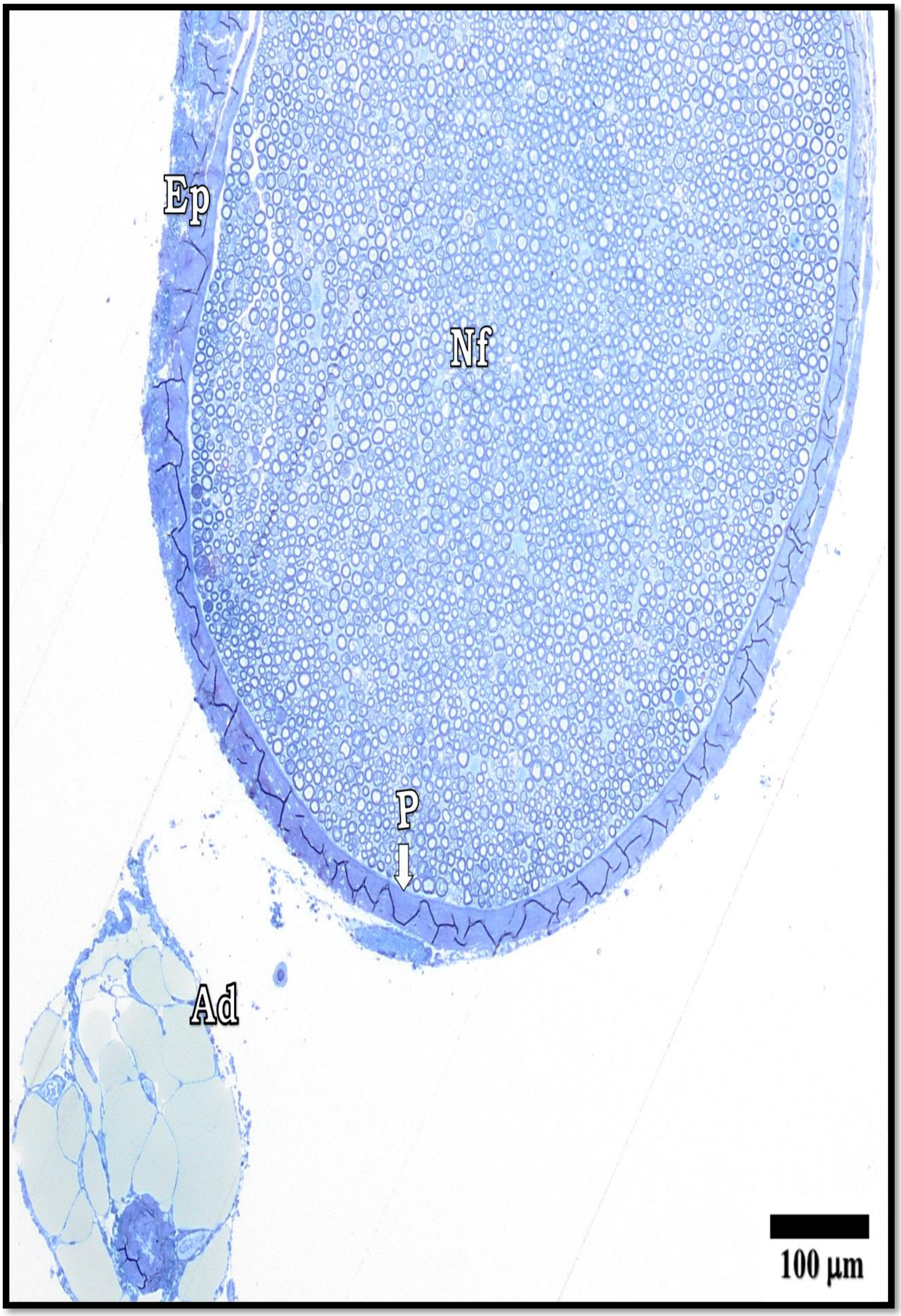


Figure 4.18. A Photomicrograph taken from the sciatic nerve of the Bet group is seen. A very healthy nerve fascicle surrounded by intact perineurium (P) is observed. Nerve fascicle (Nf) is seen and encircled by perineurium (P). The Myelinated nerve fibers in the nerve are seen as normal. A blood vessel (V) with a continuous wall can be seen in this section. Intact epineurium (Ep) around the whole nerve is clearly observed. Adipose tissue (Ad) close by the nerve is also seen

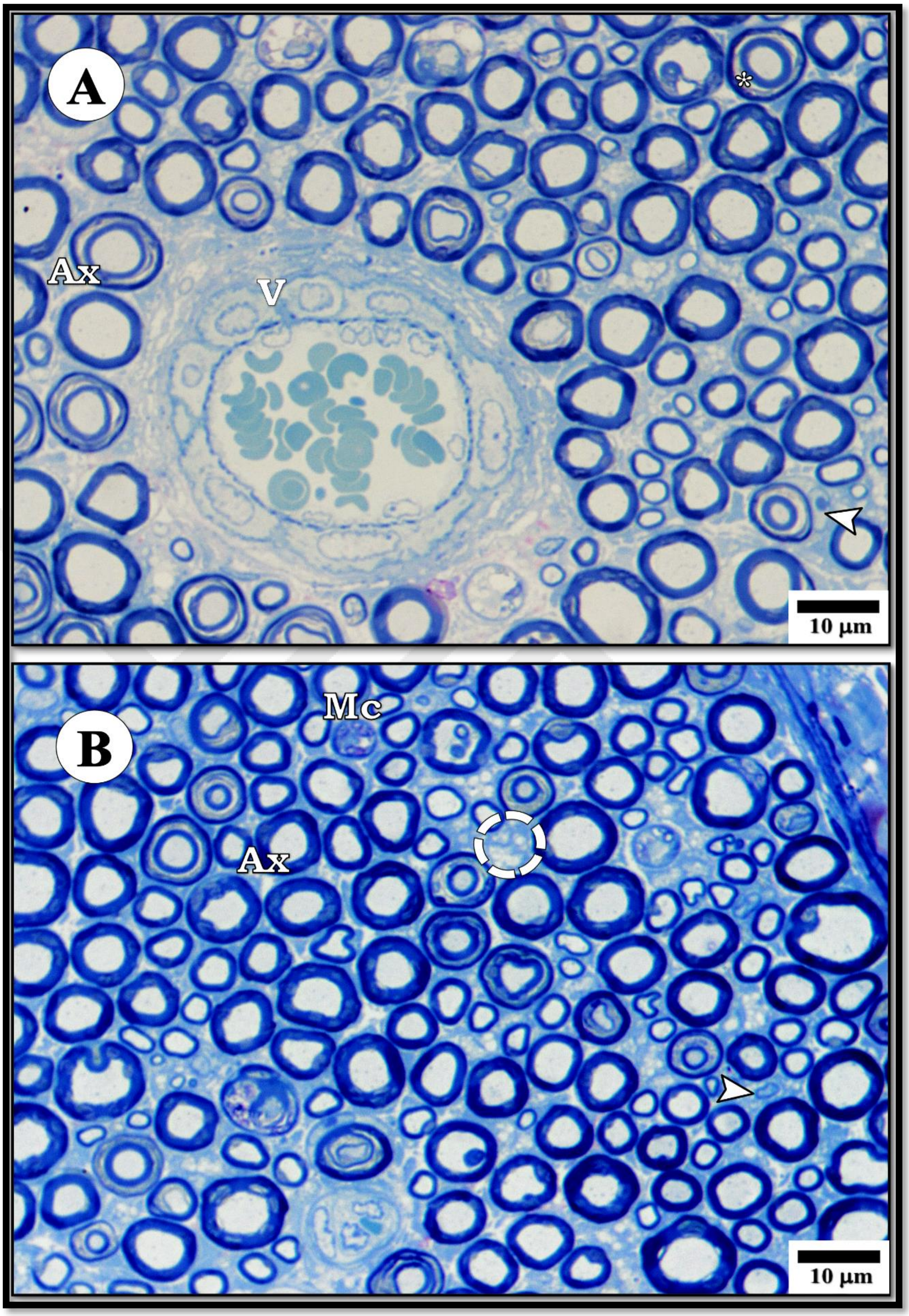


Figure 4.19. (A, B) Photomicrographs taken from the sciatic nerve of the Bet group are seen. Most of the myelinated nerve axons (Ax) and myelin sheath thickness looks normal. A thick blood vessel wall (V) is seen with many blood cells inside the lumen. Some parts of the myelin sheath were impaired Schmidt-Lanterman cleft that is pointed by (stars). A group of unmyelinated axons around the Schwann cell is also seen (interrupted white circle). The mast cell (Mc) was also seen

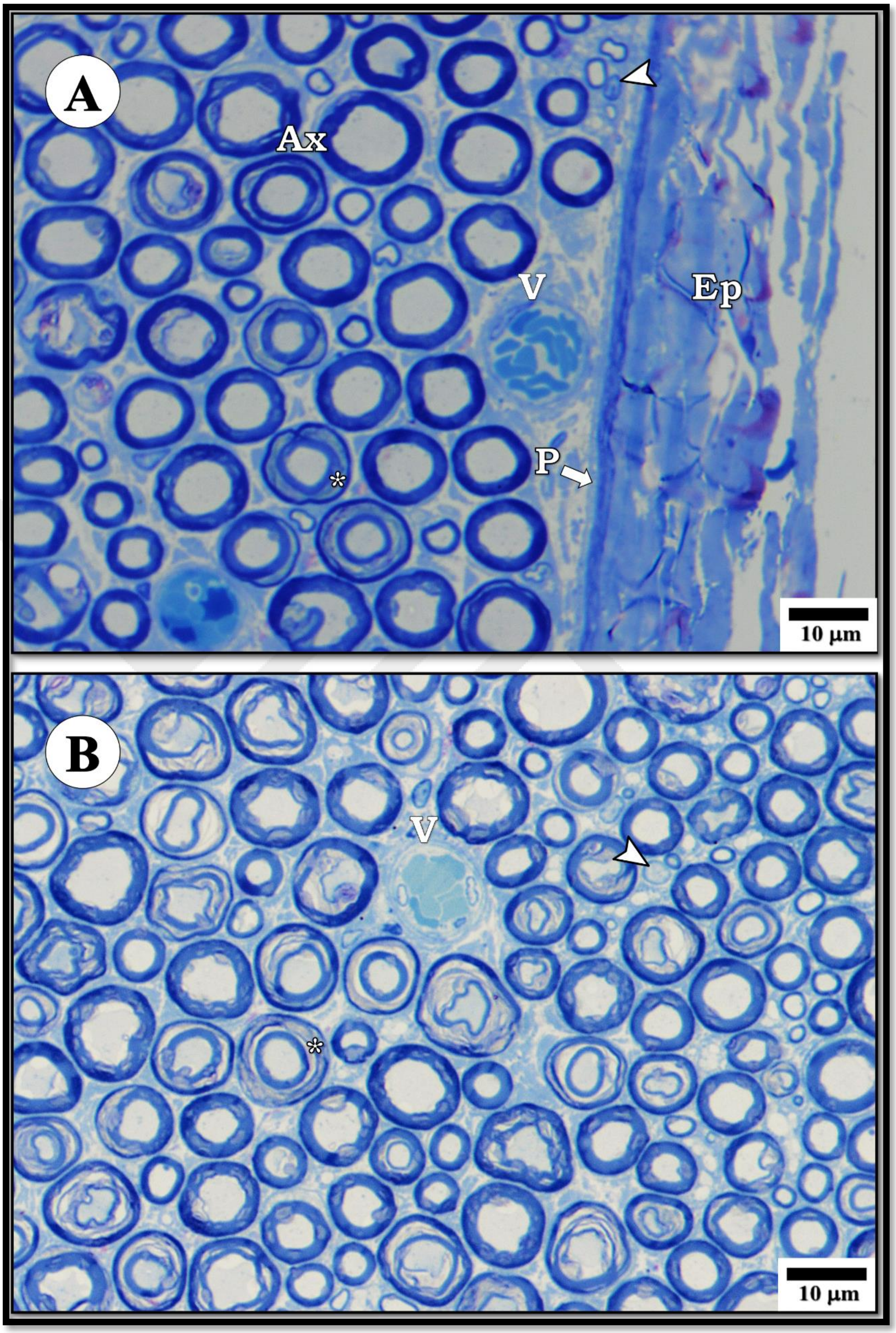


Figure 4.20. (A, B) Photomicrographs taken from the sciatic nerve of the Bet group are seen. Most of myelinated nerve axons (Ax) and myelin sheath thickness looks normal. A thick blood vessel wall (V) is seen with many blood cells inside the lumen. Some parts of the myelin sheath were impaired Schmidt-Lanterman cleft that is pointed by (stars). An intact perineurium (P) and epineurium (Ep) are observed

4.1.4.4. Light Microscopic Findings in the Mps Group

Semi-thin sections taken from the sciatic nerve of the Mps Group were seen for LM examination. The structure of the neural tissue was healthy. The nerve was enclosed by intact connective tissue, epineurium, besides some adipose tissue was seen. Also, each nerve bundle was surrounded by normal connective tissue, the perineurium. Furthermore, the thick myelin sheath was myelinated in the majority of fibers, as well the unmyelinated fibers were also seen.

On the other hand, some impairment of myelin sheath was observed. In addition, the blood cells and vessels were normal. Within the connective tissue of the myelinated axons, the mast cells were also observed. The nuclei of the Schwann cells were found beside the myelinated fibers (Figures 4.21- 25).

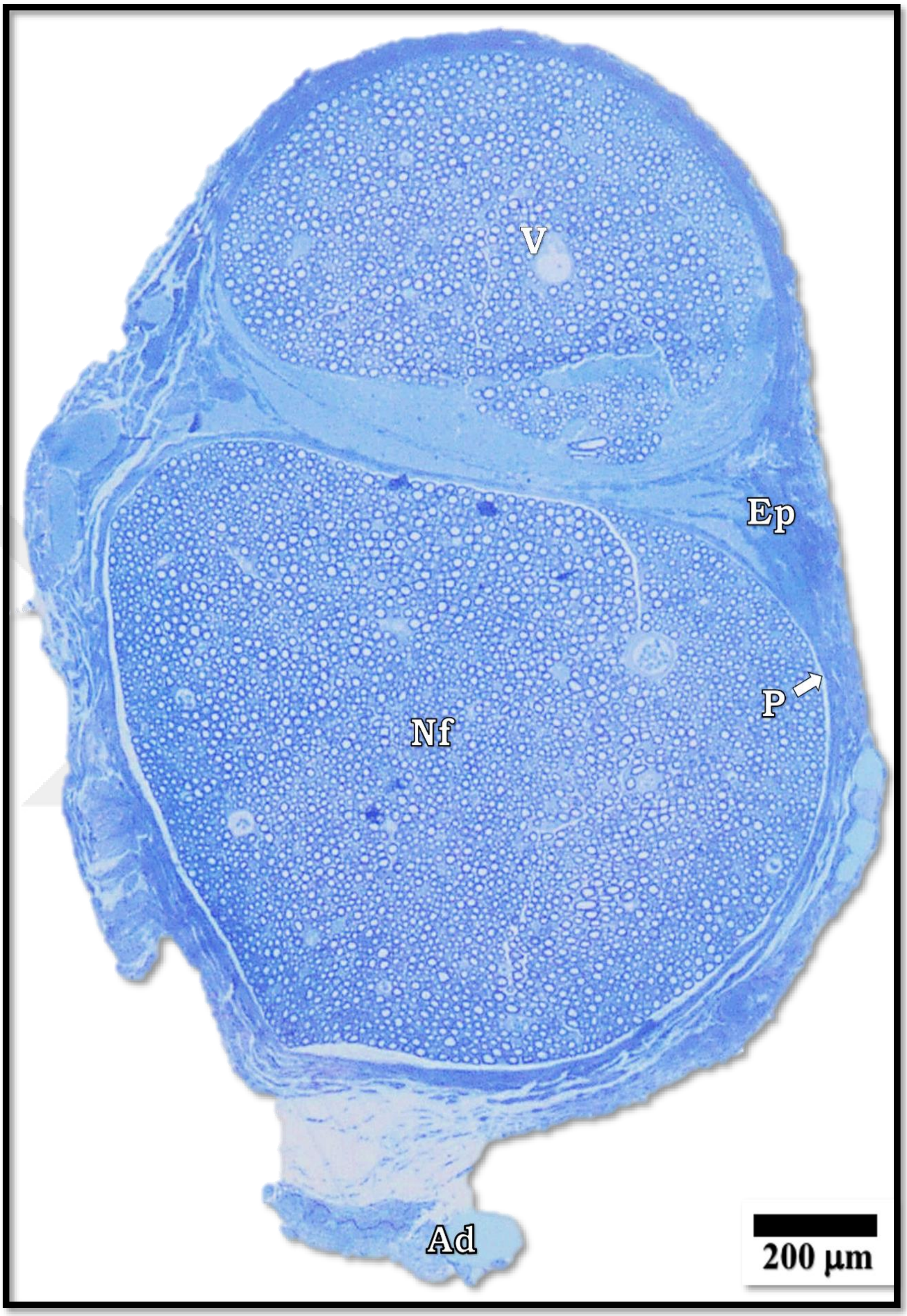


Figure 4.21. A Photomicrograph taken from the sciatic nerve of the Mps group is seen. A very healthy nerve fascicle surrounded by intact perineurium (P) is observed. Nerve fascicle (Nf) is seen and encircled by perineurium (P). The Myelinated nerve fibers in the nerve are seen as normal. A blood vessel (V) with a continuous wall can be seen in this section. Intact epineurium (Ep) around the whole nerve is clearly observed. Adipose tissue (Ad) close by the nerve is also seen

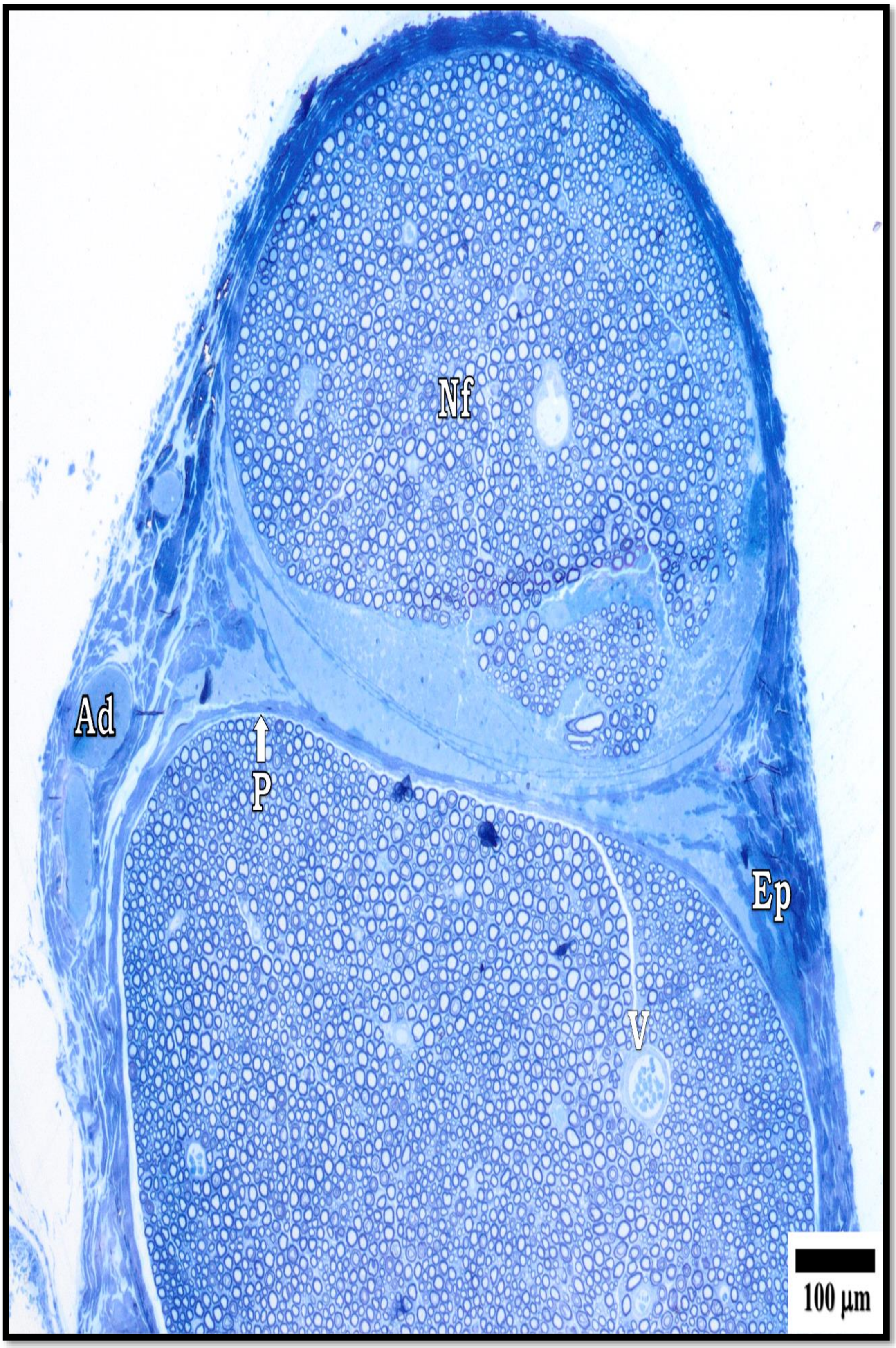


Figure 4.22. A Photomicrograph taken from the sciatic nerve of the Mps group is seen. A very healthy nerve fascicle surrounded by intact perineurium (P) is observed. Nerve fascicle (Nf) is seen and encircled by perineurium (P). The Myelinated nerve fibers in the nerve are seen as normal. A blood vessel (V) with a continuous wall can be seen in this section. Intact epineurium (Ep) around the whole nerve is clearly observed

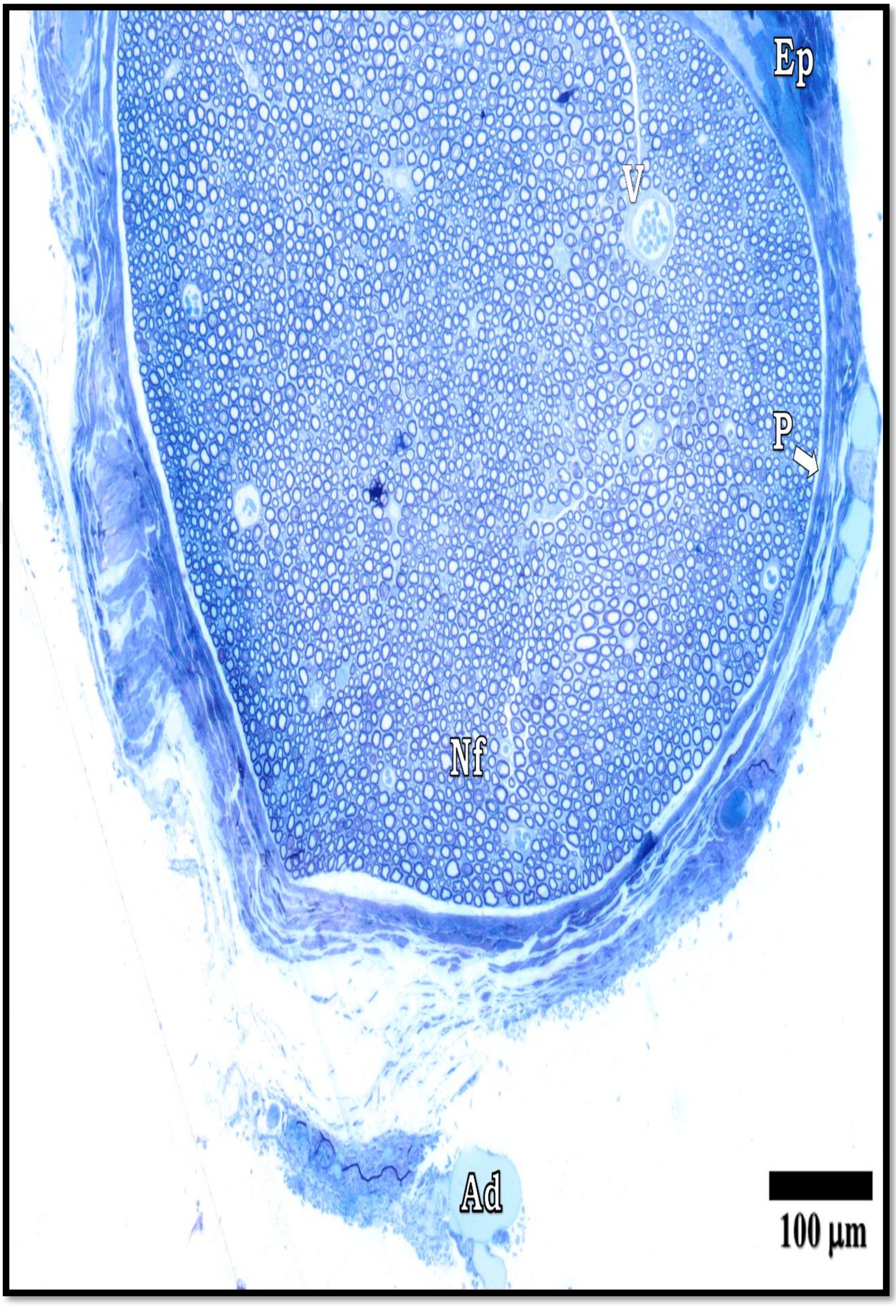


Figure 4.23. A Photomicrograph taken from the sciatic nerve of the Mps group is seen. A very healthy nerve fascicle surrounded by intact perineurium (P) is observed. Nerve fascicle (Nf) is seen and encircled by perineurium (P). The Myelinated nerve fibers in the nerve are seen as normal. A blood vessel (V) with a continuous wall can be seen in this section. Intact epineurium (Ep) around the whole nerve is clearly observed. Adipose tissue (Ad) close by the nerve is also seen

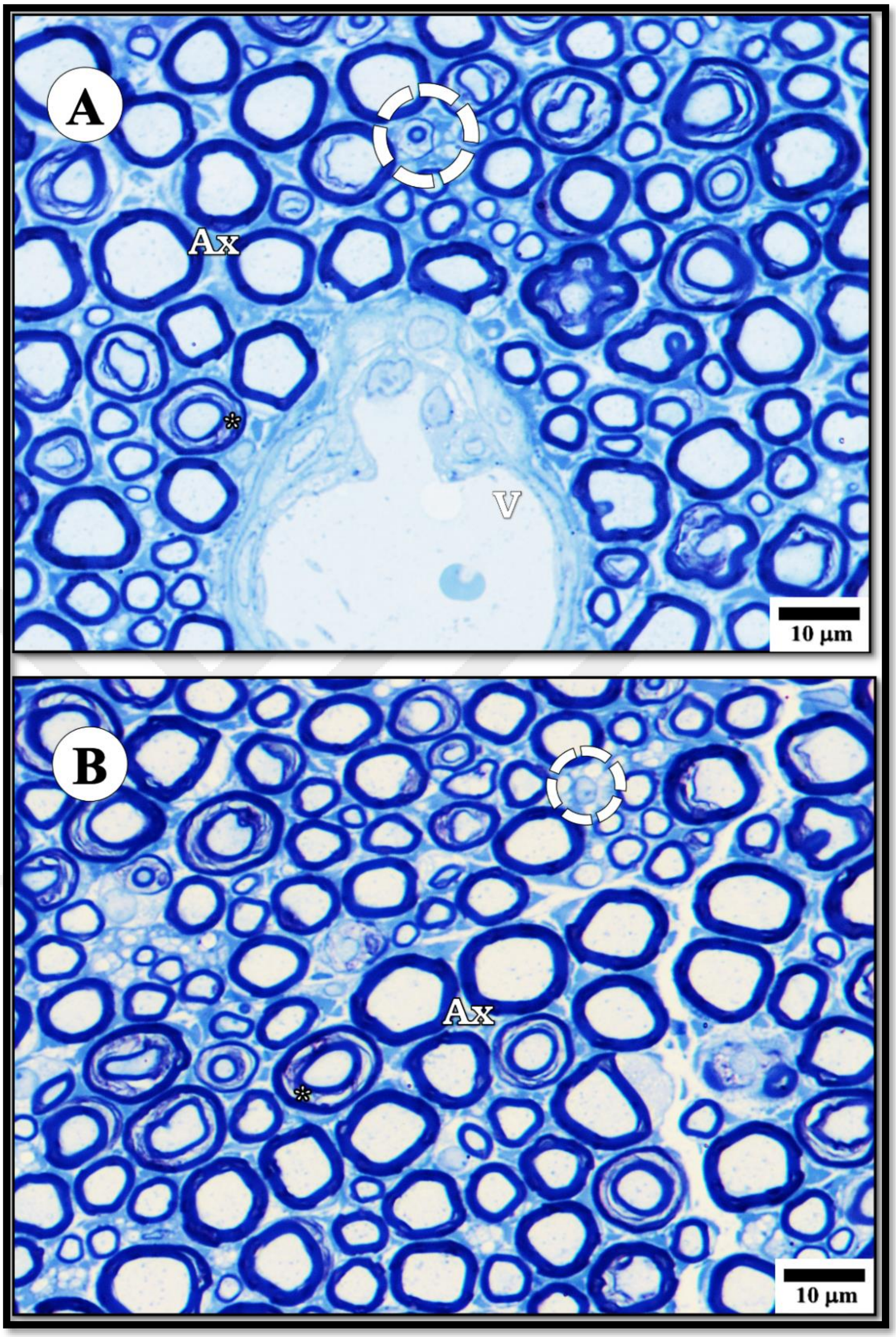


Figure 4.24. (A, B) Photomicrographs taken from the sciatic nerve of the Mps group are seen. Most of myelinated nerve axons (Ax) and myelin sheath thickness looks normal. A thick blood vessel wall (V) is seen with many blood cells inside the lumen. Some parts of the myelin sheath were impaired Schmidt-Lanterman-cleft that is pointed by (stars). A group of unmyelinated axons around the Schwann cell is also seen (interrupted white circle)

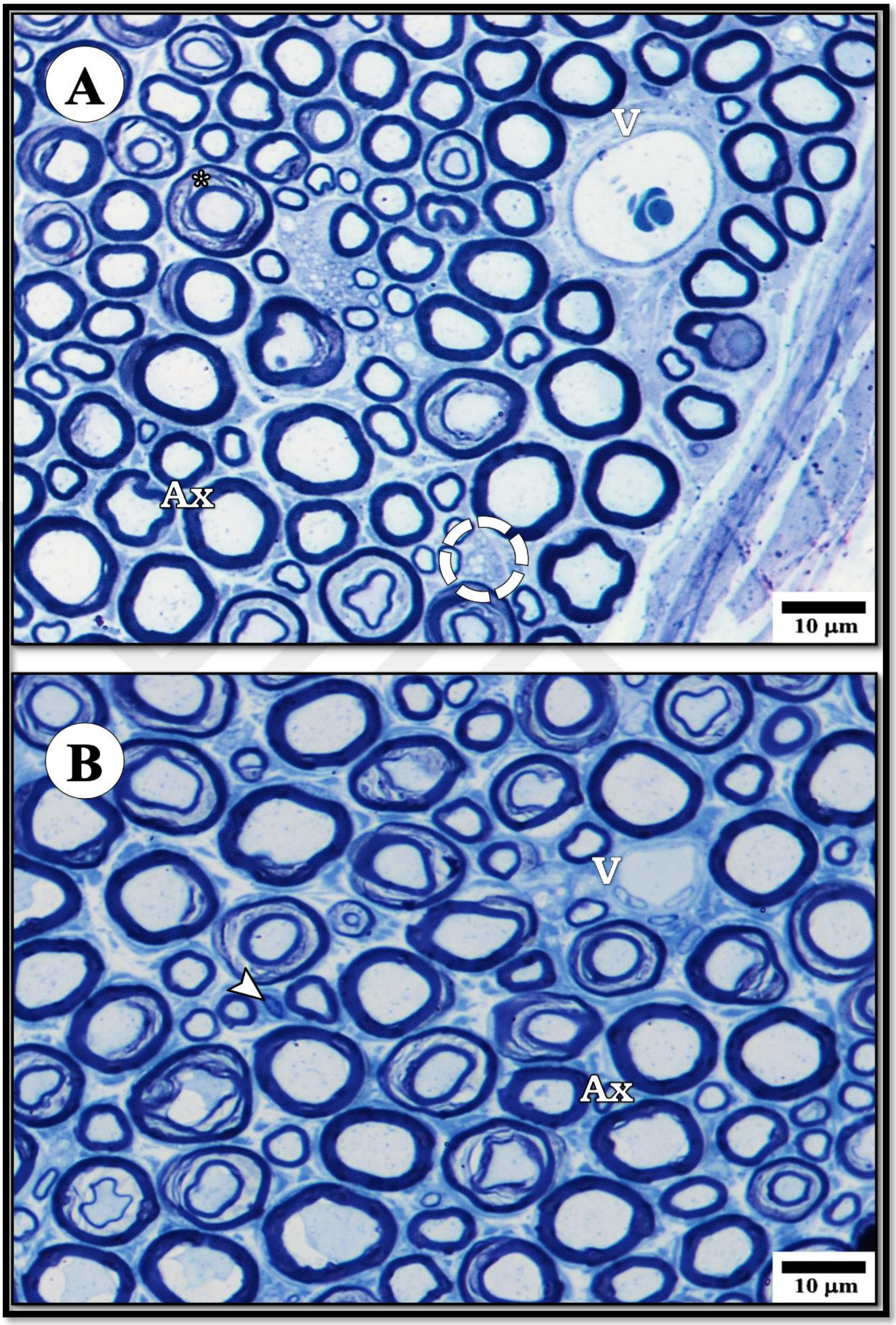


Figure 4.25. (A, B) Photomicrographs taken from the sciatic nerve of the Mps group are seen. Most of myelinated nerve axons (Ax) and myelin sheath thickness looks normal. A thick blood vessel wall (V) is seen with many blood cells inside the lumen. Some parts of the myelin sheath were impaired Schmidt-Lanterman cleft that is pointed by (star). A group of unmyelinated axons around the Schwann cell is also seen (interrupted white circle). Slight damage in the myelin sheath is seen in some myelinated axons

4.1.4.5. Light Microscopic Findings in the Inj Group

Semi-thin sections taken from the sciatic nerve of the Inj group were examined by LM. The general histological structure of the nerve appeared mostly impaired due to being affected by pathological changes. The connective tissue adipose cells around the nerve were seen healthy, with intact perineurium. Some tissue loss in the nerve cross-section was clearly seen, resulting from poor tissue processing steps. Considerable number of macrophages were observed among the degenerated nerve fibres to engulf the myelin debris (foamy appearance). Most of the fibers showed undergoing WD with tiny size and thinner myelin coats. Some of the normal thin myelinated axons are found among damaged fibres. Moreover, some blood vessels with premature endothelial cells were also observed. Also, many of the Schwann cells were seen around the newly formed myelinated axons to initiate the remyelination (Figures 4.26- 30).

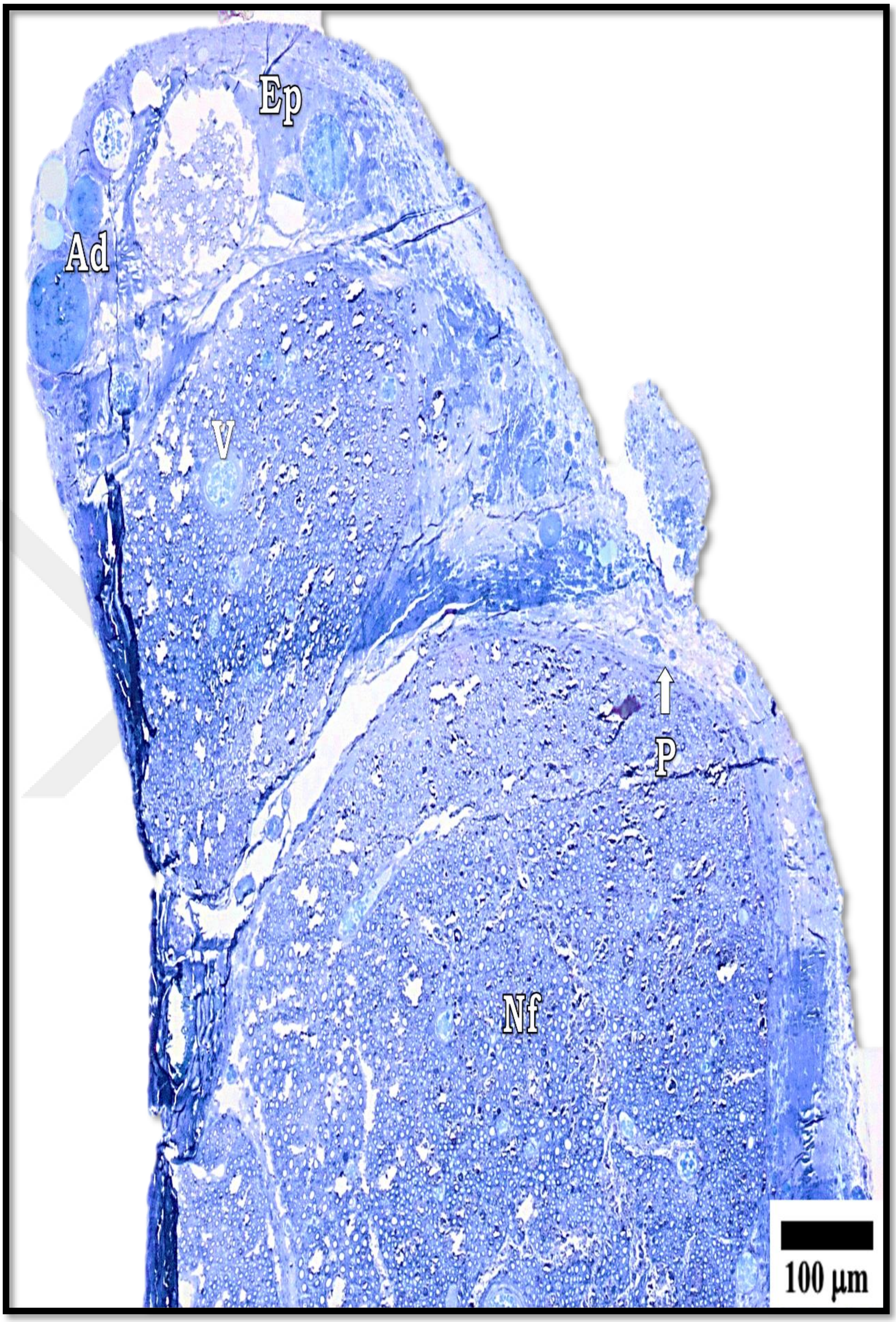


Figure 4.26. A Photomicrograph taken from the sciatic nerve of the Inj group is seen. The general structure of the nerve looks to be affected by pathological changes. An irregular connective tissue of epineurium (Ep) and perineurium (P) around nerve fascicles (Nf) is seen. Adipose cells (Ad) are seen in the connective tissue of the nerve. Blood vessels are also seen (V). Tissue loss is observed in the nerve cross-section; this may occur due to improper tissue processing

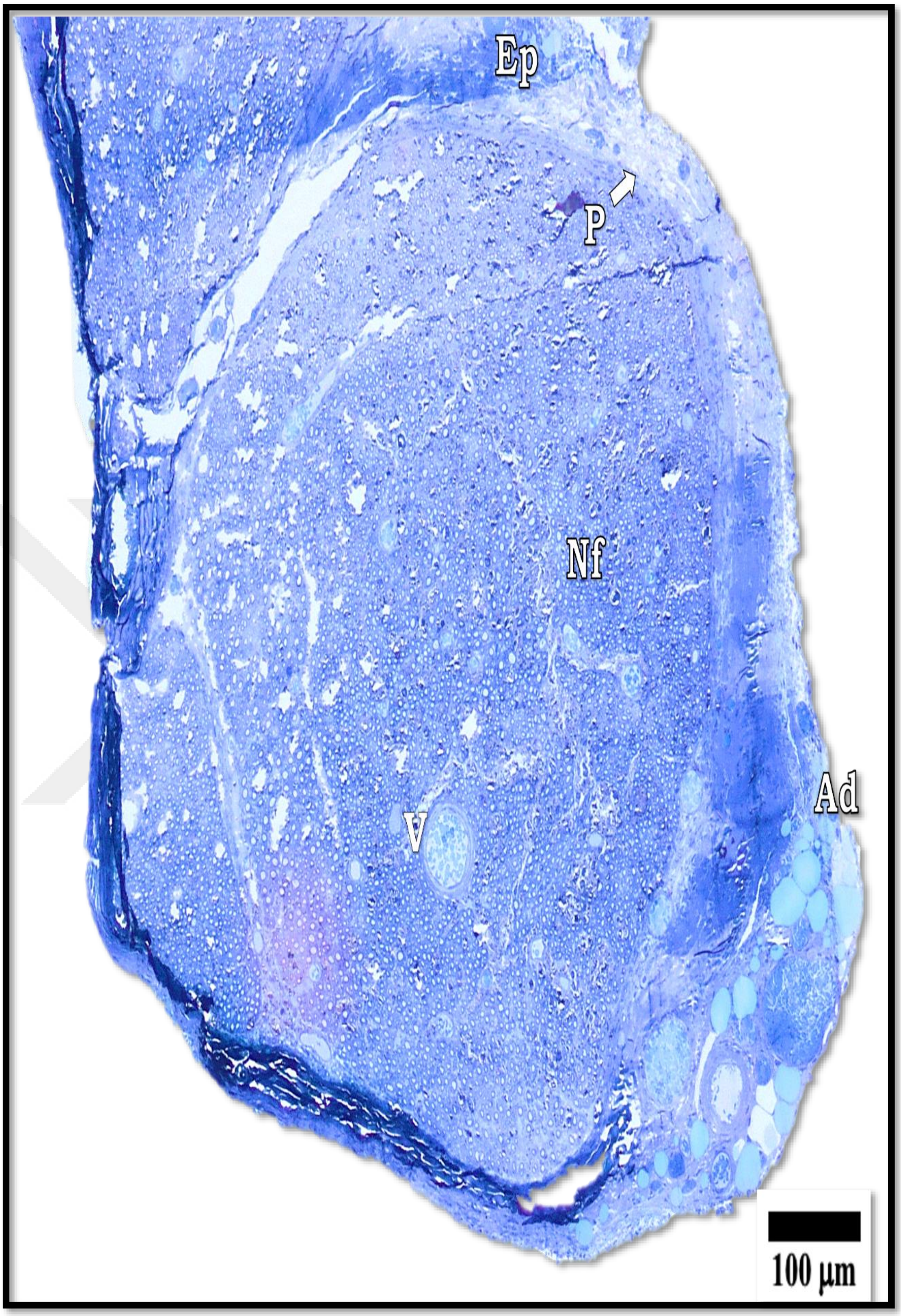


Figure 4.27. A Photomicrograph taken from the sciatic nerve of the Inj group is seen. The general structure of the nerve looks partly normal due to pathological changes. An irregular connective tissue of epineurium (Ep) and perineurium (P) around nerve fascicles (Nf) is seen. Adipose cells (Ad) are seen in the connective tissue of the nerve. A blood vessel in the middle of the nerve fascicles is also seen (V). Tissue loss is observed in the nerve cross-section; this may occur due to improper tissue processing

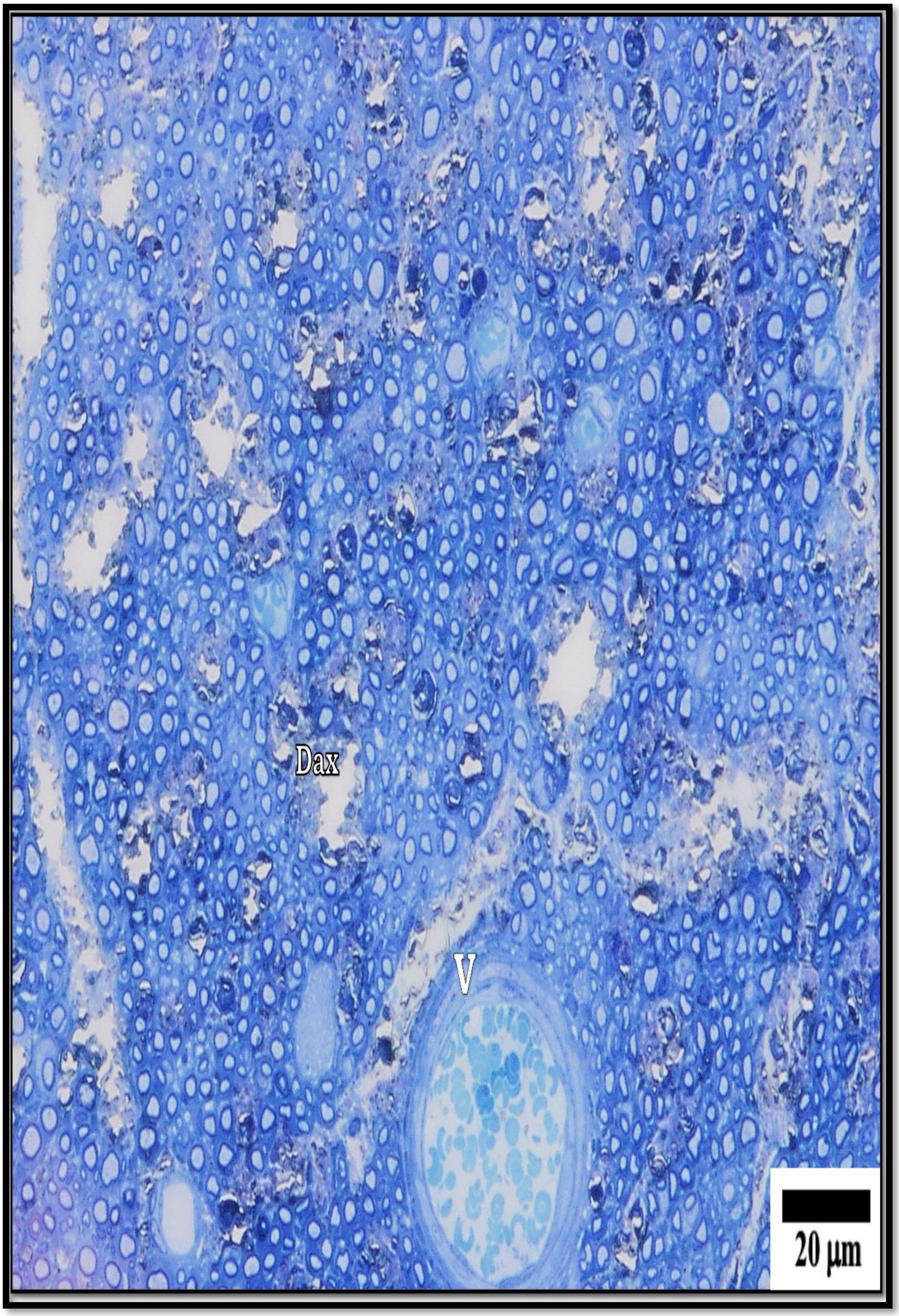


Figure 4.28. A Photomicrograph taken from the sciatic nerve of the Inj group is seen. The structure of the nerve fibers is seen mostly impaired. Degenerated nerve fibers (Dax) are observed in this section. Many myelinated axons (Ax) are seen to have normal structures with small sizes. Irregular spaces are observed in the myelin sheath. The blood vessel (V) in this section looks normal

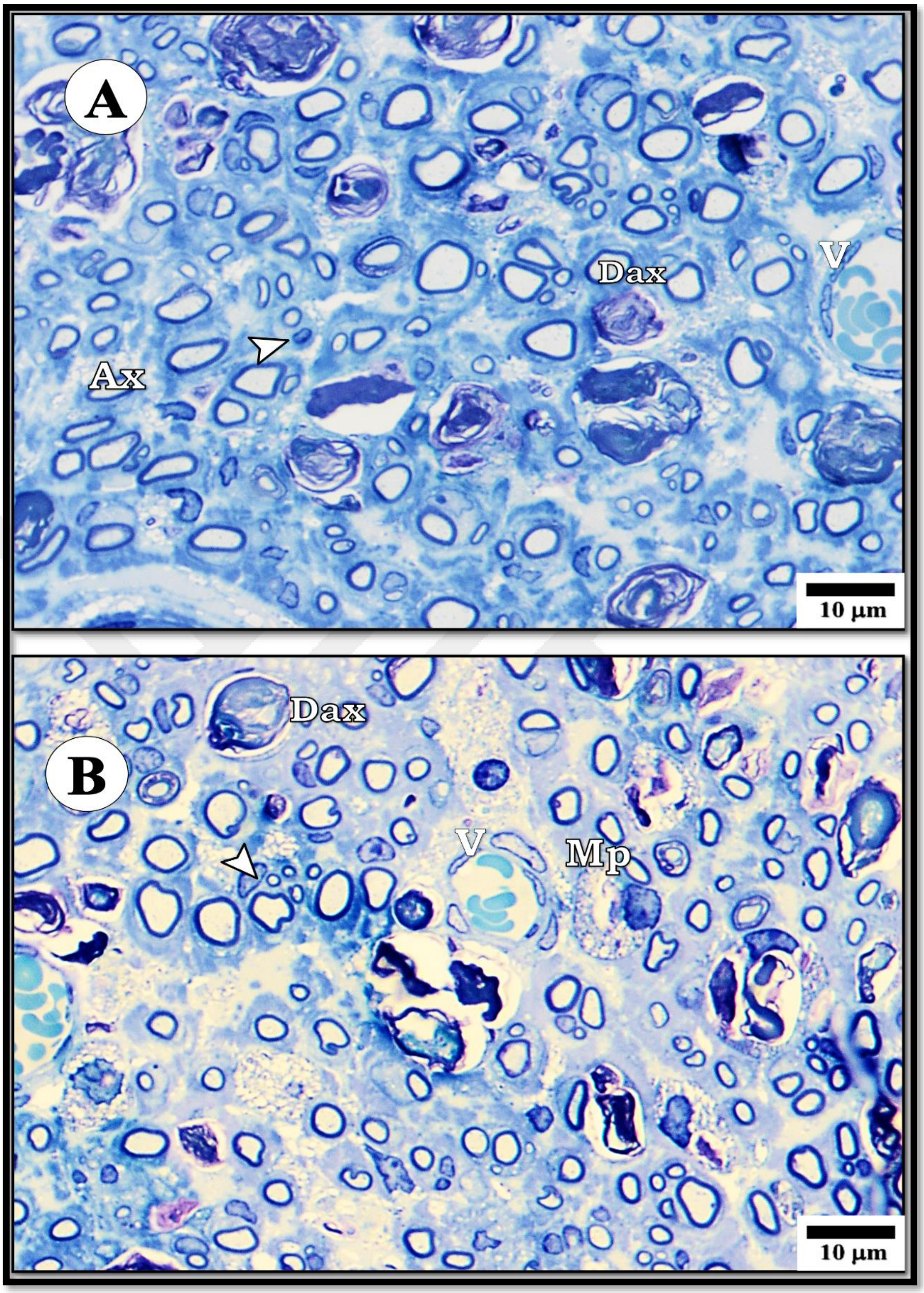


Figure 4.29. (A, B) A photomicrograph taken from the sciatic nerve of the Inj group is seen. The structure of the nerve fibers is seen mostly impaired. Degenerated nerve fibers (Dax) are observed in this section. The border of some myelinated nerve fibers cannot be seen clearly in the cross-section of the nerve. Many myelinated nerve fibers are found to have an abnormal structure. Few myelinated axons (Ax) are seen to have normal structures. Impairment of myelin sheath, myelin disorganisation is observed around many nerve fibers. A high number of Schwann cells (arrow) are observed around the myelinated axons, as well as macrophages (Mp) are observed in this section

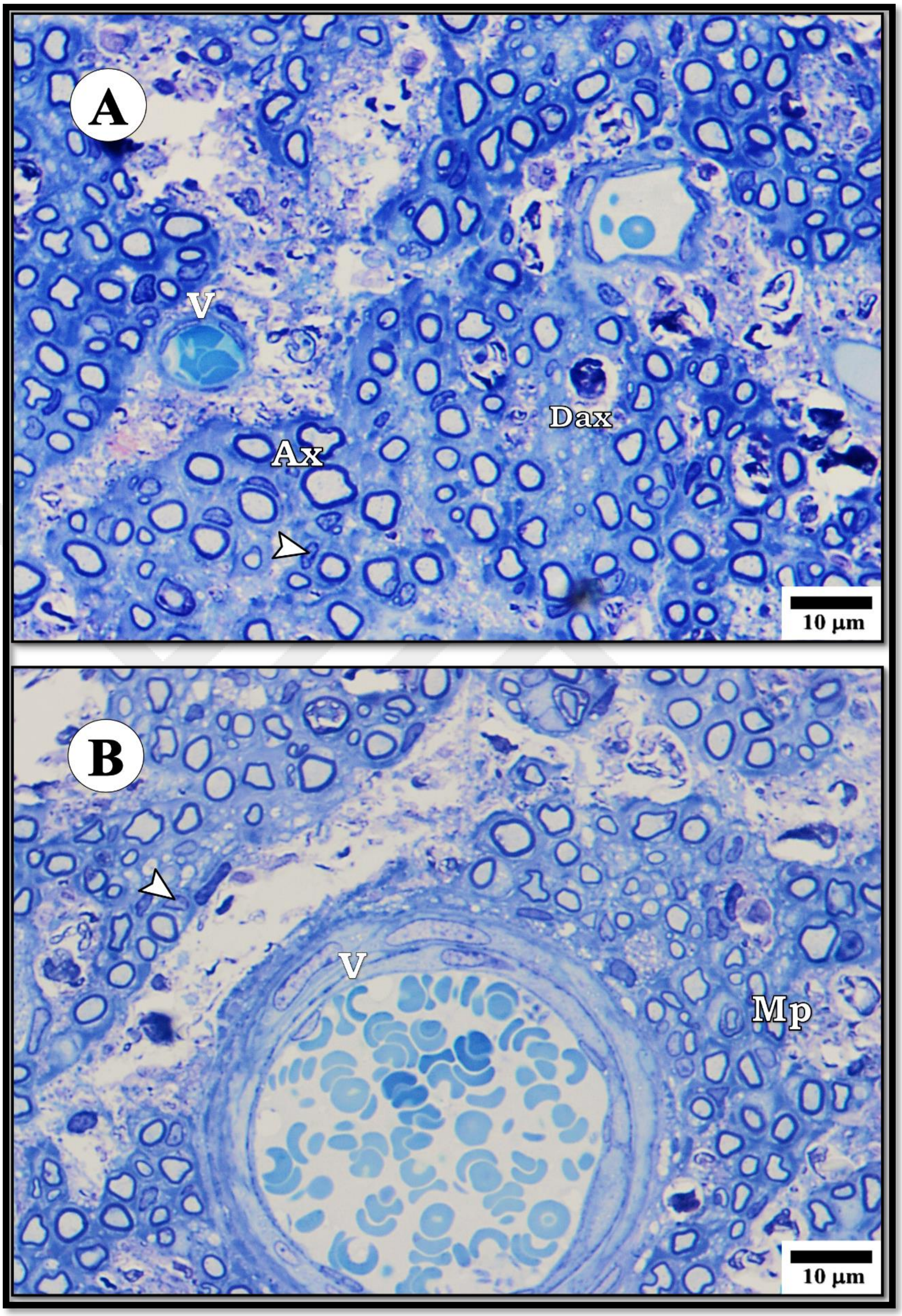


Figure 4.30. (A, B) Images taken from the sciatic nerve of the Inj group are seen. Most of the myelinated nerve fibers are seen as abnormal. A serious disintegration of the myelin sheath was observed. Irregular spaces are observed in the myelin sheath. Healthy myelinated nerve fibers (Ax) are also found surrounded by a thick myelin sheath. The Schwann cells are observed (arrowheads). Degenerated axons (Dax) and macrophages (Mp) is observed in this section

4.1.4.6. Light Microscopic Findings in the Inj+ Dex Group

Semi-thin sections taken from the sciatic nerve of the Inj+ Dex group were observed. The general histology of the nerve partly appeared normal. The connective tissue which enclosed the whole nerve, epineurium, was intact in some parts. The nerve fascicle was surrounded by normal perineurium. The adipose cells around the nerve section were also seen. The heterogeneity of the myelinated axons was observed. In addition, a considerable number of axons were enclosed by a thin myelin sheath. Some of the impaired sheaths were observed. The mast cells were found frequently in the connective tissue of the nerve. The degenerated axons were also seen. The macrophages which engulf the damaged fibres and myelin waste were observed. The blood vessels with a thicker wall and normal blood cells were found (Figures 4.31- 34).

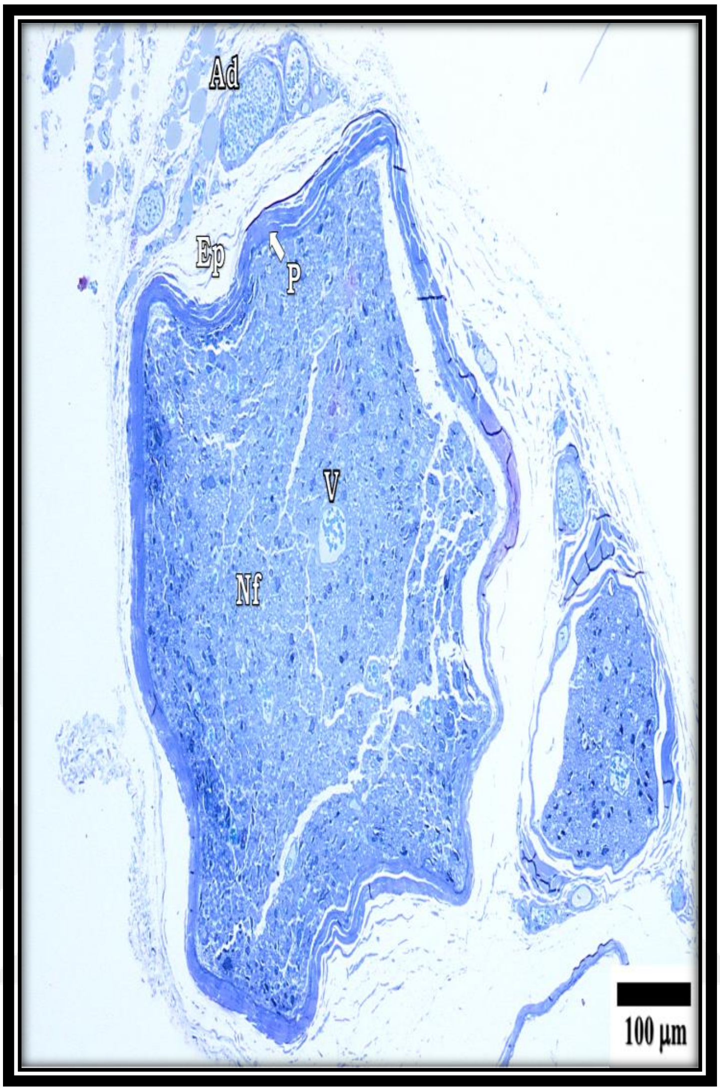


Figure 4.31. A Photomicrograph taken from the sciatic nerve of the Inj+Dex group is seen. The general structure of the nerve looks partly normal. Adipose cells (Ad) are seen in the connective tissue of the nerve. The epineurium (Ep) and perineurium (P) around nerve fascicles (Nf) are seen. A blood vessel in the middle of the nerve fascicles is also seen (V). Tissue loss is observed in the nerve cross-section; this may occur due to improper tissue processing

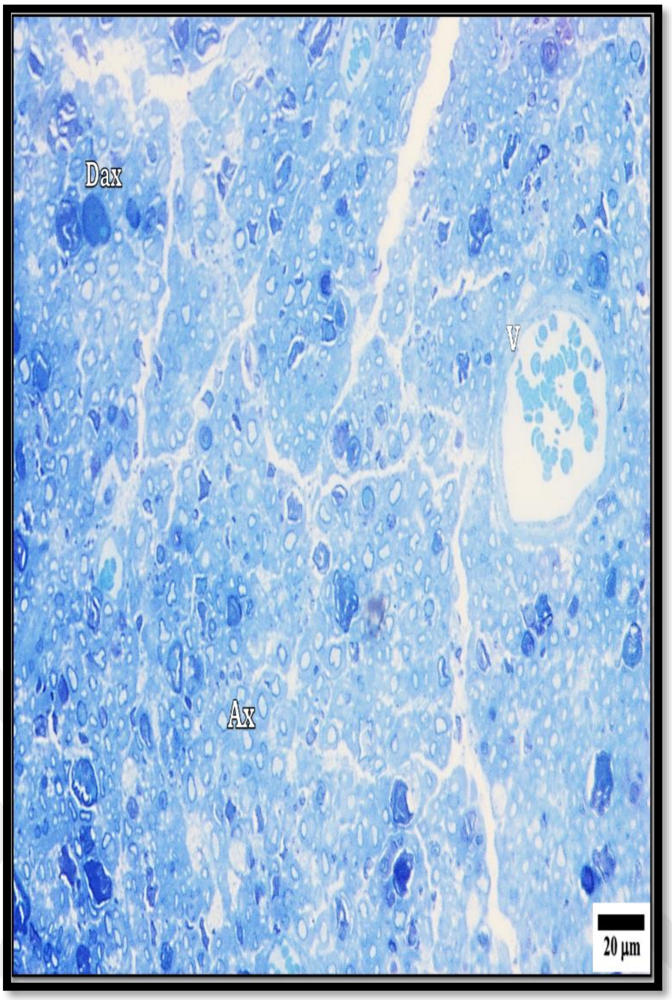


Figure 4.32. A Photomicrograph taken from the sciatic nerve of the Inj+Dex group is seen. The structure of the nerve fibers is seen mostly impaired. Degenerated nerve fibers (Dax) and macrophages (Mp) are observed in this section. Many myelinated axons (Ax) are seen to have abnormal structures. Irregular spaces are observed in the myelin sheath. The blood vessel (V) in this section looks normal

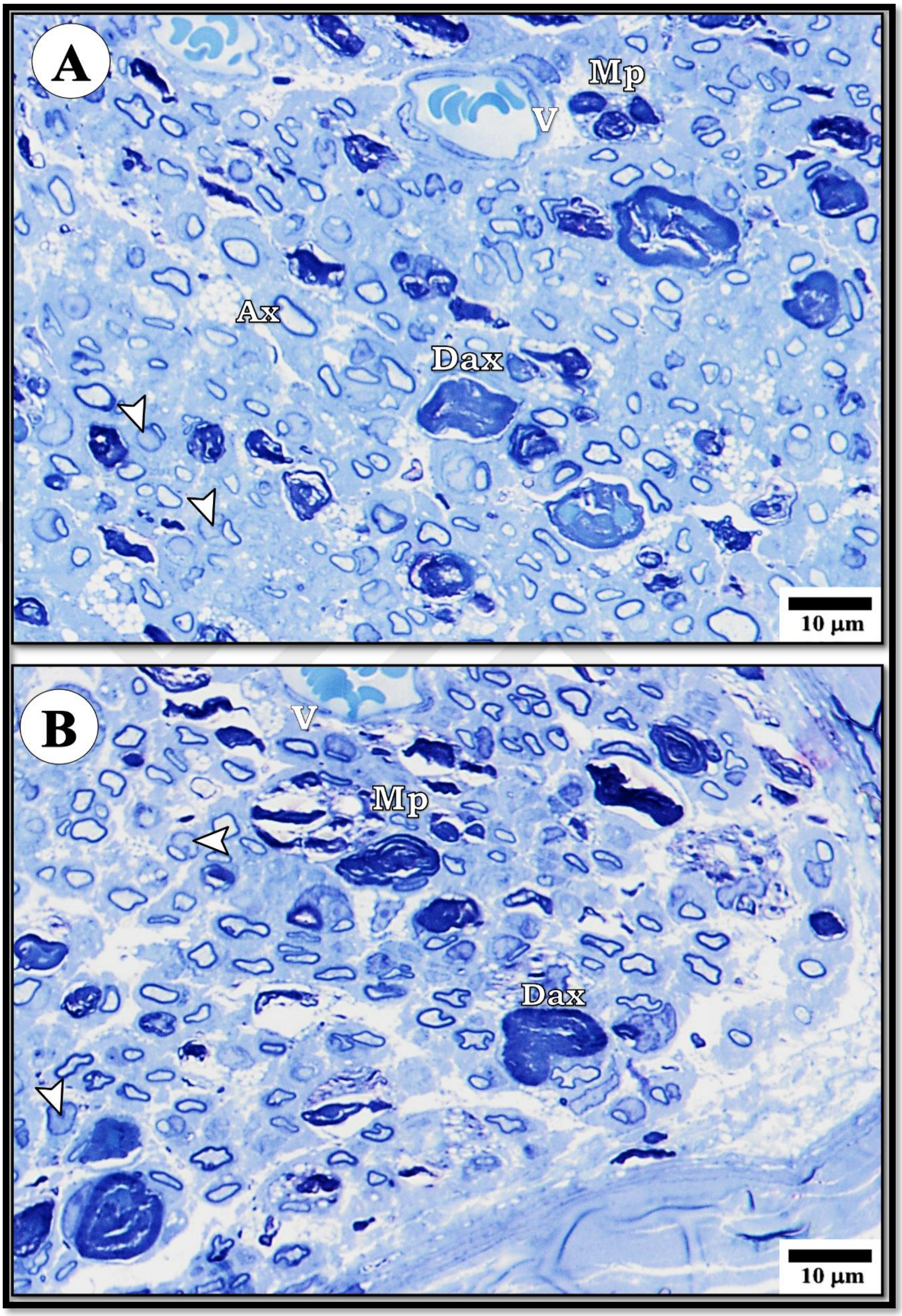


Figure 4.33. (A, B) Images taken from the sciatic nerve of the Inj+Dex group are observed. A few myelinated nerve fibers (Ax) are seen as normal. An increased number of Schwann cells are found around myelinated nerve fibers indicated by (arrowhead). Myelin sheath deterioration is common in the injured nerve fibers. A seriously impaired myelinated axon and myelin sheath are observed. Degenerated axons (Dax) and macrophages (Mp) are observed in this section. The blood vessel is also seen (V)

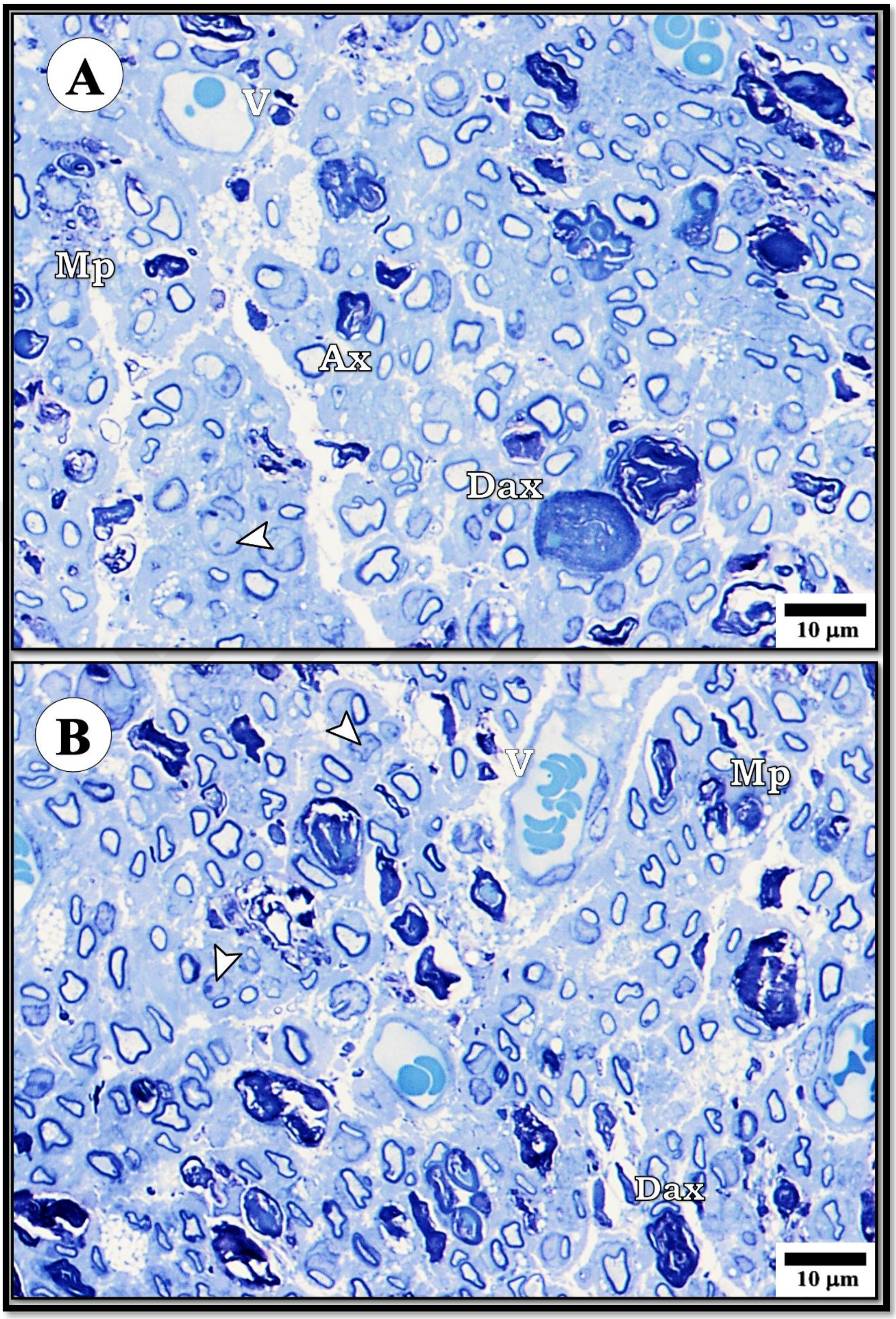


Figure 4.34. (A, B) Images taken from the sciatic nerve of the Inj+Dex group are observed. A few myelinated nerve fibers (Ax) are seen as normal. An increased number of Schwann cells are found around myelinated nerve fibers indicated by an arrowhead. Myelin sheath deterioration is common in the injured nerve fibers. A seriously impaired myelinated axon and myelin sheath are observed. Degenerated axons (Dax) and macrophages (Mp) are observed in this section. The blood vessel is also seen (V)

4.1.4.7. Light Microscopic Findings in the Inj + Bet Group

Semi-thin sections taken from the sciatic nerve of the Inj+ Bet group were examined by the LM. The histological view of the nerve was partially healthy. The intact epineurium surrounds the nerve fascicles, and normal perineurium delimits the nerve bundles were observed. Adipose cells were also seen healthy. The degenerative features were observed; the small-sized axons myelinated by thin sheath were major. The degenerated fibers were also seen. The mast cells were found frequently in the connective tissue of the nerve. The Schwann cells nuclei are observed close to the newly formed small axon due to their function.

Furthermore, a huge amount of the macrophages was found to phagocytose the myelin debris and degenerated fibers. A thick myelin coat ensheathed the protected myelinated axons with some impairments of the myelin sheath in a few axons. The normal blood vessels with blood cells were abundant as one of the features following crush surgery (Figures 4.35- 38).

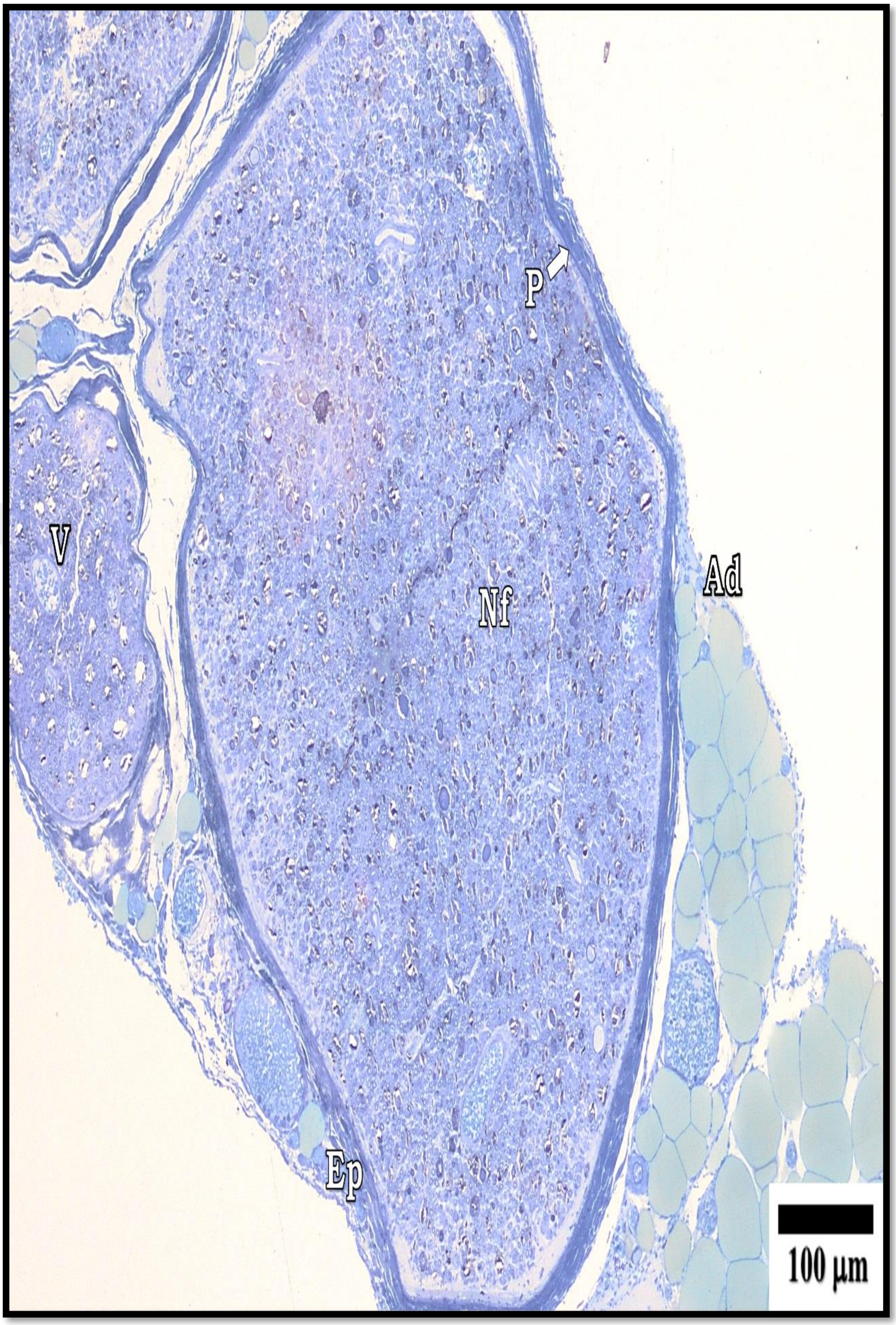


Figure 4.35. A Photomicrograph taken from the sciatic nerve of the Inj+Bet group is seen. The general structure of the nerve looks partly normal. The connective tissue of epineurium (Ep) and perineurium (P) around nerve fascicles (Nf) are seen. Adipose cells (Ad) are seen in the connective tissue of the nerve. A blood vessel in the middle of the nerve fascicles is also seen (V). Tissue loss is observed in the nerve cross-section; this may occur due to improper tissue processing

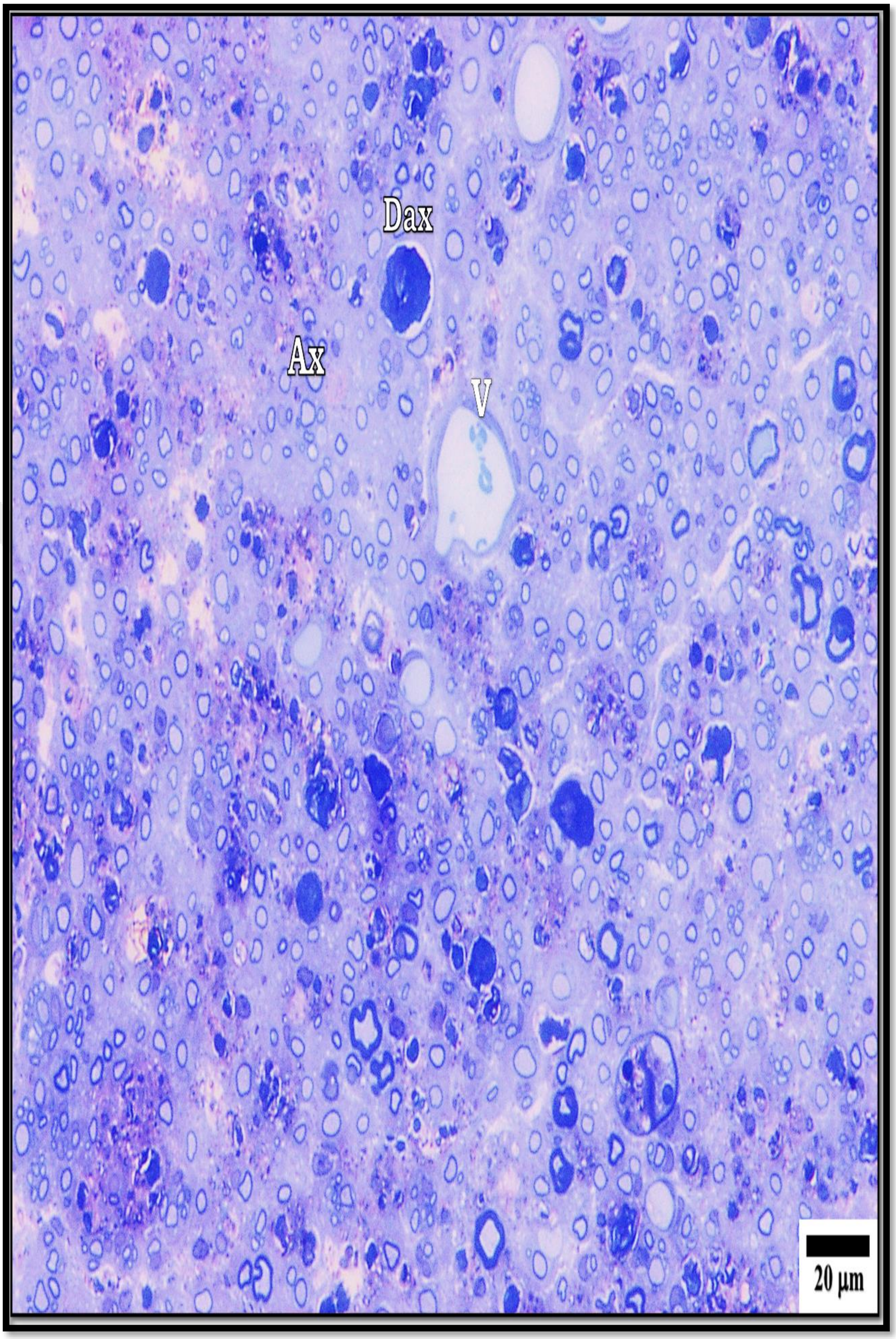


Figure 4.36. A Photomicrograph taken from the sciatic nerve of the Inj+Bet group is seen. The structure of the nerve fibers is seen mostly impaired. Degenerated nerve fibers (Dax) is observed in this section. Many myelinated axons (Ax) are seen to have normal structures. Irregular spaces are observed in the myelin sheath. The blood vessel (V) in this section looks normal

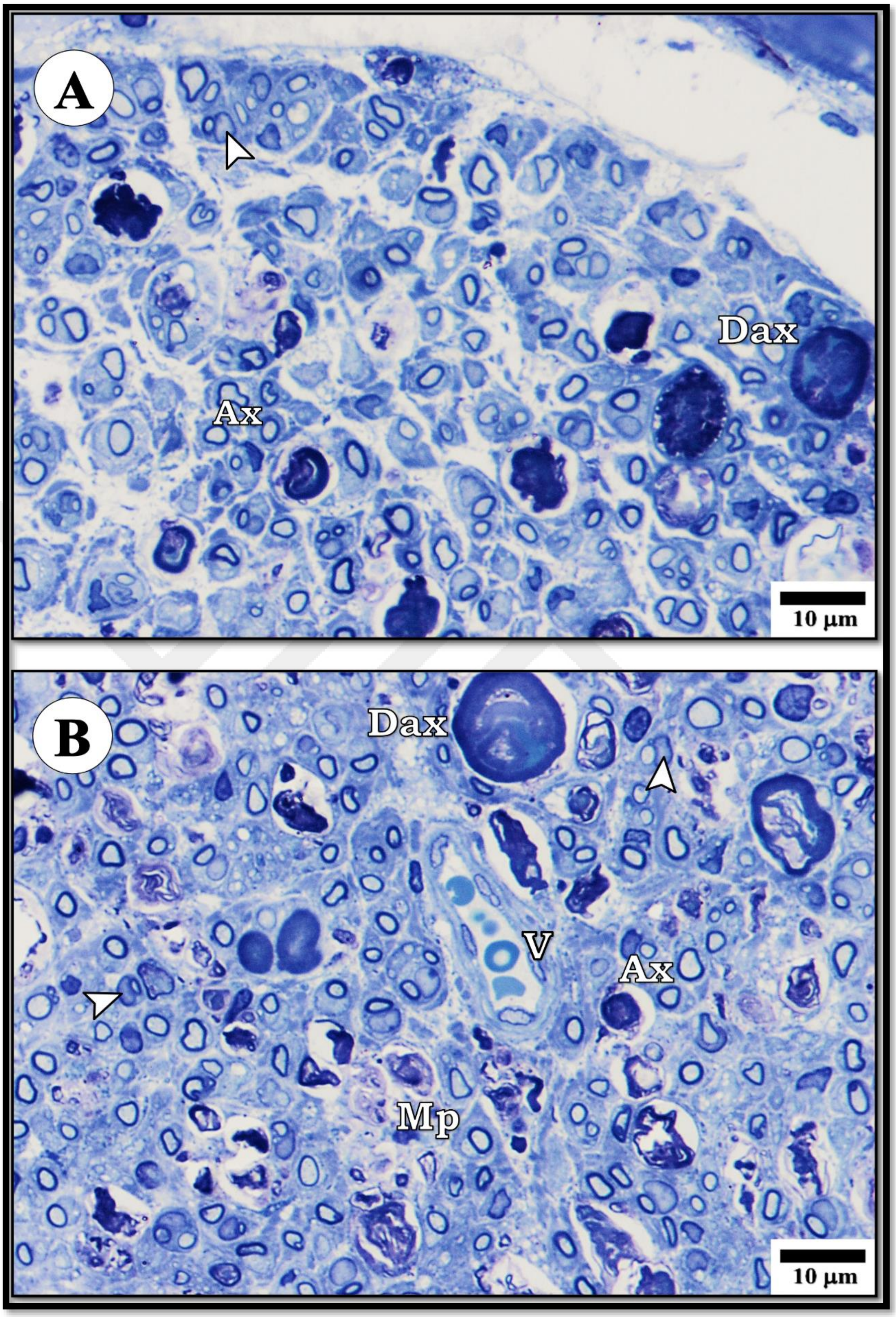


Figure 4.37. (A, B) Images taken from the sciatic nerve of the Inj+Bet group are observed. A few myelinated nerve fibers (Ax) are seen as normal. An increased number of Schwann cells are found around myelinated nerve fibers indicated by (an arrowhead). Myelin sheath deterioration is common in the injured nerve fibers. A seriously impaired myelinated axon and myelin sheath are observed. Degenerated axons (Dax) and macrophages (Mp) are observed in this section. The blood vessel is also seen (V)

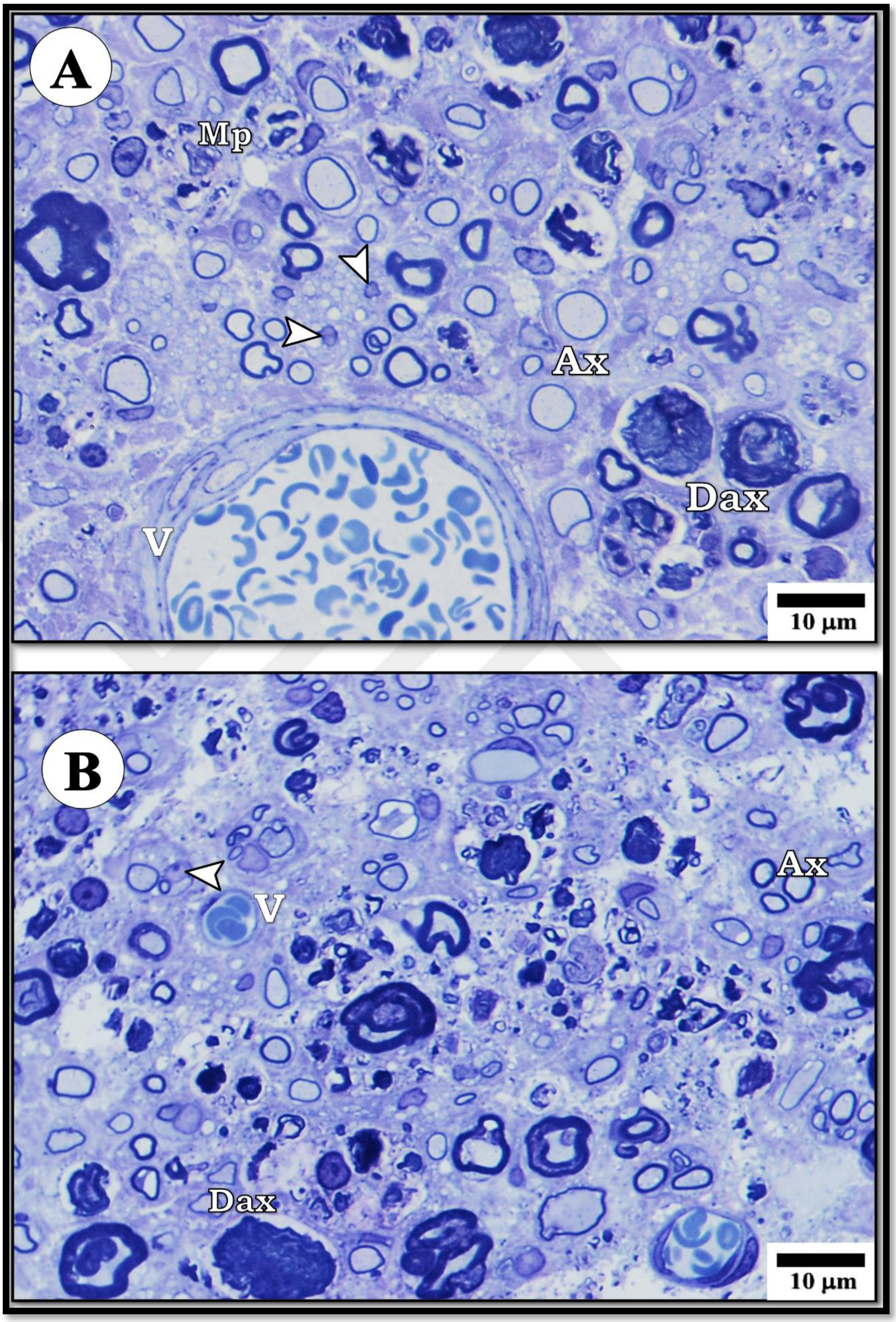


Figure 4.38. (A, B) Images taken from the sciatic nerve of the Inj+Bet group are observed. A few myelinated nerve fibers (Ax) are seen as normal. An increased number of Schwann cells are found around myelinated nerve fibers indicated by an arrowhead. Myelin sheath deterioration is common in the injured nerve fibers. A seriously impaired myelinated axon and myelin sheath are observed. Degenerated axons (Dax) and macrophages (Mp) are observed in this section. A blood vessel is also seen (V)

4.1.4.8. Light Microscopic Findings in the Inj+Mps Group

Semi-thin sections were taken from the sciatic nerve of the Inj+ Mps group for LM examination. The general histological appearance of the neural tissue is partly protected. The connective tissue of epineurium and perineurium around nerve fascicles are seen regularly in appearance. A thin myelin sheath delimited the degenerated fibers, and small-sized myelinated axons were seen. The Schwann cells were observed closed to nerve fibers due to their function. A remarkable number of the macrophages that remove the degenerated fibres, myelin debris, and thicker blood vessels with normal blood cells were also observed. The mast cells were found frequently in the connective tissue of the nerve. Some of the regenerative observations, such as protected thicker myelinated axons, were found due to the positive effect of treatment Mps in terms of axon number and myelin thickness. Additionally, unmyelinated axons were also shown. On the other hand, some impaired myelin sheaths were also seen (Figures 4.39- 44).

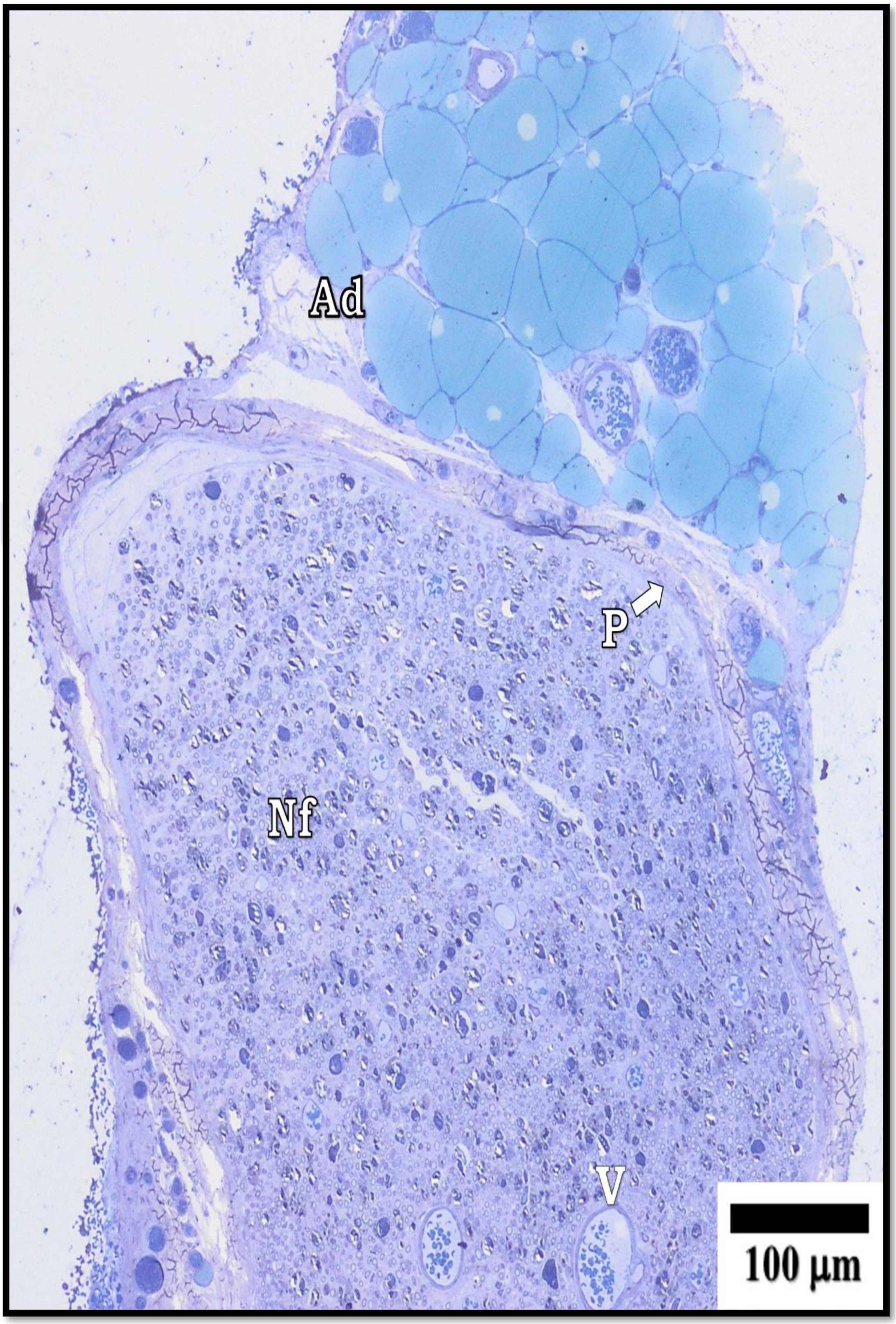


Figure 4. 39. A Photomicrograph taken from the sciatic nerve of the Inj+Mps group is seen. The general structure of the nerve looks partly normal. An irregular connective tissue of epineurium and perineurium (P) around nerve fascicles (Nf) is seen. Adipose cells (Ad) are seen in the connective tissue of the nerve. A blood vessel in the middle of the nerve fascicles is also seen (V). Tissue loss is observed in the nerve cross-section; this may occur due to improper tissue processing

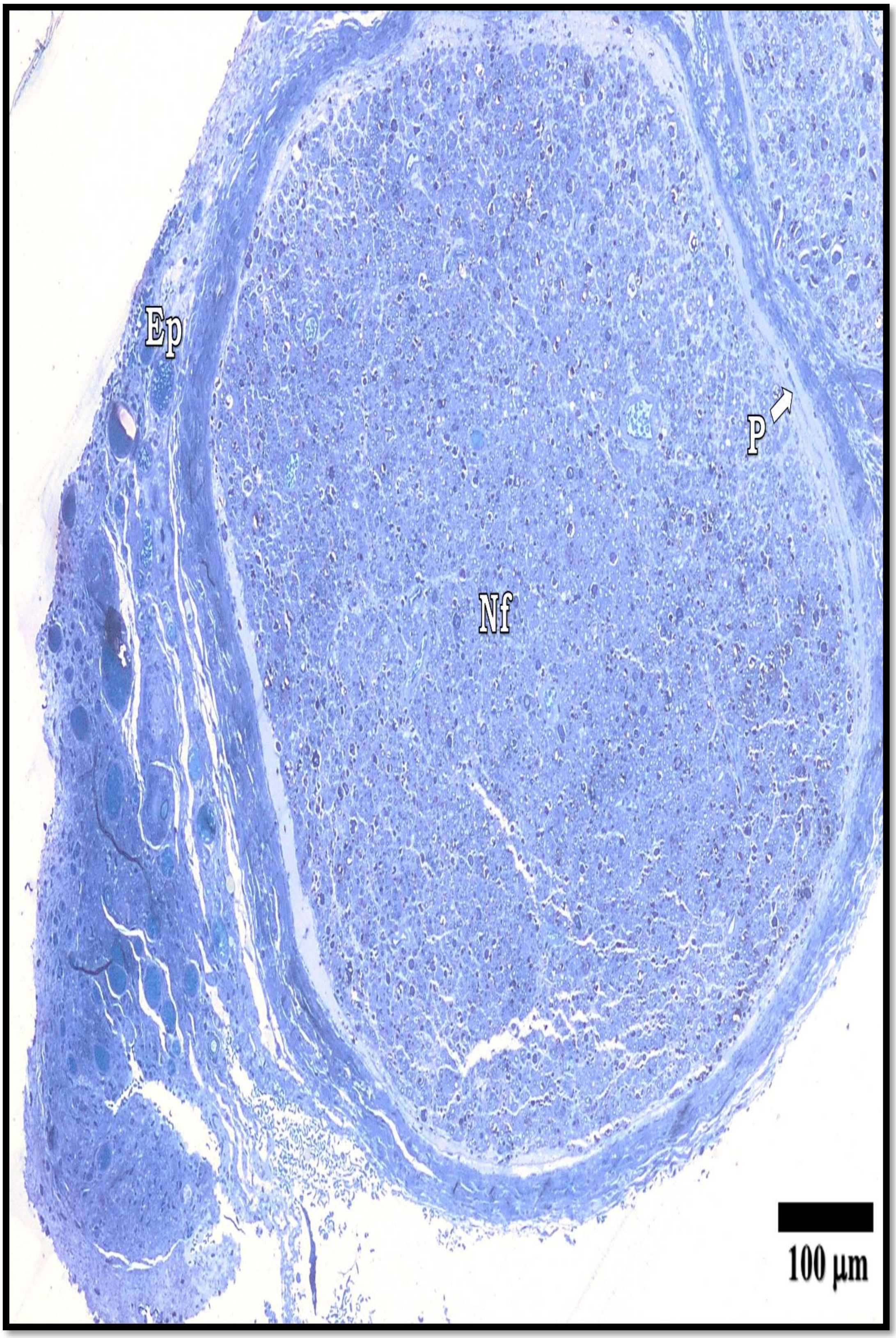


Figure 4.40. A Photomicrograph taken from the sciatic nerve of the Inj+Mps group is seen. The general structure of the nerve looks partly normal. The connective tissue of epineurium (Ep) and perineurium (P) around nerve fascicles (Nf) are seen regularly. Adipose cells (Ad) are seen in the connective tissue of the nerve. Tissue loss is observed in the nerve cross-section; this may occur due to improper tissue processing

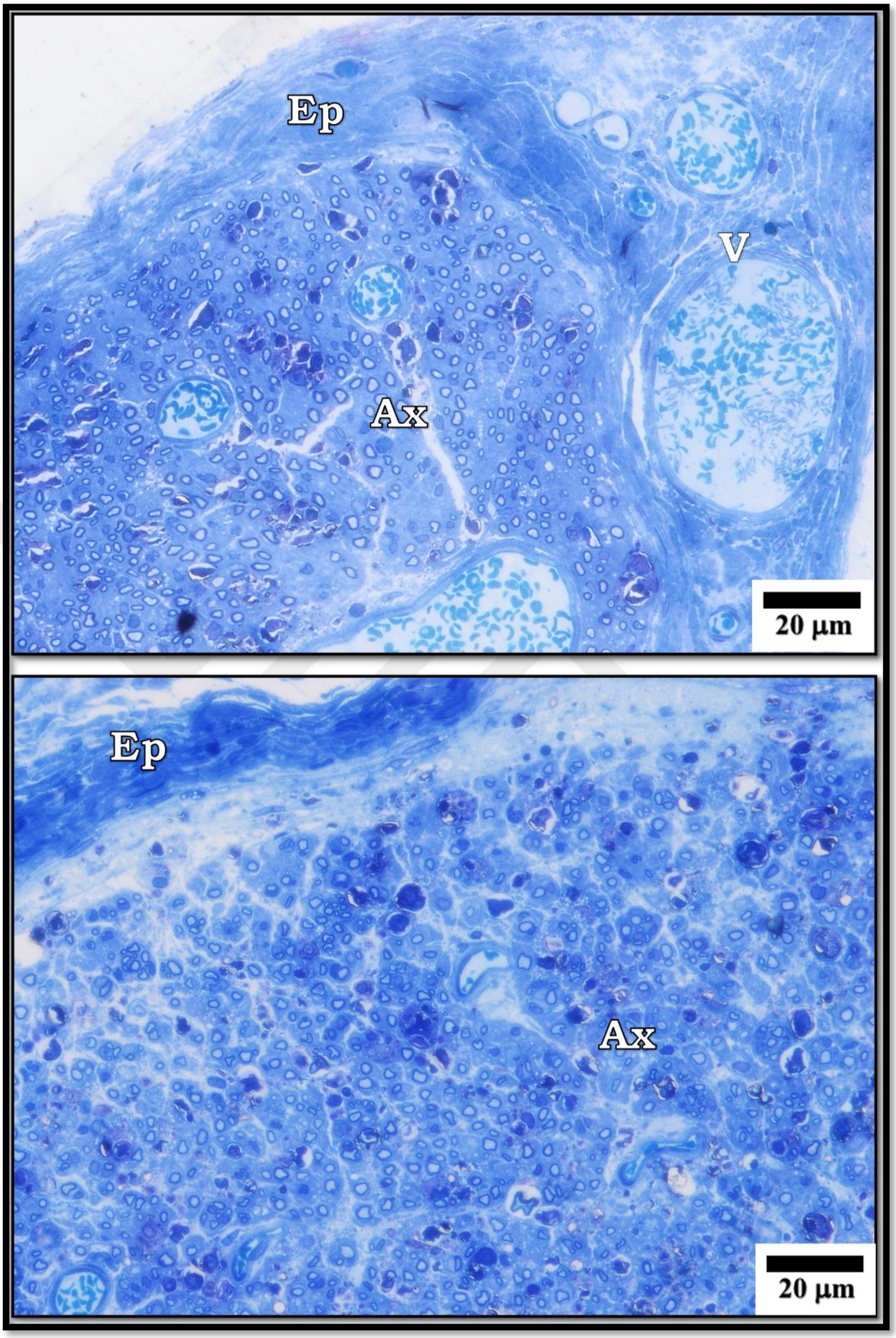


Figure 4.41. (A, B) A Photomicrograph taken from the sciatic nerve of the Inj+Mps group is seen. The structure of the nerve fibers is seen as partly impaired. Degenerated nerve fibers (Dax) are observed in this section. Many myelinated axons (Ax) are seen to have normal structures. Irregular spaces are observed in the myelin sheath. The blood vessel (V) in this section looks normal. The epineurium (Ep) is also seen in this section

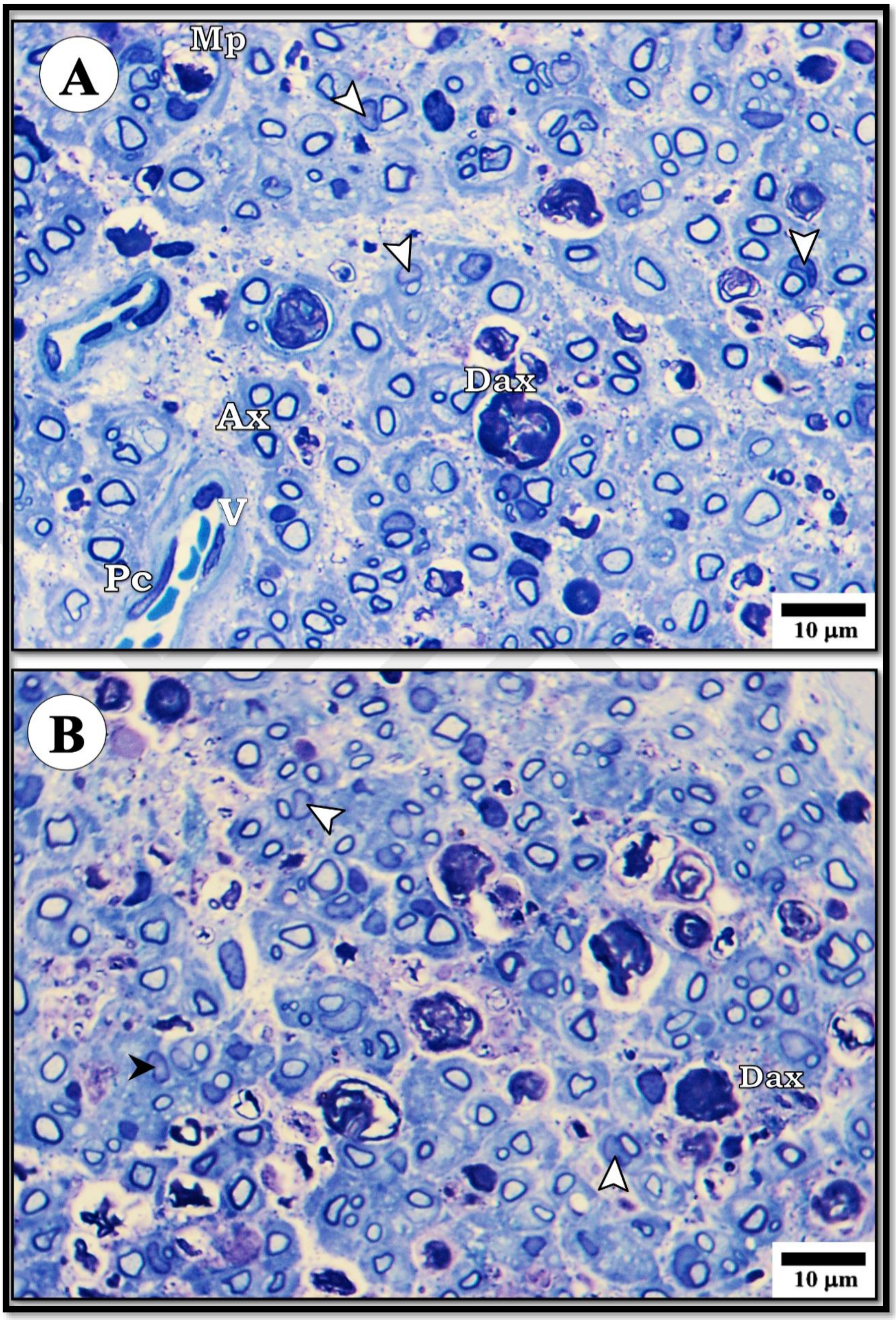


Figure 4.42. (A, B) Images taken from the sciatic nerve of the Inj+Mps group are observed. A few myelinated nerve fibers (Ax) are seen as normal. An increased number of Schwann cells are found around myelinated nerve fibers indicated by an arrowhead. Myelin sheath deterioration is common in the injured nerve fibers. A seriously impaired myelinated axon and myelin sheath are observed. Degenerated axons (Dax) and macrophages (Mp) are observed in this section. Blood vessels (V) and pericytes (Pc) are also seen

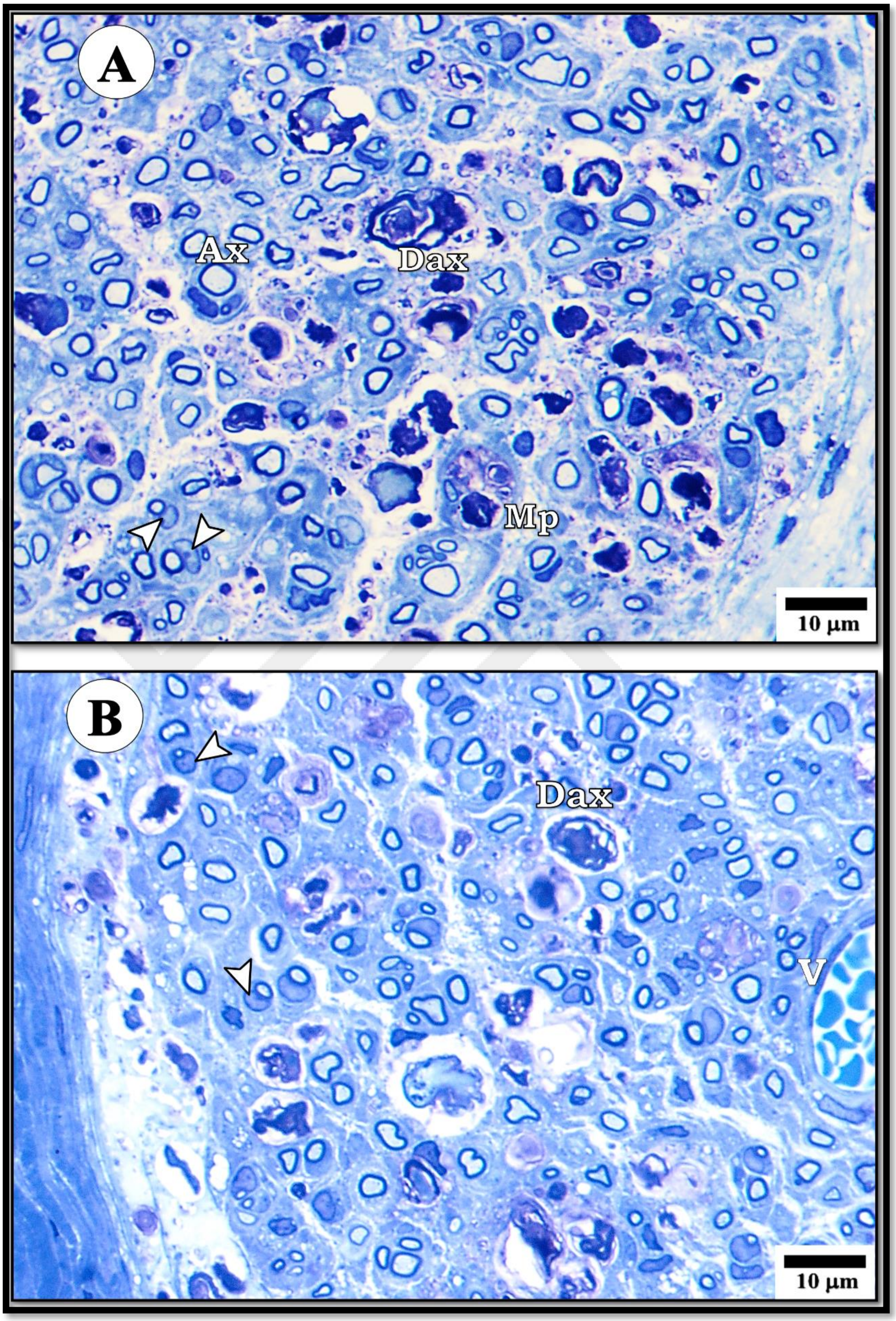


Figure 4.43. (A, B) Images taken from the sciatic nerve of the Inj+Mps group are observed. A few myelinated nerve fibers (Ax) are seen as normal. An increased number of Schwann cells are found around myelinated nerve fibers indicated by an arrowhead. Myelin sheath deterioration is common in the injured nerve fibers. A seriously impaired myelinated axon and myelin sheath are observed. Degenerated axons (Dax) and macrophages (Mp) are observed in this section. A blood vessel is also seen (V)

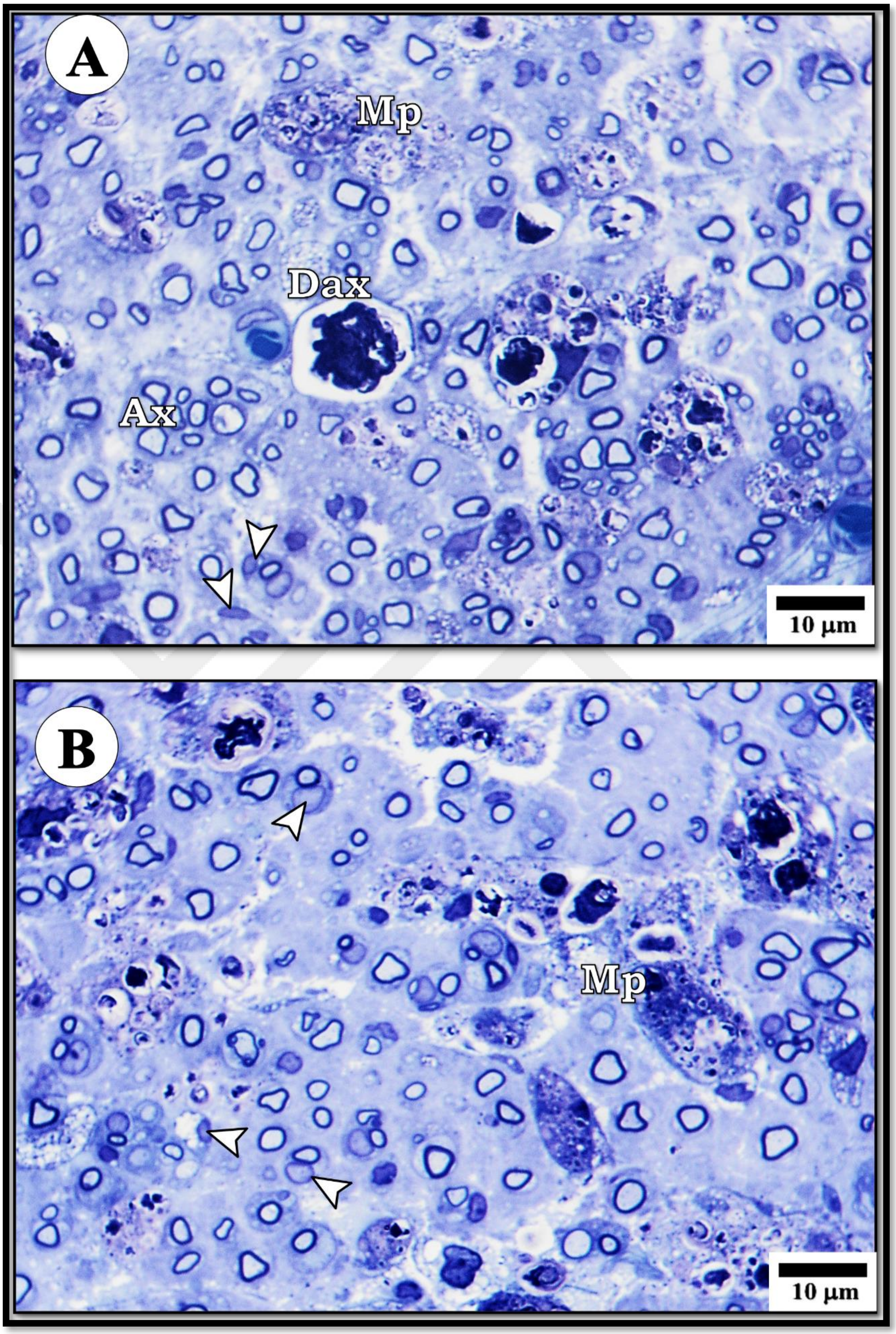


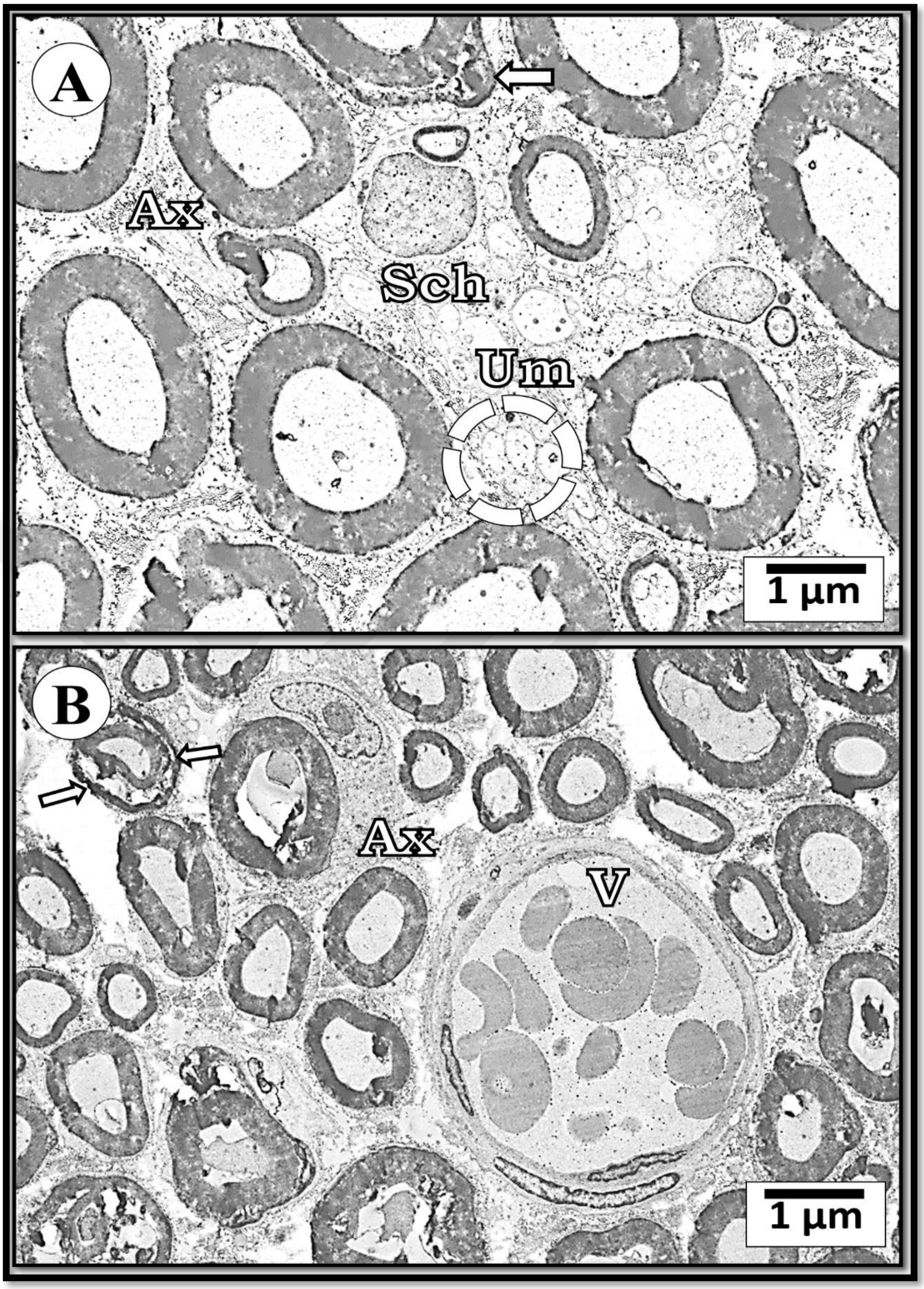
Figure 4.44. (A, B) Images taken from the sciatic nerve of the INJ Mps group are observed. A few myelinated nerve fibers (Ax) are seen as normal. An increased number of Schwann cells are found around myelinated nerve fibers indicated by an arrowhead. Myelin sheath deterioration is common in the injured nerve fibers. A seriously impaired myelinated axon and myelin sheath are observed. Degenerated axons (Dax) and macrophages (Mp) are observed in this section

4.1.5. Electron Microscopic Findings

Thin cross-sections taken from the sciatic nerve were stained with uranyl acetate and lead citrate and evaluated by transmission electron microscope (TEM). The obtained results are revealed below

4.1.5.1. Electron Microscopic Findings in the Cont Group

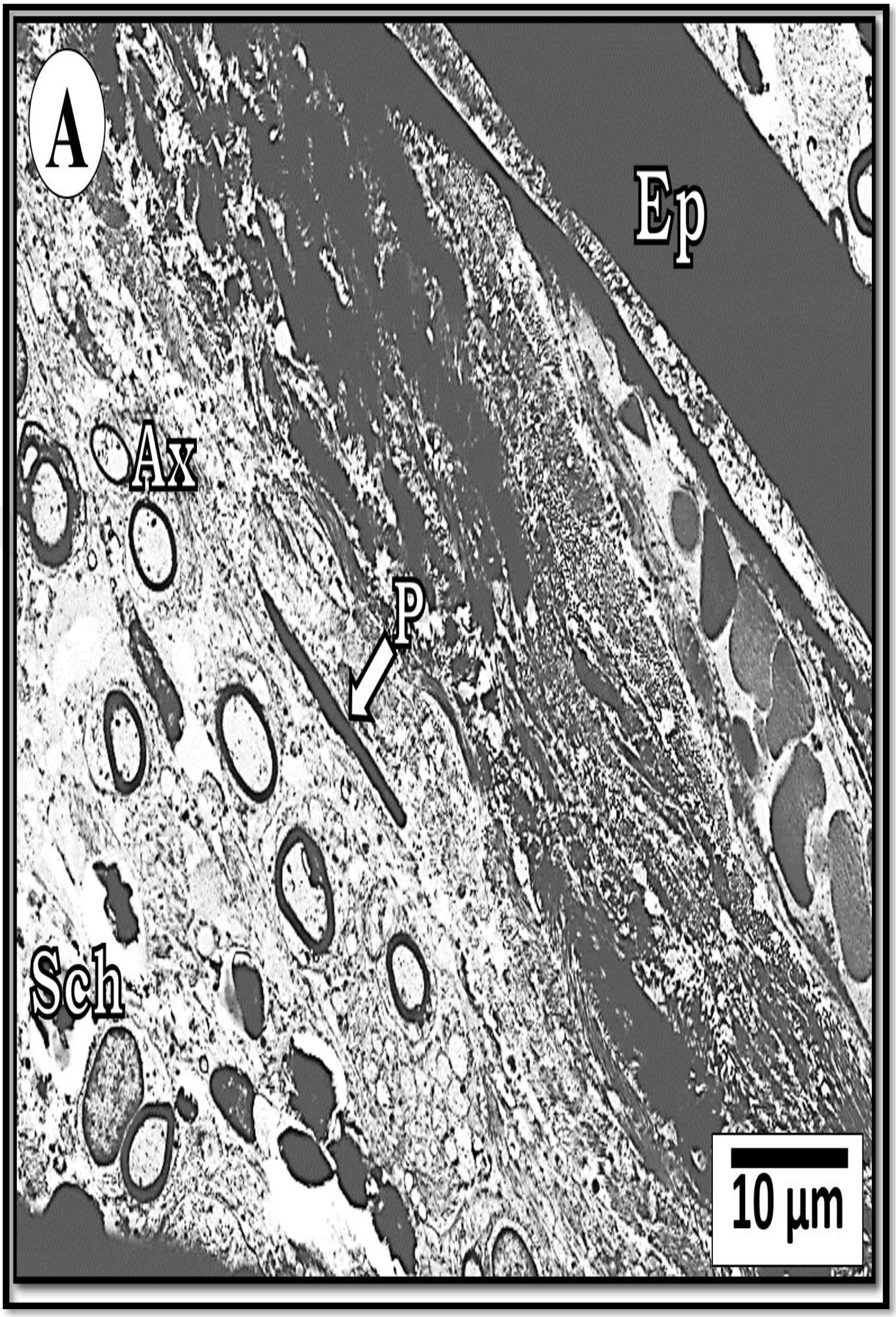
The thin cross-sections taken from the sciatic nerve of the Cont group was evaluated by the TEM. The general structural view of the neural tissue looks healthy. Endoneurial collagen fibril can be found between the different types of nerve fibers. Aggregations of healthy unmyelinated nerve fibres were found with a well-defined border of each unmyelinated axon was detected. Myelinated axons in the sciatic nerve cross-section were observed with normal structures. The size heterogeneity of myelin sheath was seen around the different sizes of myelinated axons. Some physical impairment of myelin sheath; vacuolization was seen, and physiological impaired nerve fibers; Schmidt-Lanterman cleft was also found. In some sections, blood vessels with mature walls and normal blood cells were also observed (Figure 4.45).



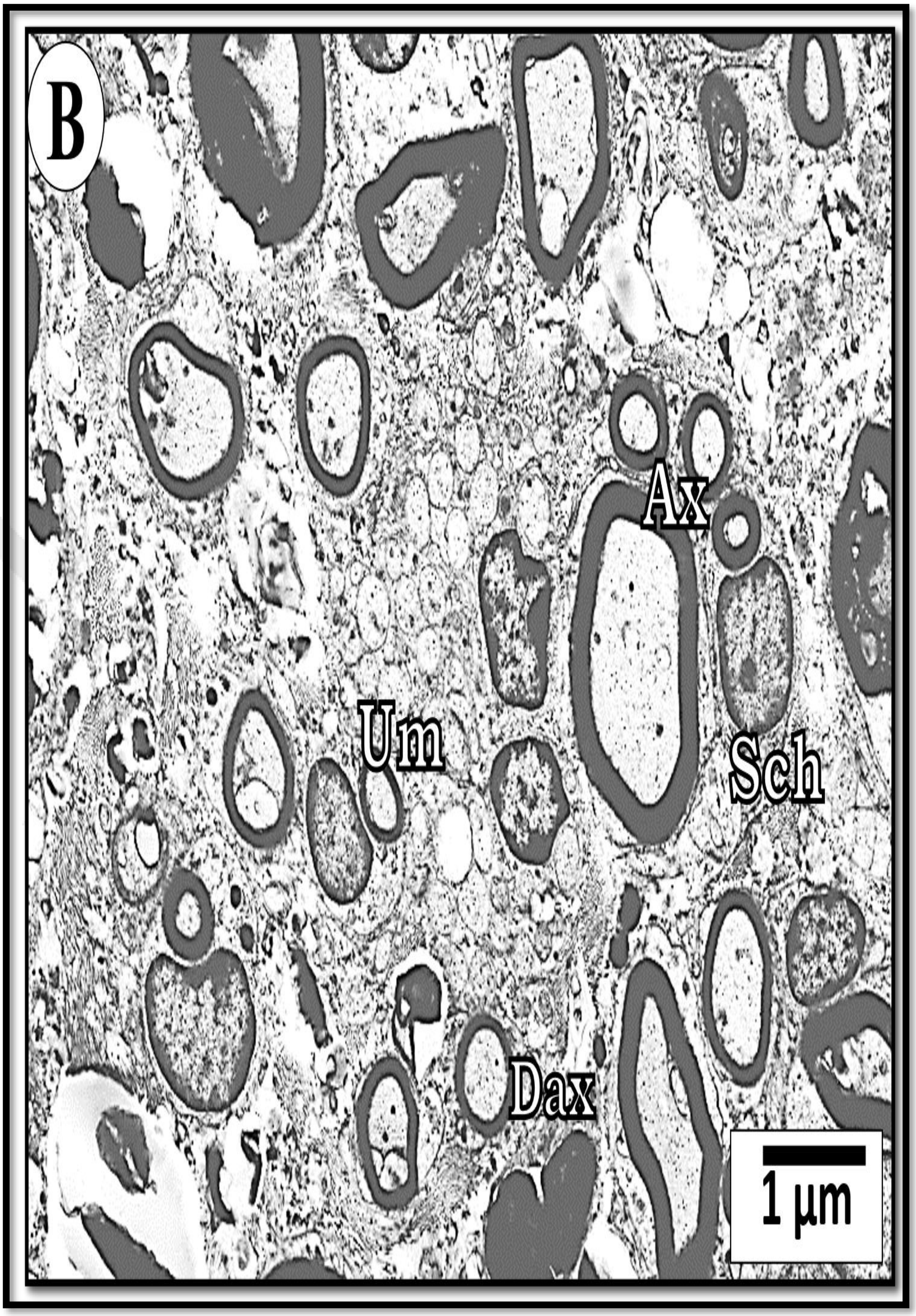
Figures 4.45. (A, B) TEM images of the sciatic nerve thin cross-section are belonged to the Cont group were seen. The general structural view of the nerve looks healthy. Aggregations of healthy unmyelinated nerve fibres (Um) were found with well-defined borders. The myelinated axons (Ax) were observed as normal structures. The size heterogeneity of myelin sheath was seen around the different sizes of myelinated axons. (Sch) indicating to Schwann cell nuclei. Some physical impairment of myelin sheath, vacuolization (arrow), and physiological impaired nerve fibres; Schmidt-Lanterman cleft (double arrows) was also found. The (V) indicating to a blood vessel with a mature wall and normal blood cell

4.1.5.2. Electron Microscopic Findings in the Inj Group

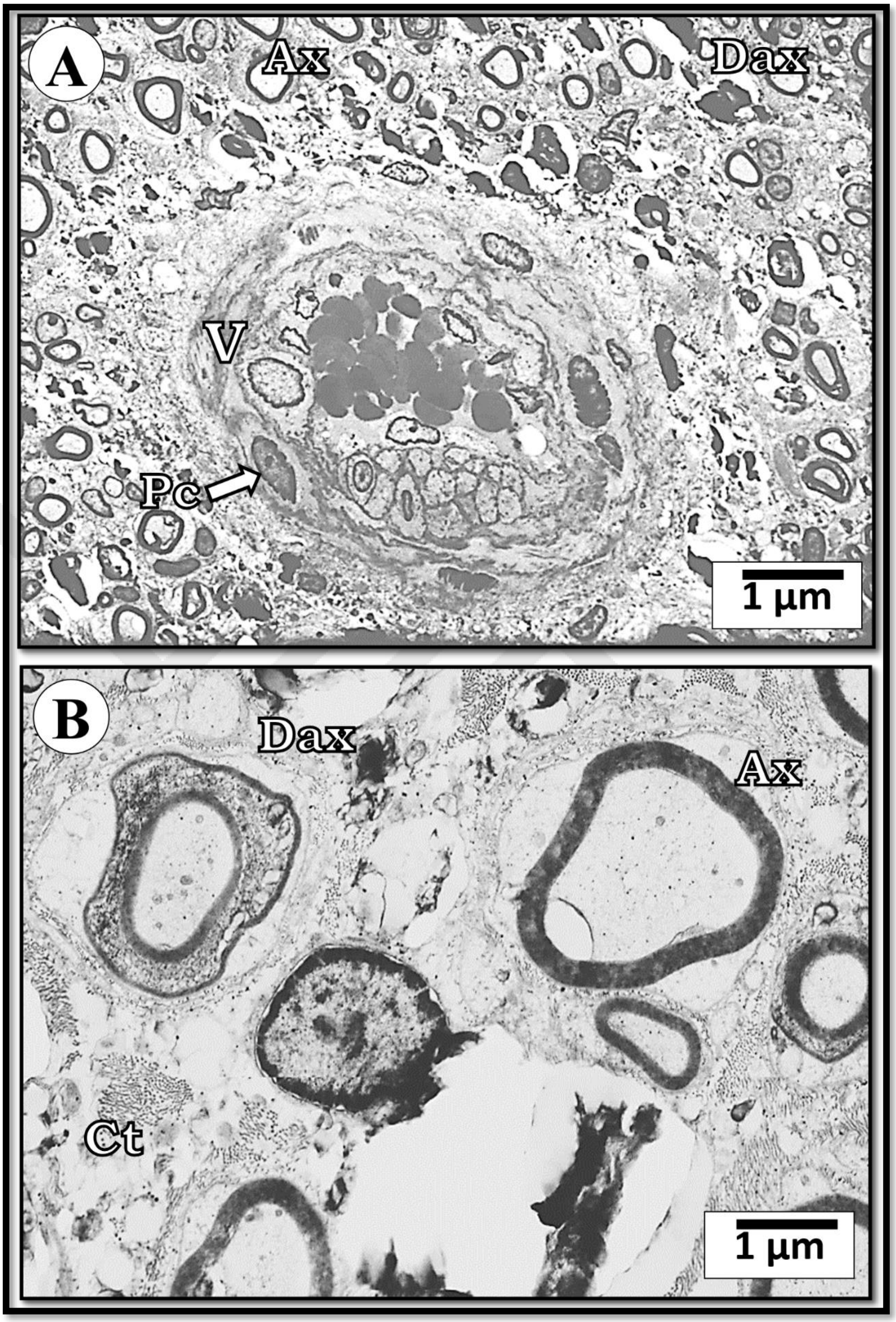
The thin cross-sections taken from the sciatic nerve of the Inj group was examined by the TEM. The histological nerve structure was seriously damaged. Perineurium around the nerve bundles is seen partly normal, with an obvious flattened nucleus. Many degenerated axons with unclear myelin sheaths were seen, an increased number of macrophages that are clean myelin debris from the injured area was found, and a remarkable nerve integrity loss was observed; these are the significant features of this group. At the site of the injury, macrophages with distended cytoplasm (foamy appearance) were seen. It was observed that the amount of connective tissue and its cells were obviously enlarged. The endoneurium around and between the myelinated axons was observed healthy. The numbers of myelinated axons were intensely increased. We observed the small size of myelinated axons and thin myelin sheath; these features are common features following the performing of crush injury. The normal myelin sheath was found in a few nerve fibres. The Schwann cells nuclei were seen close to the myelinated axons. The unmyelinated axons clusters with a poorly defined border were also noticed. Some blood cells were found in the lumen of the blood vessels with premature endothelial cells, and the pericyte was also seen (Figure 4.46-48).



Figures 4.46. (A) Different magnifications of TEM images of the sciatic nerve thin cross-section are belonged to the Inj group were seen. The histological nerve structure was seriously affected. Perineurium (P) around the nerve bundles with a flattened nucleus and epineurium (Ep) that enclose the nerve were seen normal. Some protected axons with thick sheath (Ax) were found. The unmyelinated axons clusters with a poorly defined border were also noticed



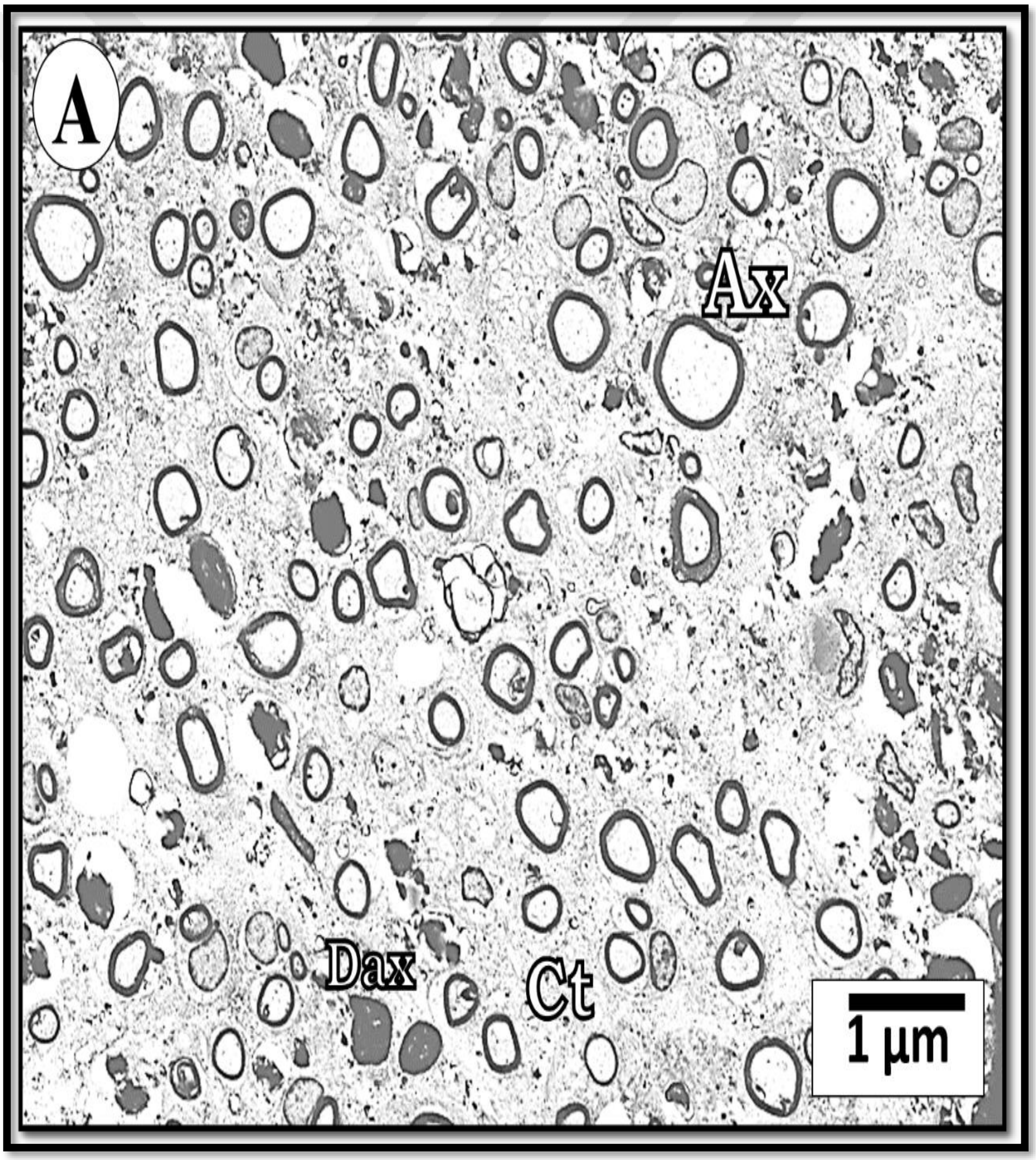
Figures 4.47. (B) Different magnifications of TEM images of the sciatic nerve thin cross-section are belonged to the Inj group were seen. The histological nerve structure was seriously affected. Many degenerated axons (Dax) with unclear myelin sheaths were seen. The Schwann cells (Sch) were seen closed the nerve fibres due to their function. Most nerve axons are small-sized with a thin sheath due to nerve fibres sprouts. Some protected axons with thick sheath (Ax) were found. The unmyelinated axons (Um) clusters with a poorly defined border were also noticed

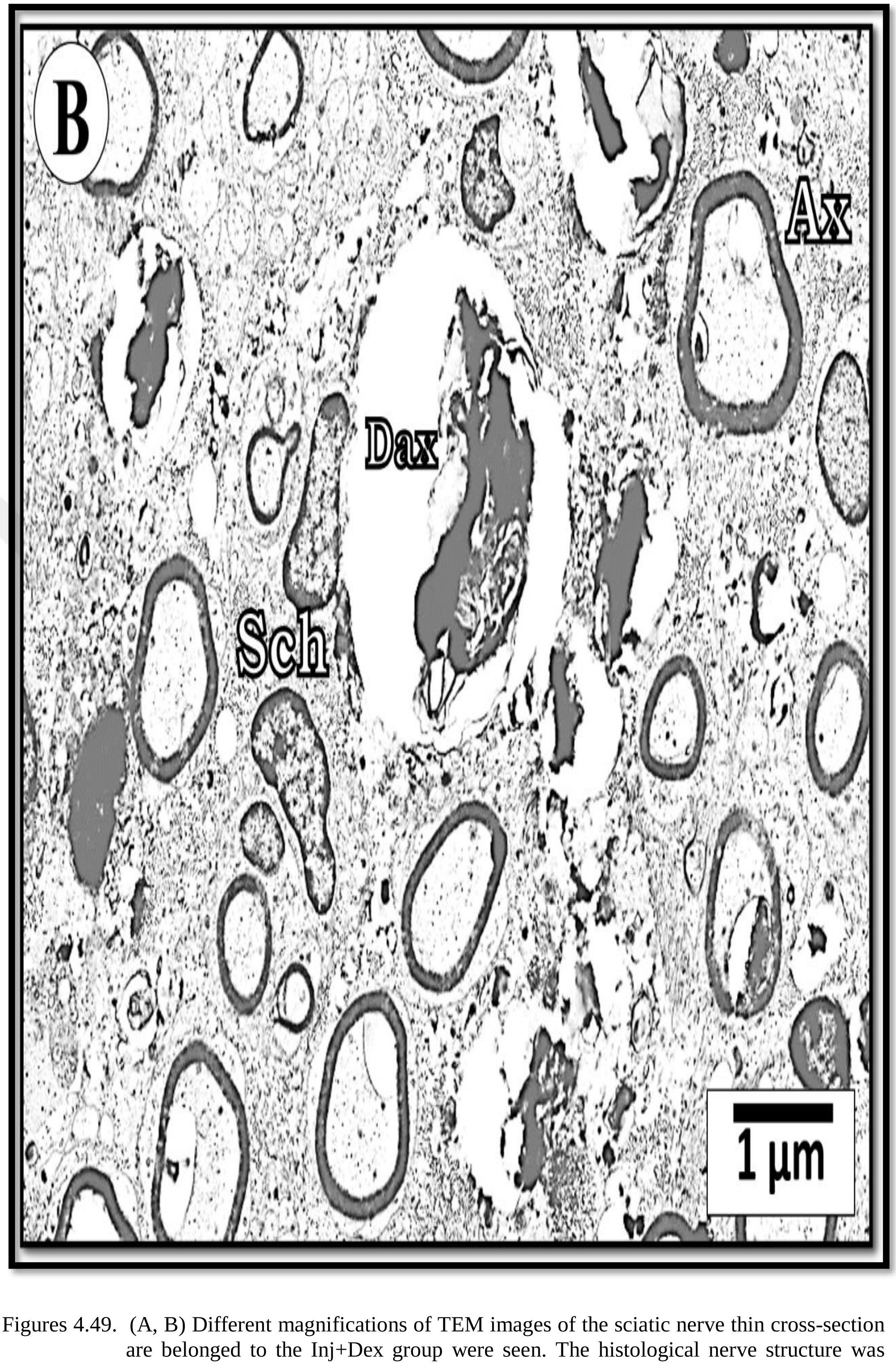


Figures 4.48. (A, B) TEM images of the sciatic nerve thin cross-section are belonged to the Inj group were seen. Many degenerated axons (Dax) with unclear myelin sheaths were seen. Most of the nerves are small-sized with a thin sheath as a result of nerve fibres sprouts. Some protected axons with thick sheath (Ax) were found. The connective tissue (Ct) among the axons was increased. The pericyte (Pc) and newly formed capillaries (V) with premature endothelial cells were detected

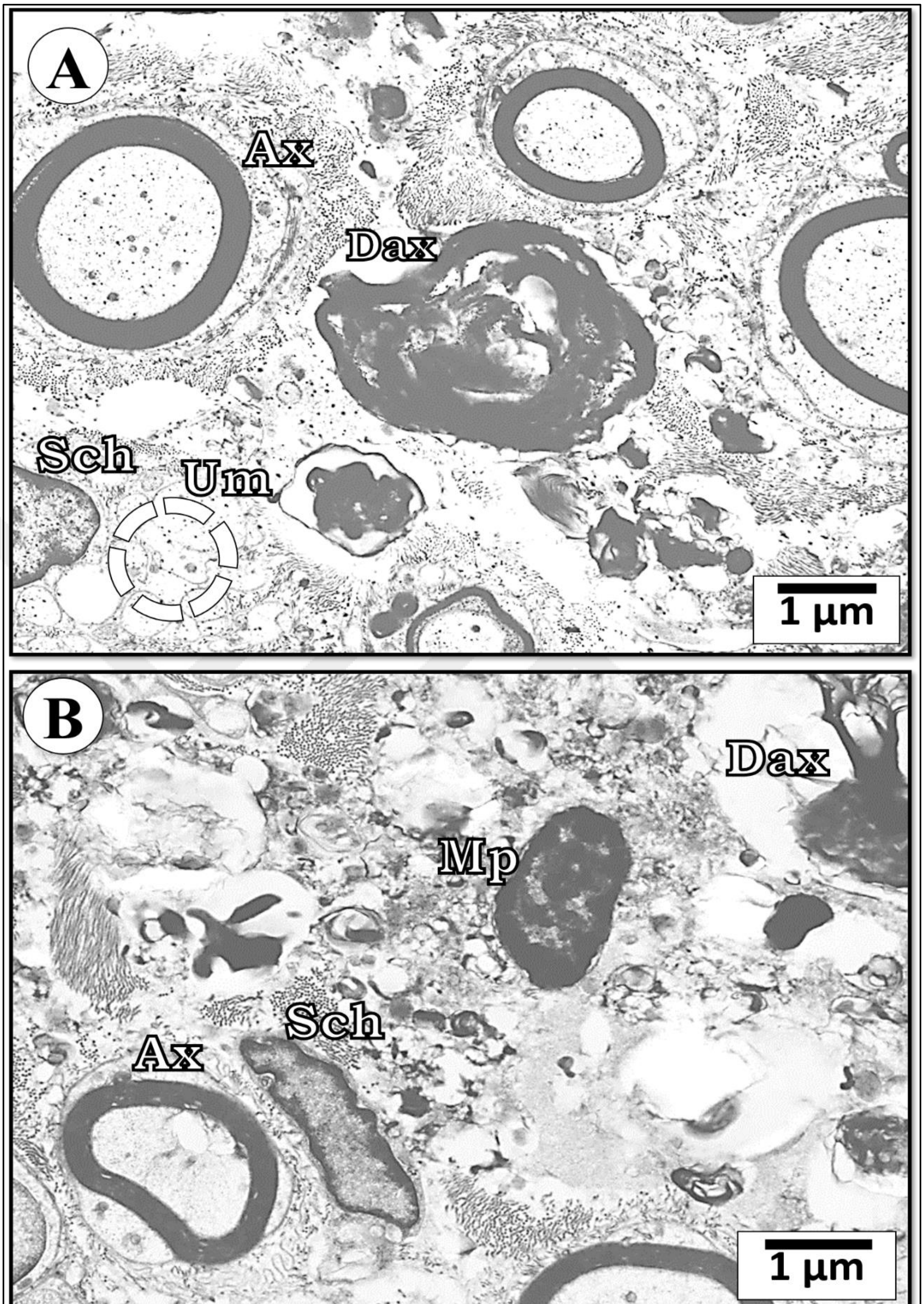
4.1.5.3. Electron Microscopic Findings in the Inj+Dex Group

The thin cross-sections taken from the sciatic nerve of the Inj+Dex group was examined by the TEM. After the injured nerve was exposed to Dex, it is seen that the general structures of the nerve fibres look normal. The degenerated axons and macrophages were seen abundantly in the injury site. The newly formed small-sized axons were also observed. Myelin coat of the large and small-sized myelinated nerve fibres was observed well protected, most of the axons with thick myelin sheath due to the positive impact of Dex. Delineated Schwann cells nuclei were observed around the nerve fibers. Aggregation of unmyelinated axons was identified clearly. The connective tissue with its fibroblast was intact and observed (Figure 4.49-50).





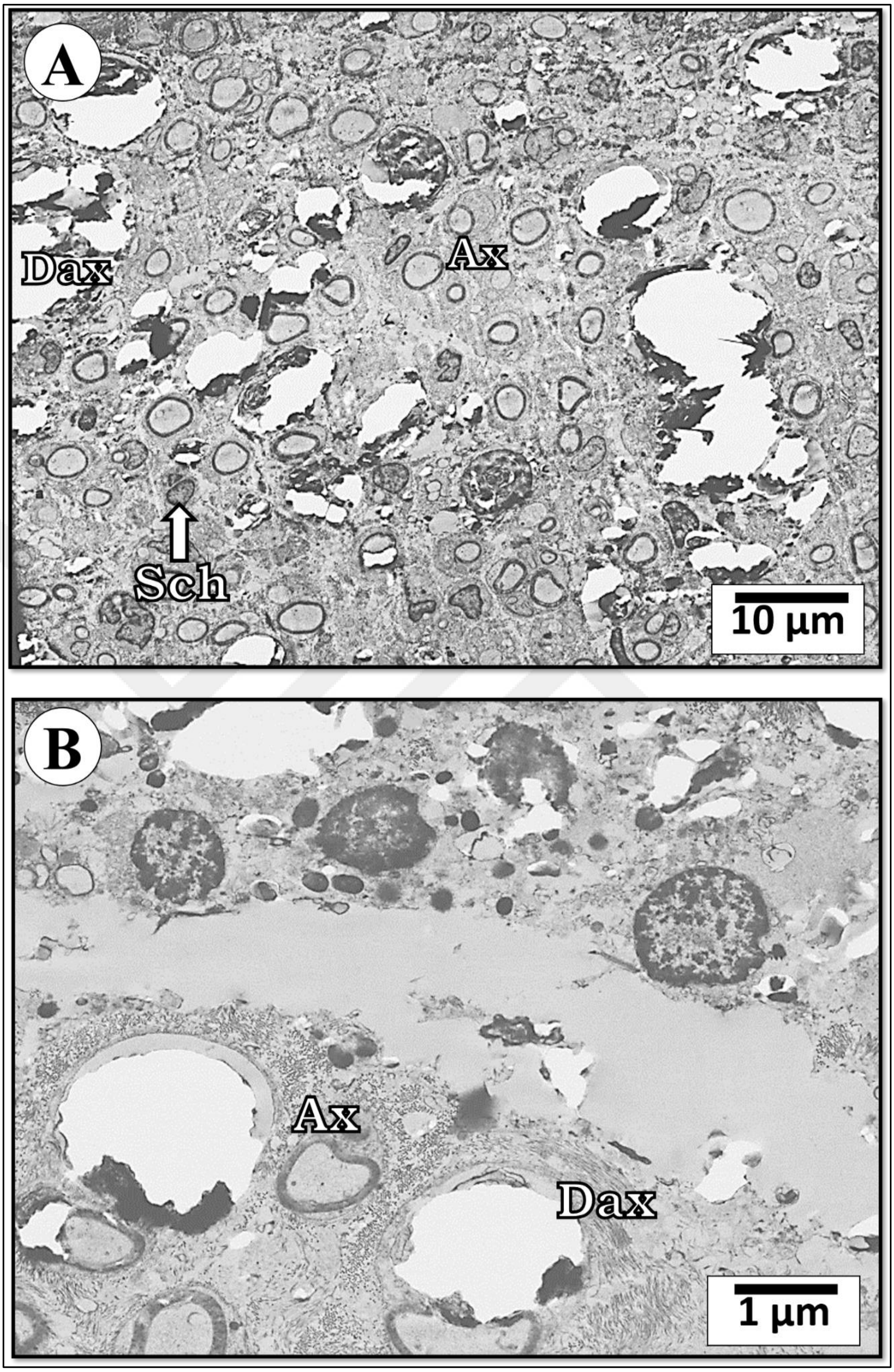
Figures 4.49. (A, B) Different magnifications of TEM images of the sciatic nerve thin cross-section are belonged to the Inj+Dex group were seen. The histological nerve structure was partially damaged. Many degenerated axons (Dax) were seen. The connective tissue (Ct) among the axons was detected. The Schwann cells (Sch) were seen closed the nerve fibres due to myelination function. Most of the nerves are small-sized with a thin sheath due to nerve fibres sprouts. Some protected axons with thick sheath (Ax) were found due to the positive impact of Dex



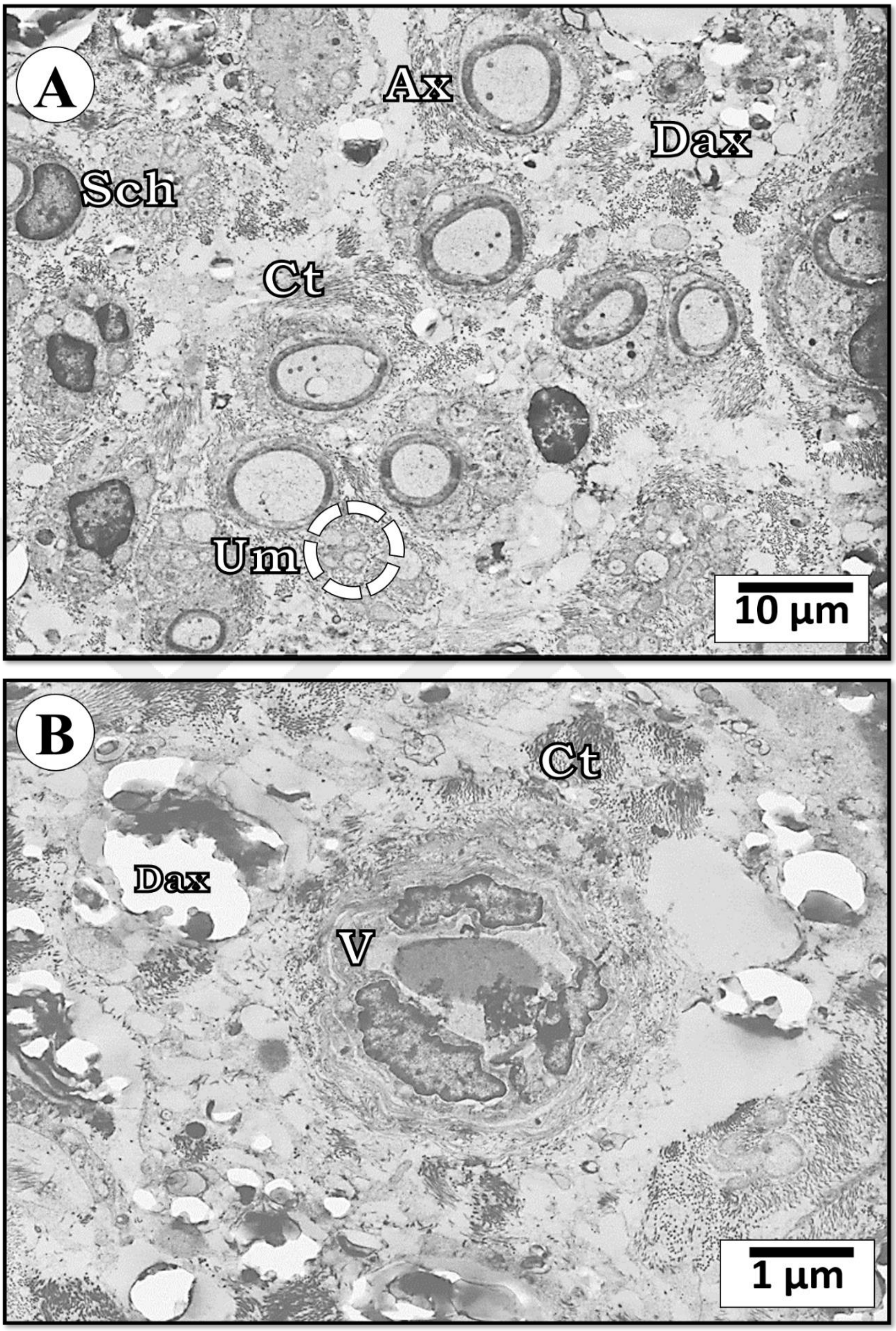
Figures 4.50. (A, B) TEM images of the sciatic nerve thin cross-section are belonged to the Inj+Dex group were seen. The Schwann cells (Sch) were marked close to axons due to myelination function. Many macrophages (Mp) and degenerated axons (Dax) were seen in the injured area. Besides, a considerable loss of nerve integrity was observed. Most of the nerves are small-sized with thin sheaths due to axons sprouts. Some protected axons with thick sheath (Ax) were found due to the positive impact of Dex. (Um) indicates unmyelinated nerve fibers

4.1.5.4. Electron Microscopic Findings in the Inj+Bet Group

Thin sections taken from the sciatic nerve of the Inj+Bet group were examined by the TEM. After treating the injured nerve by Bet, it was observed that the overall structure of the axons appears healthy. A thick and well-preserved myelin sheath around the axons was seen. Some myelinated axons were well protected; the usual round shape of the axons and myelin sheath is lost in some sections. A group of unmyelinated axons with a clear border were found. Schwann cells nuclei were observed at the center of unmyelinated axons. Macrophage with extensive cytoplasm of phagocytotic materials and rich myelin debris was also observed. The mast cell was seen in the same section as expected due to its recruiting the macrophages to the injury site. The intact connective tissue was observed around the myelinated nerve fibres. The blood cells within the blood vessel surrounded by premature endothelial cells were seen (Figure 4.51-52).



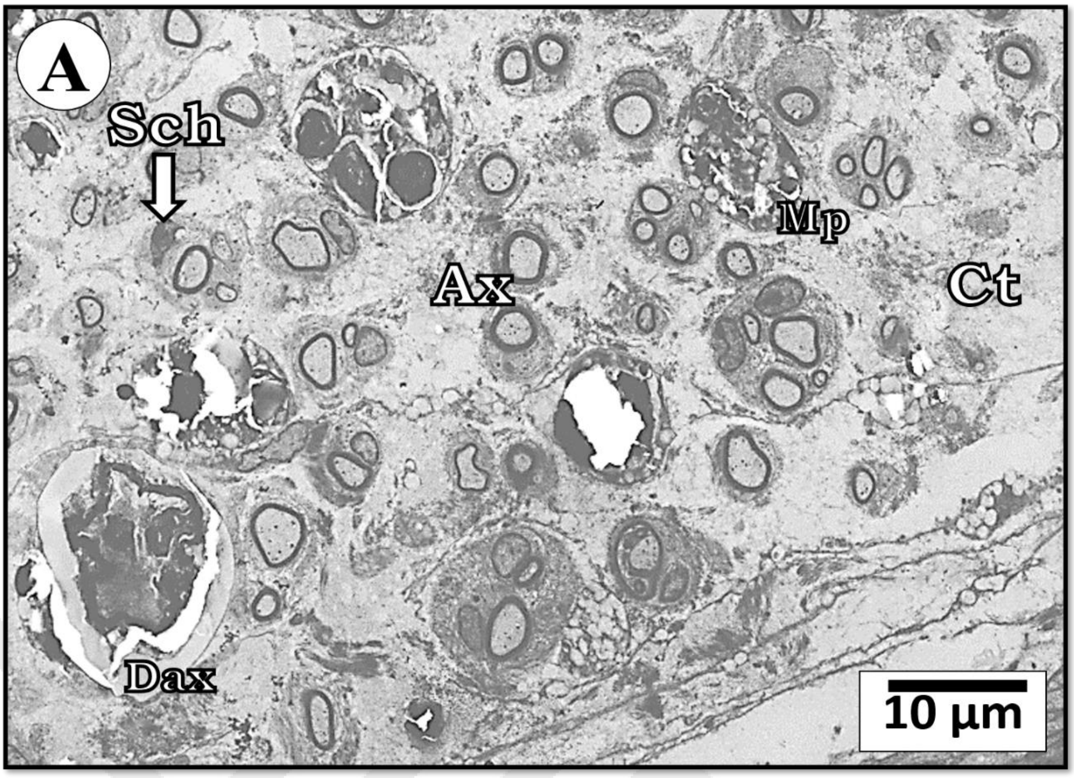
Figures 4.51. (A, B) Different zooms of TEM images of the sciatic nerve thin cross-section belonged to the Inj+Bet group. After crush application, the general structural view of the nerve was partially damaged. A degenerated axons (Dax) were seen in the injured area. (Mc) indicates the mast cell. Besides, the loss of nerve integrity was clearly observed. The Schwann cells (Sch) were marked and seen. Most of the nerves are small-sized with thin sheath due to WD

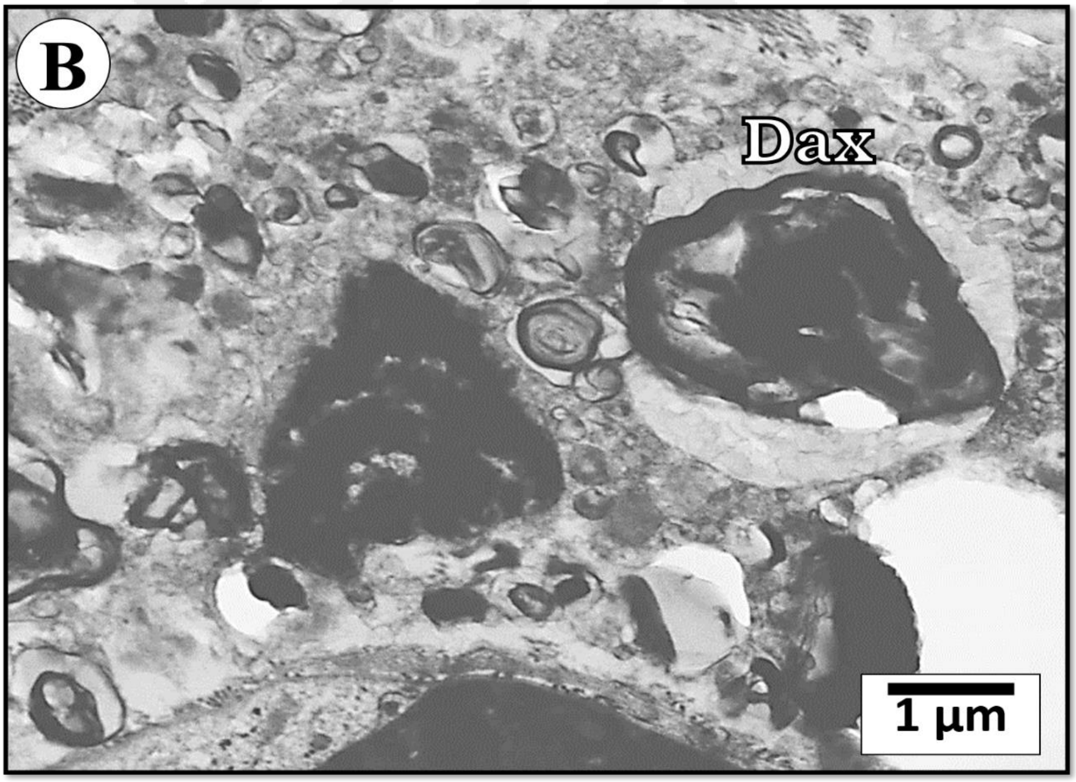


Figures 4.52. (A, B) TEM images of the sciatic nerve thin cross-section are belonged to the Inj+Bet group were seen. After crush application, the general structural view of the nerve was partially damaged; as the loss of the nerve integrity was clearly observed, and the connective tissue (Ct); degenerated axons (Dax) were seen in the injured area. (V) indicates blood capillary with premature endothelial cells. Besides, the Schwann cells (Sch) were marked and seen. Most of the nerves are small-sized with thin sheath due to WD. (Um) indicates unmyelinated axons

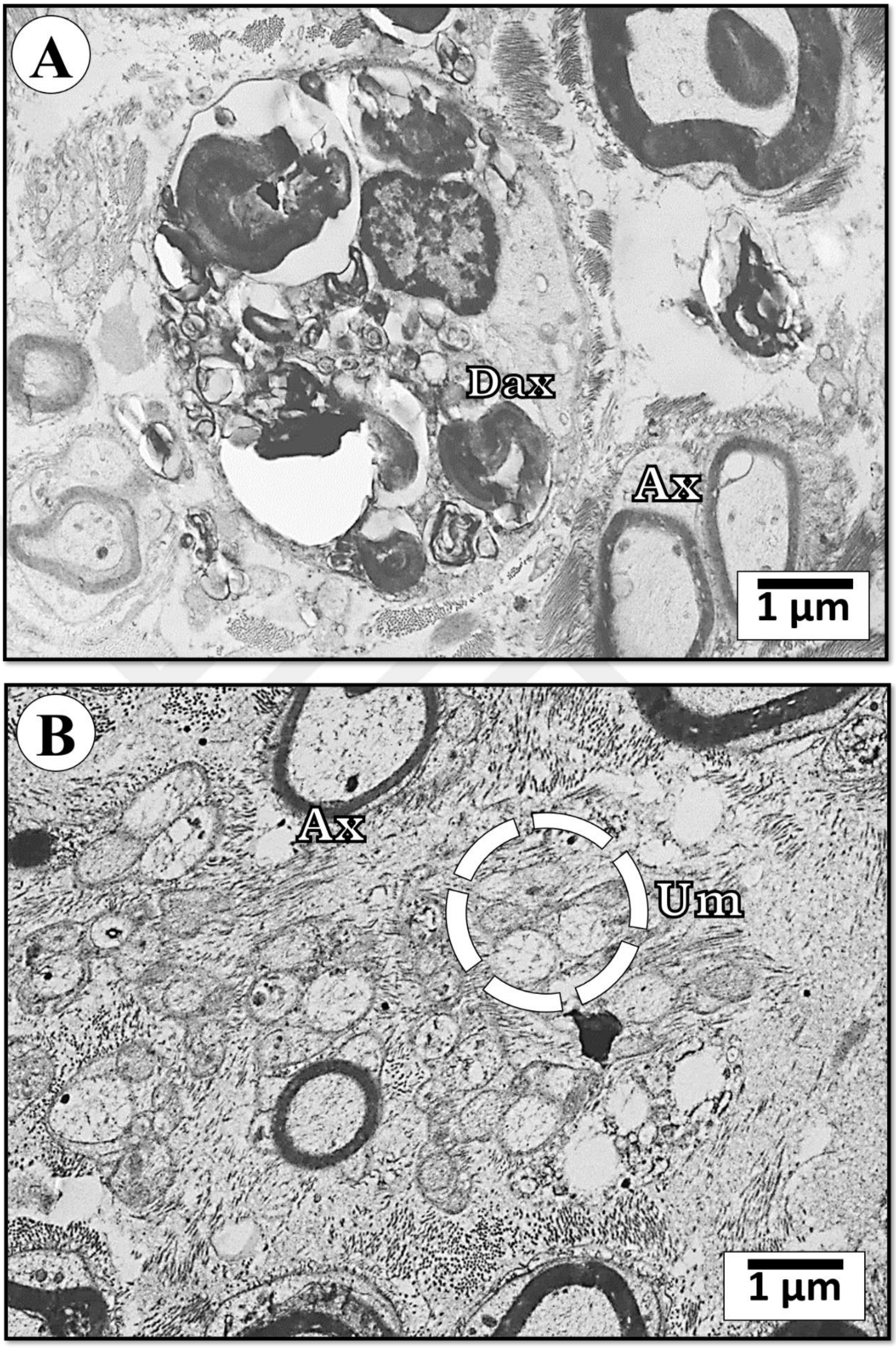
4.1.5.5. Electron Microscopic Findings in the Inj+Mps Group

Thin sections belonging to the Inj+Mps group examined by TEM showed that the general architecture of the nerve appears partially preserved. The size heterogeneity of the myelinated nerve fibres was found normal. The majority of axons in this group are small-sized and ensheathed by a thin coat, reflecting a high degree of myelination around the axonal sprouts. Schwann cell nucleus was found around the axons and in the center of unmyelinated axons in this group. A considerable number of the macrophages with the remnant of myelinated nerve fibres in their cytoplasm (foamy appearance), and degenerated axons were found in the tissue sections. An increased amount of connective tissue was a remarkable feature of the nerve in this group. Some protected axons were seen; some myelin sheath physical defects, such as fragmentation was seen (Figure 4.53-54).





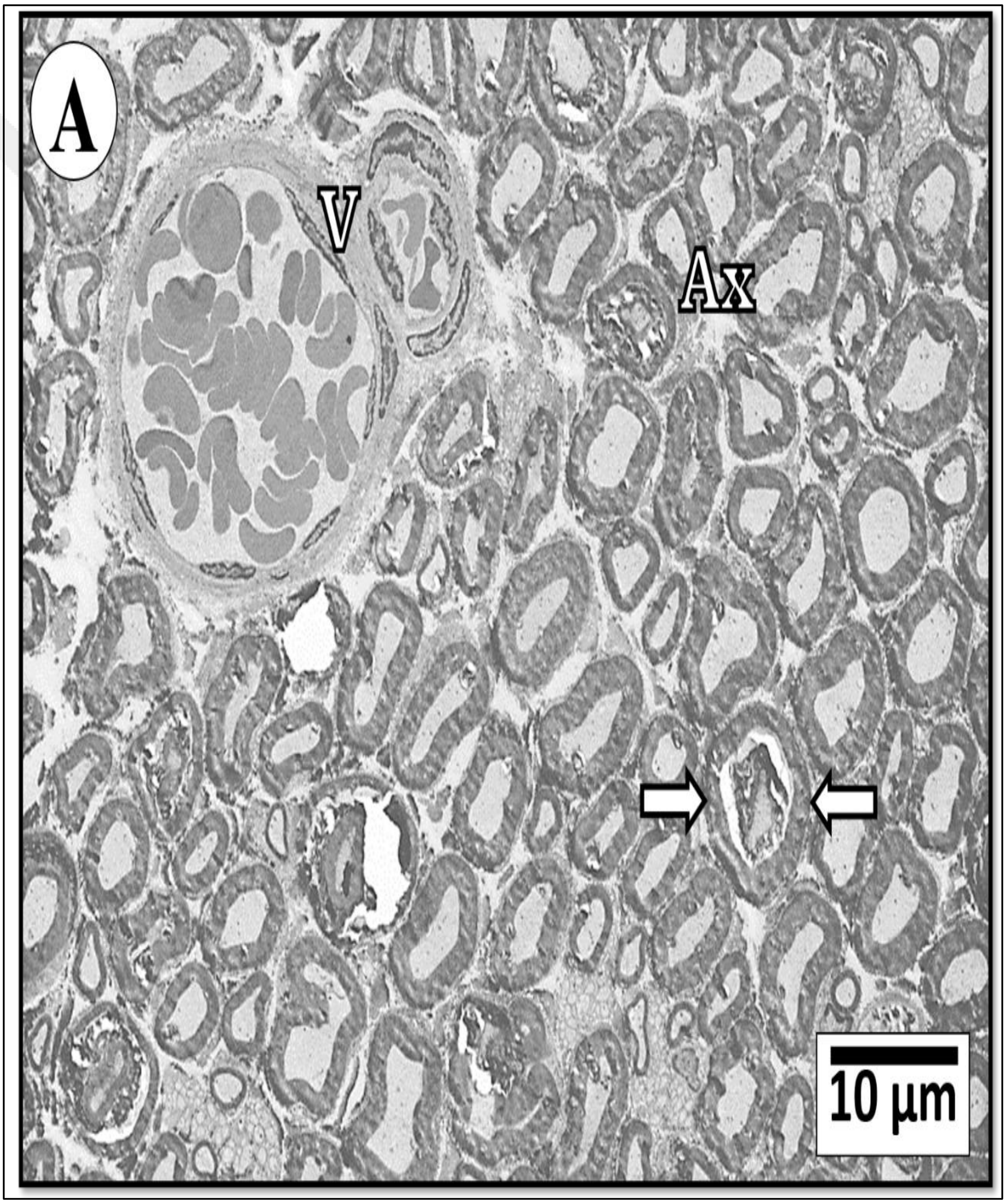
Figures 4.53. (A, B) Different magnifications of TEM images of the sciatic nerve thin cross-section belonged to the Inj+Mps group. The general nerve view of the nerve was partially damaged. Many degenerated axons (Dax) were seen. A huge number of macrophages (Mp) that are clean myelin droplets from the injured area was seen, and a remarkable nerve integrity loss was observed. The connective tissue (Ct) among the axons was detected. The Schwann cells (Sch) were seen closed the nerve fibres due to myelination function. Most of the nerves are small-sized with a thin sheath as a result of WD stages

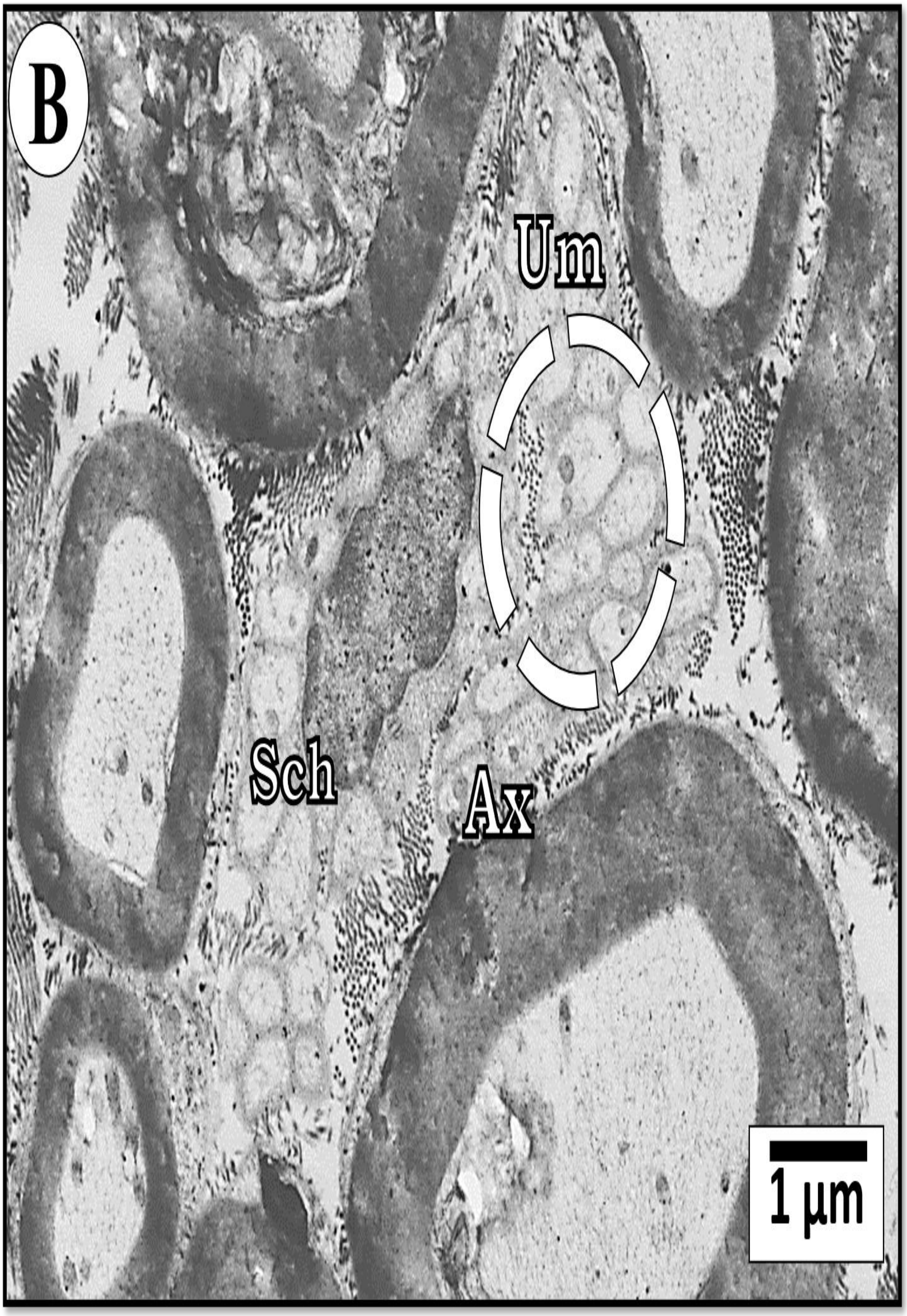


Figures 4.54. (A, B) TEM images of the sciatic nerve thin cross-section are belonged to the Inj+Mps group were seen. The nerve structural view of the nerve was damaged. Most of the nerves are small-sized with a thin sheath as a result of axon sprouts. Many degenerated axons (Dax) were seen, a remarkable nerve integrity loss was observed (Um), indicating myelinated axons. Some impaired myelin sheath; fragmentation. (Ax) for healthy axons

4.1.5.6. Electron Microscopic Findings in the Dex Group

The thin cross-sections taken from the sciatic nerve of the Dex group was examined by the TEM. The histological structure of the nerve tissue was healthy. The connective tissue of the nerve was intact. Different sizes of myelinated nerve fibers were observed. The myelin sheath impairments, such as cleft in some nerve fibers, were also seen. The nuclei of the Schwann cells were also observed. In addition, the aggregation of unmyelinated axons was found. The blood cells within the lumen of the mature vessel wall were also observed normally (Figure 4.55).

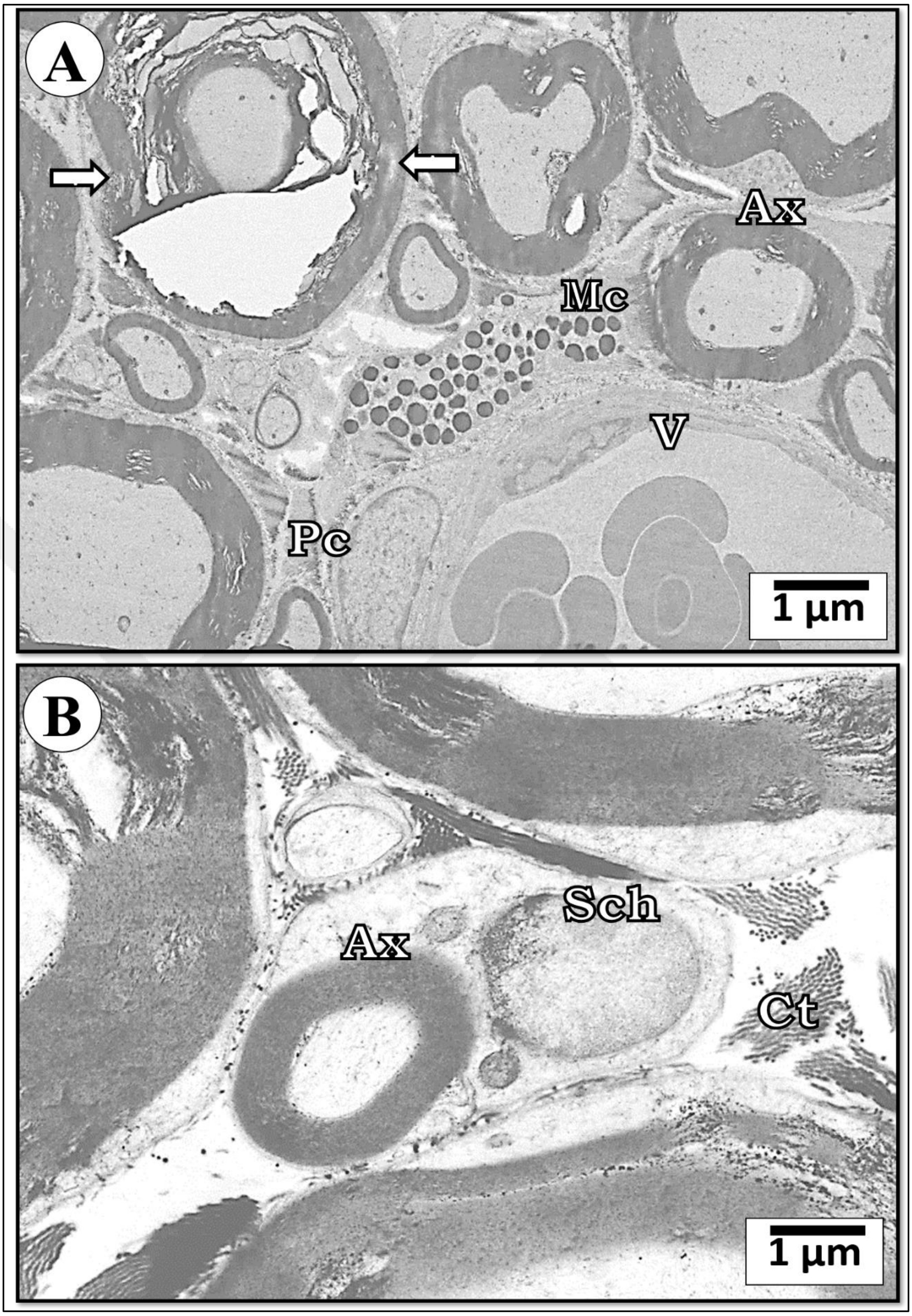




Figures 4.55. (A, B) Different zooms of TEM images of the sciatic nerve thin cross-section are belonged to the Dex group were seen. The overview structure of the nerve appeared normal. The myelinated axons (Ax) were observed healthy. The size heterogeneity of myelin sheath was seen around the different sizes of myelinated axons. (Sch) indicating to Schwann cell nuclei. Some physiological impairment of myelin sheath; Schmidt-Lanterman cleft (double arrows) was seen. The (V) indicating to a blood vessel with a mature wall and normal blood cells. (Um) indicating for unmyelinated axons

4.1.5.7. Electron Microscopic Findings in the Beta Group

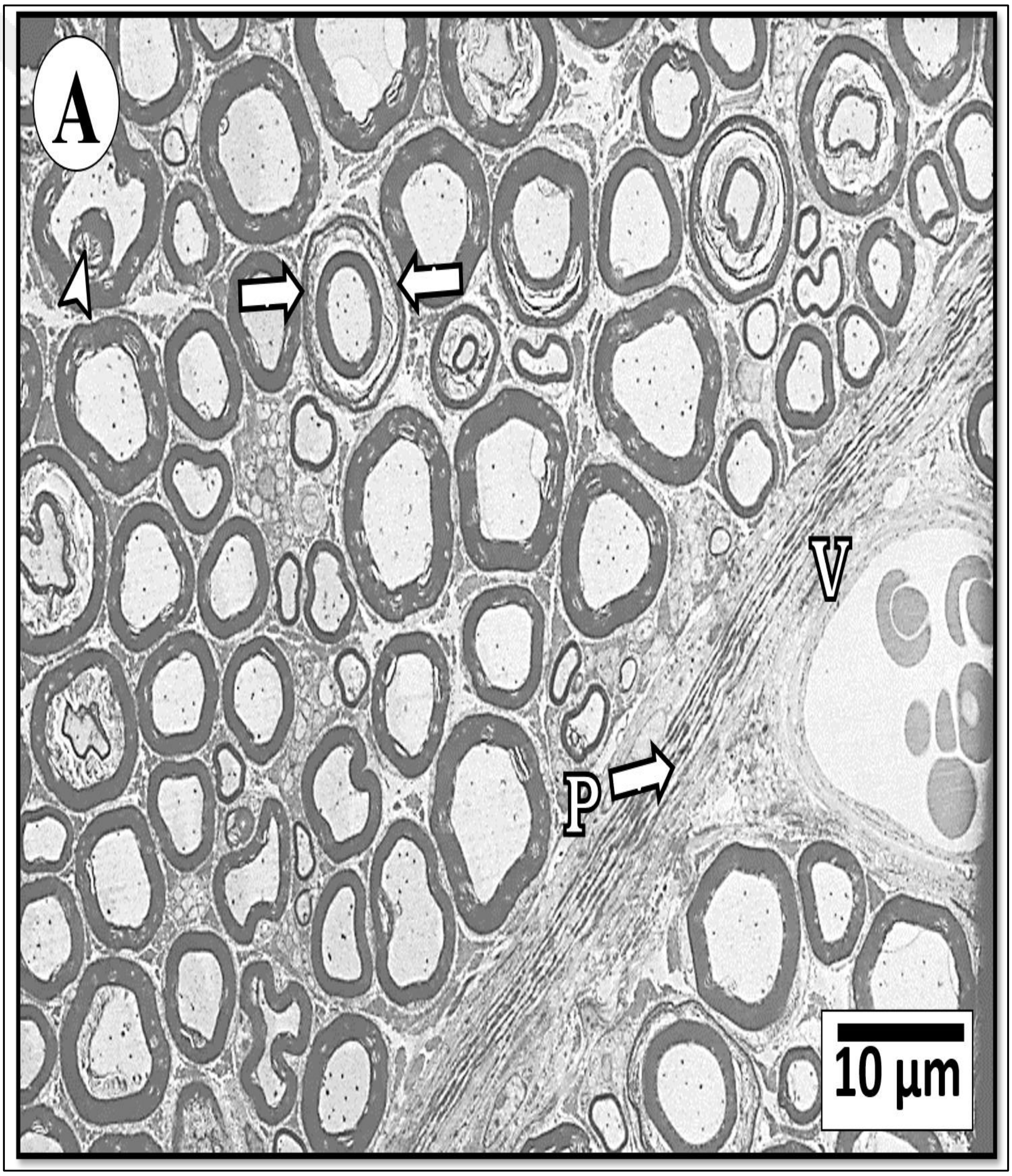
TEM examination of the Beta group thin section showed that the general structure of the nerve has a healthy structural view. A thick and normal myelin sheath was seen around the myelinated fibres. Some myelinated nerve fibers display double rings of a sheath that would contribute to the appearance of the Schmidt-Lanterman cleft, as seen in the LM. As well, some tissue was lost, and vacuolization of nerve fibers was found. A group of healthy unmyelinated nerve fibres with well-defined borders were found among the myelinated axons. The nuclei of Schwann cells are observed with clear borders in the center of the unmyelinated axons clusters. The mast cell was observed in the connective tissue of the nerve (Figure 4.56).

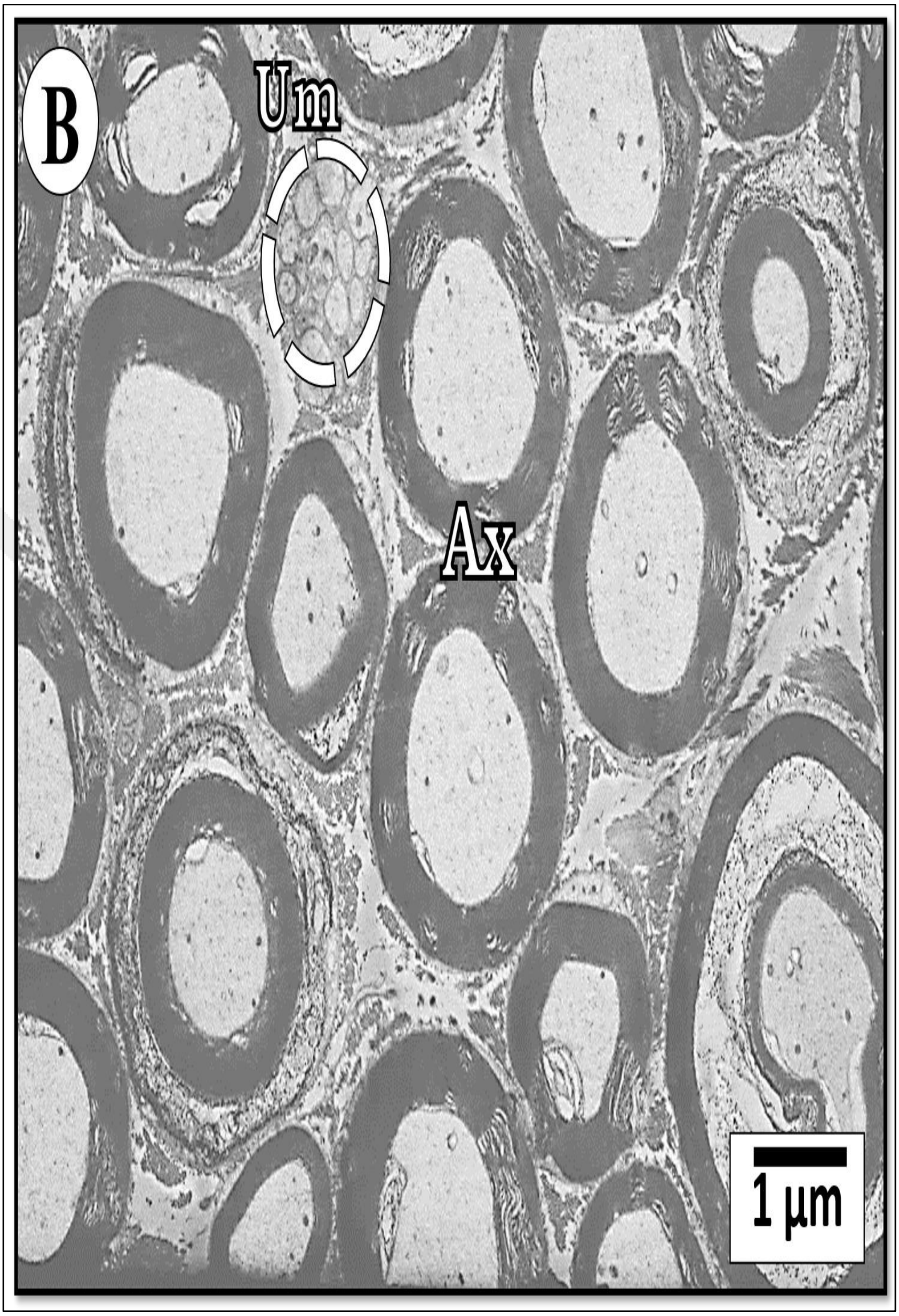


Figures 4.56. (A, B) TEM images of the sciatic nerve thin cross-section are belonged to the Bet group were seen. The general structure of the nerve appeared normal as the connective tissue (Ct) was healthy. The myelinated axons (Ax) were observed healthy. The size heterogeneity of myelin sheath was found around the different sizes of myelinated axons. (Sch) indicating to Schwann cell nuclei. Some impairments of the myelin sheath, Schmidt-Lanterman cleft (double arrows), and vacuolization were seen. The (V) indicating to a blood vessel with a mature wall and normal blood cells. (Mc) for mast cell. While the (Pc) for pericyte

4.1.5.8. Electron Microscopic Findings in the Mps Group

Thin sections taken from the sciatic nerve of the Mps group were evaluated by the TEM. The general architecture of the neural tissue appeared normal. A healthy connective tissue, the perineurium that encloses the nerve fascicles, was seen. A thick normal myelin sheath around myelinated axons was seen. Clusters of unmyelinated nerve axons were also observed; the border of each unmyelinated axon was seen. Some myelinated nerve fibers display double rings of a sheath that would contribute to the appearance of the Schmidt-Lanterman cleft Schwann cells with a prominent nucleus were found close to the myelinated axons. The physical and physiological impairments of the myelin sheath; vacuolization/cleft were observed (Figure 57).





Figures 4.57. (A, B) Different zooms of TEM images of the sciatic nerve thin cross-section are belonged to the Mps group were observed. The overview structure of the neural tissue looks normal; the perineurium (P) was healthy. The myelinated axons (Ax) were observed healthy. The size heterogeneity of myelin sheath was seen around the different sizes of myelinated axons. Some impaired sheath, Schmidt-Lanterman cleft (double arrows), and vacuolization were indicated by an arrowhead. The (Um) indicating to unmyelinated axons

4.2. Discussion

This study aims to investigate the effects of some corticosteroids medications named: Dex, Bet, and Mps (varying in the period of acting; long-acting and intermediate-acting corticosteroids) in promoting peripheral nerve regeneration based on the histological, sciatic functional index, and electromyography factors; in addition to stereological factor as a fundamental criterion in the evaluation since it provides sensible and exact findings, which allow the researchers to analyze subtle changes with precision in peripheral nerve tissues (D. L. Brown et al., 2020).

During the American Civil War, neurologist S. Weir Mitchell conducted the first systematic study of peripheral nerve injury (Seddighi et al., 2016b). In the United States, nerve injuries account for approximately $150 billion in annual healthcare spending (Taylor et al., 2008).

Accordingly, the present study conducted in 64 healthy adults Wistar albino rats; by exposing them to surgical intervention through creating a longitudinal incision between the musculature of gluteal and femoral regions; then, the sciatic nerve was freed and exposed to crushing injury by applying crushing force equalizing 50 Newton, 5 mm distal from the sciatic notch, and the crushing time was 60 seconds by using a clamp forceps.

In the first part of the current study, the stereological techniques were used to achieve a precise and unbiased estimate of the number of myelinated axons, myelin sheath thickness, and axonal area in the sciatic nerves. The 2D fractionator method was used for stereological analysis to approximate the number of myelinated axons; In contrast, the nucleator was being used to calculate the axonal area and thickness of the myelin sheath of the sciatic nerve.

The stereological findings revealed a substantial improvement in the mean of myelinated axon number in the injured groups treated with Dex (Inj+Dex) compared with the Inj group; this confirms that Dex promotes the number of axons of the injured sciatic nerve. On the other hand, comparing the same previous criterion in the evaluation of the injured group treated with Dex, it was noted that there is no significant difference in the mean of the axonal area and mean myelin sheath thickness. That means that Dex has no therapeutic effect in promoting the myelination in the axons of the injured sciatic nerve. It is worth noting that when comparing Inj and the Cont groups, a substantial decrease in mean myelin sheath thickness was observed in the Inj group as a result of crush injury induced in the sciatic nerve; meanwhile, no significant difference between the injured groups that treated with Bet and Mps was observed in terms of the mean number of myelinated axons and mean axonal area as well as mean axonal area comparing to Inj group.

Physiological examinations that included SFI, and EMG, on the other hand, were used to assess the functional recovery of muscle and sciatic nerve regeneration. The rat's two-sided footprint was analyzed by comparing the normal sciatic nerve in the lower-left limb with that of the crushed sciatic nerve in the lower-right limb. The SFI findings showed positive findings among the injured groups treated with Dex and Mps reflected in significant differences compared with the Inj group. On the other hand, no therapeutic effect was shown in the injured group treated with Bet. Amplitude has been used to measure muscle contraction rapidly, and latency tests measured the duration of nerve pulses from nerve to muscle. The physiological experiments generally partly come in the same line as the stereological findings, especially in SFI.

This study also included microscopic analyses in order to observe the histological variations that occur in the sciatic nerve formation among the various study groups. As a result, the positive and therapeutic results of Dex have directly impacted the crushed sciatic nerve structural framework, which appears in the increase of myelinated axon number. Similarly, regarding Bet and Mps, positive therapeutic results were affirmed in the microscopic analyses, which is unable to be achieved by the statistical values.

4.2.1. Peripheral Nerve Injury

Peripheral nerve injuries may be severe and cause chronic weak. About one-third of the peripheral nerve injuries show incomplete recovery with inadequate functional reconstruction despite a careful armamentarium of microsurgical techniques like direct healing, auto-graft defect grafting, and cadaveric allografting is in use. This can involve absolute loss or partial motor and/or sensory rehabilitation, chronic pain, atrophy of the muscles, which may lead to permanent morbidity. Many of these affected nerve regeneration can be due to perineural scarring and fibrosis at the damage and reconstruction site; Up to date, this daunting clinical issue has not been appropriately resolved (M. L. Wang et al., 2019).

As previously mentioned, peripheral nerve injury is represented in three major classes: neurapraxia, axonotmesis, and neurotmesis. According to *Seddon's* classification, neurapraxia is described by local myelin injury, which is generally caused by compression. Axon continuity is maintained, and thus the nerve does not degenerate distally. In contrast, the lack of continuity of axons with varying preservation of nerve connective tissue components is known as axonotmesis. Concerning the neurotmesis is the worse damage to the whole nerve and corresponds to physiological disruption; actual transection of the nerve may or may not occur (S. K. Lee and Wolfe, 2000; Seddon and Wilkins, 1972). *Sunderland's* diagnosis of peripheral nerve injury was based on another factor; he focused on the classification of degree of connective tissue involvement; neurapraxia corresponds to an injury of type-1, this injury type will take weeks to months for complete rehabilitation.

While in the case of type-2 injury, endoneurium, perineurium and epineurium remain constant; but physiologically, the axons are impaired or disrupted. However, regenerating axons are guided along the original path due to the intact endoneurium, and full functional rehabilitation can be predicted.

The endoneurium is also disrupted in a Sunderland type-3 injury, but the perineurium and epineurium remain unchanged; for a variety of causes, healing in this class of injury is incomplete.

Just the epineurium is stable in respect of the Sunderland type-4 injury; neuronal and intrafascicular fibrosis retrograde trauma has been intensified, providing for minimum useful recuperation (S. K. Lee and Wolfe, 2000; Sydney Sunderland and Williams, 1992).

A series of pathological events in the cell body and axon arises during the axonic transaction in case of nerve degeneration. A mechanism where the granules of Nissl are dispersed, and the cell body is bloated, and chromatolysis appears relatively eosinophile. The cell nucleus is shifted to a peripheral position. This represents a shift in the metabolism of the development of neurotransmitters to structural materials essential for axon repair and development. The proximal axon would have traumatically degenerated inside the damaged region immediately after the axon transaction (S. K. Lee and Wolfe, 2000).

In most cases, the injury zone stretches proximately from the damage site toward the next node at Ranvier; however, it is possible to destroy the cell body itself based on the injury's mechanism and intensity. The distal segment to the site of injury is undergoing a gradual process of degeneration. This degeneration mechanism is referred to as WD. That follows myelin degrading, and the axon becomes disorganized. Myelin and axonal debris are phagocytosed by Schwann cells, which proliferate. It should be considered that nerve trauma has the ability to interrupt the nerve-blood barrier (Geuna et al., 2009; S. K. Lee and Wolfe, 2000).

In diagnosing and treating acute peripheral nerve injury, both nerve conduction tests and needle electromyography provide valuable information (Aminoff and Weiskopf, 2004). The potential for compound muscle activity and nerve action potential in neurapraxia is retained indefinitely in stimulus distal to the lesion. Stimulation which occurs proximal to the lesion, exposes partial or full conduction block, with differing degrees of reduction of compound muscle activity potential amplitude. Depending on the characteristics of a specific lesion, compound muscle activity potential configuration and conduction velocity slowing can occur. These irregularities should increase or vanish when remyelinating is complete, as long as nerve pressure is not persistent. In a total neurapractical lesion, needle EMG does not show a voluntarily regulated motor unit action potential, but fibrillations do not exist. In partial neurapraxia, irregular recruitment is the most prominent EMG abnormality. The electrodiagnostic image in axonotmesis and neurotmesis is determined by the period of time that has occurred between the damage and the assessment (Seddighi et al., 2016b).

The method of peripheral nerve regeneration is formed when the effector organ or tissue is denervated and happens in two ways depending on the degree of injury that includes the axon being subjected to damage or not; the first direction in the former type of injury is collateral branching, which is called the primary method for regeneration; this process takes place frequently. To achieve complete healing or recovery, the nerve must go through three major processes: WD, axon regeneration, and end or effector organ innervation, as previously mentioned. Failure of these processes can result in poor physical and clinical function, which is common in patients with peripheral nerve injury (Geuna et al., 2016; Menorca et al., 2013).

4.2.2. Dexamethasone

It is a synthetic long-acting corticosteroid medication often used for analgesic and anti-inflammatory disorders such as arthritis, blood/hormone disturbances, allergic reactions, skin conditions, retinal issues, and respiratory difficulties. In addition to these therapeutic uses, it is also used for postoperative nausea and vomiting (Ciobotaru et al., 2019; D. M. Williams, 2018). According to the stereological findings, the mean of the number of myelinated nerve fibers in the Inj+Dex group was substantially more significant than in the Inj group, and the mean of the myelin sheath thickness as well as the mean of the axonal area in Inj+Dex was significantly less than the Inj group. Thus, it is possible that Dex prevents nerve fibers that have been damaged that are about to be removed.

Based on the literature review have shown that Dex has a curative effect on sciatic nerve regeneration; according to *Uzun,* regional application of gelatin sponges of Dex to the injured right sciatic nerve and compared its effect with powdered Artesunate and Tacrolimus by using the same route of administration; this study findings statistically demonstrated the positive effect of Dex on nerve regeneration. Histopathologic analysis revealed that Artesunate reduced fibrosis and inflammation while raising the diameter of myelinated axons; on the other hand, Tacrolimus reduced the fibroblast scores; in the same manner, Dex only reduced fibrosis scores. According to immunohistochemical results, Dex and Artesunate groups had more favourable immunoreactivity to nerve growth factors (Uzun et al., 2019).

Another study was supportive of the hypothesis of the therapeutic effect of Dex in improving the electrophysiological and histological result of the injured left sciatic nerve. In this study, the Dex was tropically administrated by using a silicon bridge and compared with another group treated with phosphate-buffered saline; the results of the study came in the same line with the previous study, that electrophysiological confirmed the positive and faster recovery of nerve axon in the group treated with Dex; also it showed more positive reaction to S-100 location, regarding the morphometric indices it revealed that Dex treated group substantially higher nerve fiber, axon diameter and thickness of myelin sheath compare with Silicon group. The author justified this result by knowing that Dex blocks arachidonic acid metabolism and all subsequent metabolites through cyclooxygenase and lipoxygenase tracts; Dex has also been documented in the laboratory studies on rat gliomas (brain and spinal cord tumors) to decrease tumor-alpha necrosis factor control of subcutaneous tissue and to reduce transcapillary permeability. It has been shown that local steroid injection in the site of nerve compression can promote the regeneration, but not the regeneration of a demyelinating lesion in animals with experimentally induced compression neuropathy (Mohammadi, Azad-Tirgan, et al., 2013).

Another research was carried out to determine the relationship between the dosage of Dex used in treating injured sciatic nerve by intramuscular injection around the injury site. The effect of various Dex doses on functional rehabilitation in rats with the sciatic nerve; regarding the dose-dependent effects, the beneficial effect was seen as the time of therapy increased. Furthermore, the immunohistochemistry result for CD3 expression based on T-cell penetration confirms that Dex therapy resulted in a decrease in CD3-positive cells, owing primarily to its immunosuppressive impact. Histologically, the analysis confirms that an increase in myelinated axon counts is directly proportional to the Dex does increase (Feng and Yuan, 2015).

Another comparative study came to support and reinforce what preceded that Dex exposed a positive therapeutic outcome in promoting the functional recovery of peripheral nerve regeneration. According to a medication comparison, the following drugs are implicated: Dex, Tacrolimus, Artesunate, and Petrolatum. The study was carried out by performing a crush injury in the sciatic nerve of the selected groups and examining the impact of each treatment separately. In terms of administration, gelatine sponges were soaked and applied to the affected nerve regions with primed drug dilution for each particular drug class. The study results revealed that Artesunate has diminished fibers, reduced inflammation, and improved myelination axon diameter following the histopathology test; fibroblasts decreased while Dex reduced Tacrolimus fibrosis rating and reduced fibroblast numbers. In addition, immunohistochemistry analysis showed that Dex and Artesunate induced the growth factor (Uzun et al., 2019).

In another view, when paired Dex with another pharmaceutical drug, it can have a more significant therapeutic effect; that was proven by the experimental rat's sciatic nerve was subjected to crush damage in the study. Dex (1 mg/kg) was given to one group of rats, while vitamin B12 (2 mg/kg) was given to another. Both groups were given the same dosage of the two drugs. To evaluate the topical effect, the drugs were inserted into the injury site. The study's results were impressive in that the group of rats undergoing integrative treatment, which included Dex and vitamin B12, had a better clinical outcome. The outcome is justifiable since the two drugs act synergistically to increase the number of Schwann cells. The regeneration of myelinated nerve fibers and the growth of Schwann cells illustrate the beneficial outcome, which contributes to a positive influence on afferent and efferent conduction and the enhancement of the brain-derived neurotrophic factor countenance (Sun et al., 2012).

According to experiments that investigated the effects of topical and systemic route administration of Dex on facial nerve regeneration in rabbits. It was shown that the systemic route of Dex therapy significantly improved nerve regeneration and better healing results based on electrophysiological examinations in comparison with the topical route (Seth et al., 2012). Based on this, we recommend the intraperitoneal administration route, which is a form of systemic route in the current study.

Regarding previous studies that used stereological approaches to determine the beneficial effect of Dex in the nervous system, the emphasis was on the central nervous system rather than the peripheral nervous system; mentioned below are some examples of these studies.

In this context, previous studies worthy of attention are conducted by *Virginia,* which describes the anatomical consequences of Dex on the adrenals of adult male guinea pigs and adrenocorticotrophic hormone, considering the quality and quantity in assessment. The study results showed increases in external fasciculate zone cells and decreases in the reticular zone; concerning the amount of the Golgi apparatus decreases dramatically after using Dex throughout all cells but increases except in the zona fasciculate interna as well as zona reticularis. Furthermore, the volume and amount of lysosomes and mitochondria in all cells following Dex decreased, and their function in steroidogenesis are maintained (Black and Russo, 1980).

Another study demonstrates the influence of Dex on the CNS, precisely the fetal rat hypothalamic paraventricular nucleus. The study was conducted in adult fertilized female Wistar rats. The study's females were selected implementing a regular 4-day estrus period confirmed by vaginal smears checked and showed spermatozoa as pregnancy indicator. The targeted group was administered with 0.5 mg of Dex on days 16, 17, and 18 of gestation. The embryonic brain and pituitary glands (hypophysis) were extracted along with the sphenoid bone for tissue processing. The evaluation is based on the stereological technique by estimating volume and total cell by applying the Cavalieri principle measure theory and the optical fractionator. The study results demonstrated that after maternal Dex administration, the number of cells in the hypothalamic paraventricular nucleus decreases, which in turn illustrates the negative impact (Manojlović-Stojanoski et al., 2016). According to the findings of the studies reported above, most results seem to be compatible with the stereological findings of the current study; in this sense, Dex has a therapeutic effect in promoting nerve regeneration.

4.2.3. Betamethasone

This medication identified as a synthetic long-acting corticosteroid, which treats a broad spectrum of diseases and disorders primarily for the anti-inflammatory and immunosuppressive effects of glucocorticoids and their effects on circulatory and lymphatic system processes in palliatives treating a wide-ranging of diseases such as; dermatitis, allergic disorders, including asthma, angioedema, and premature labour, to accelerate the growth of the infant's respiratory system (Drugs.com). According to the literature, corticosteroids are used to treat peripheral nerve injury, but their action mechanisms are unknown (Dahlin et al., 1996). According to the stereological results, the mean of myelin sheath thickness and axonal area in the Inj+Bet group has no significant statistical difference compared to the Inj group; by the same manner, the mean of myelinated nerve fibers in the Inj+Bet group also had no significant statistical difference in term of the number of myelinated when compare with the Inj group Depending on stereological findings of the current study it proved that Bet is acting negatively to enhance the peripheral nerve regeneration. Several previous reports affirmed and came in line with the current findings. *Sadraie* reported that Bet and the amniotic membrane act synergistically to enhance the peripheral nerve regeneration in a rat model study. The study conducted by administrating (4 mg/mL) tropically to the injured sciatic nerve; according to the results of the study when comparing the group that treated by amniotic membrane solely and the group treated with Bet solely, the best results were revealed in the group that received the both proposed medications synergistically. These results are inferred through the morphometric findings, Where a significant difference was observed in the number and thickness of the sciatic nerve fibers treated with Bet and the amniotic membrane (Sadraie et al., 2016). An additional study does not come in favour with the current findings to prove the beneficial impact of Bet in promoting sciatic nerve recovery. Bet dosage was measured according to plasma clearance in the experimental rats and administrated via a subcutaneous route (2 mg/kg/day) every 8 hours, beginning at 20 minutes before the operation for the first 24 hours. The effect of the medication evaluated depends on the immunohistochemistry factor by the usage of macrophage marker antibodies and p75. Furthermore, the study results exhibited that the beneficial effect of a mild perioperative dosage of Bet on nerve regeneration is expressed in the recruitment of macrophages but only to a limited degree in the expression of p75 (Al-Bishri et al., 2008).

It has also been reported the therapeutic effect of topical administration of Bet in promoting peripheral nerve regeneration. The study was conducted by injecting the medication in the vein graft taken from doner rats external jugular vein, washed with a physiological solution, and prepared to be used as a conduit for the transected nerve. The groups of rats that were treated topically by Bet 0.1 mg/kg loaded into the conduit with an additional group of rats that considered as control which treated by phosphate-buffered saline, the result of the experiment showed that Gastrocnemius muscle mass in the group treated with Bet was found to be substantially more significant than in the Cont group; also the morphometric fiber indexes revealed that the myelinated fiber numbers and diameters of the group treated with Bet were significantly higher than those of the Cont groups, as well as the location of the S-100 reactions in the group treated with Bet was slightly more optimistic than the Cont group through the use of immunohistochemistry factor. That can be summarized as follows; Bet improved the sciatic nerve's functional regeneration and quantitative morphometric indexes (Mohammadi, Amini, et al., 2013).

Another study, performed in rats, supported the previous authors findings in terms of the beneficial therapeutic role of Bet in promoting peripheral nerve regeneration, which contradicts the results of our current study. The study was conducted by administering chitosan membrane and Bet; each of them is administered separately; another group of rats received both medications by chitosan membrane impregnated with Bet. The study's findings after eight weeks that followed the surgical approach showed that the group of rats that obtained both chitosan membrane impregnated with Bet according to histological assessments improved significantly compared to other rats in the nervous fibers, fiber sizes, and myelin sheath thickness. Eventually, the study demonstrated that Bet and Chitosan membrane have beneficial effects when used synergistically on the transected sciatic nerve by accelerating regeneration (Moattari et al., 2018).

Another spellbound study aimed at testing the role of Bet and nerve growth factor (NGF) in promoting nerve regeneration; it focused on studying the exact ultrastructural effects under an electron microscope in rat model study. The rats were first subjected to a crush nerve injury, after which they were divided into groups based on the therapeutic's design. The experiment findings showed that as compared to the experimental control and other treatment groups, the number of axons, axon diameters, and myelin thickness was significantly higher in the combined therapy group (Bet 2 mg/kg/day administrated subcutaneously and NGF); hence, Bet and NGF have a synergistic effect on fostering peripheral nerve regeneration (Sencar et al., 2020). Thus, according to the findings of the studies reported above, Bet has a beneficial therapeutic effect in promoted nerve regeneration and preserved myelination when used synergistically with other medications, and that justified the broadly incompatible results with our study's stereological findings, which proved that there is no curative effect in promoting the nerve regeneration.

4.2.4. Methylprednisolone

It is considered the intermedia synthetic glucocorticoid, which for its anti-inflammatory and immunosuppressive action, belongs to the pregnane steroid hormone resulting from hydrocortisone and prednisolone (Timmermans et al., 2019). Mps is used to treat symptoms such as arthritis, blood disorders, acute allergic reactions to certain tumours, skin diseases including dermatitis, and intestinal and pulmonary diseases, as well as immune system defects; according to recent studies, Mps nowadays has been used extensively to treat serious COVID-19 infections (J. Liu et al., 2020).

According to the stereological results, the mean of myelin sheath thickness and axonal area in the Inj+Mps group has no significant statistical difference compared to the Inj group; That is similar to the mean of myelinated nerve fibers also had no significant statistical difference in terms of the number of myelinated when compare with the Inj group depending on stereological findings of the current study it proved that Mps is acting negatively to enhance the peripheral nerve regeneration.

A number of studies have been performed to examine the impact of Mps on the promotion regeneration of the injured peripheral nerve; the first study results contravene with the results of the current study, which aimed basically to histopathologically assess the effects of Mps and Ozone on promoting functional recovery of the sciatic nerve after crush injury. The study was conducted on the rat model, where animal groups are divided according to the treatment used in the experiment. The first group received 2 mg/kg Mps intraperitoneally and compared the result with other groups treated with Ozone solitary and duel medication groups formed by the previous medications together. The results of the study revealed significant differences in the beneficial effect on nerve sheath atrophy, cellular intraneural inflammatory infiltration, and granulation of perineural tissue growth, perineural vascular supply proliferation, inflammatory perineural neuronal invasion, and peripheral tissue inflammation in the group of rats who received the combined medications (Mps and Ozone) (Ozturk et al., 2016).

Another study with a different administration route gave contraindicated results compared to current findings, proving that Mps promotes peripheral nerve regeneration; the study was conducted using a chitosan conduit loaded with an aquatic Mps solution. Furthermore, after measurements for regenerated fibers in the group obtaining Mps, the morphometric findings suggest a comparatively high number and diameter of myelinated fibers. Thus, the outcome of these studies can be perfectly described in that Mps can contribute to the therapeutic effect of peripheral nerve regeneration when used topically (Mehrshad et al., 2017).

Another exciting comparative study aimed to evaluate the effect of steroid medication paralleling to traditional or herbal drugs in promoting peripheral nerve regeneration. The study was conducted by administrating 1-mg/kg Mps as a steroid medication in the cont group; the other groups received traditional drugs, namely Propolis and Curcumin, separately. It is worth noting that Propolis and Curcumin have antioxidant, anti-inflammative, immunomodulatory, and neuroprotective features. The findings of the study are based on the histomorphometric, electron microscopic as well as functional factors. The histomorphometric results of the Myelin thickness, axon diameter, nerve diameter revealed no significant difference between groups treated with Mps and other groups treated with traditional medicine compared to the injured group. Thus, according to the findings of this study, traditional medications acting as an antioxidant can be used to supplement routine steroid medication (Mps) in promoting the regeneration of the injured peripheral nerve (Yüce et al., 2015). Therefore, this study might conclude that Mps has no therapeutic benefit when used solely, which confirms the study's present findings.

An experimental study conducted (*Serdar et al)* in line with the current study that manifests the limited effect in promoting myelin and axonal regeneration. The study was essentially directed to evaluate the impact of aminoguanidin, melatonin, and Mps on peripheral facial nerve neurorrhaphy. This study was relatively different from its predecessors, where it proved that the therapeutic effect of Mps is relatively limited compared with aminoguanidin and melatonin. The experiment was carried out on the buccal branch of the facial nerve after exposure to crushing injury. The study was conducted by administrating 1 mg/kg of Mps, and the other comparative groups received aminoguanidin and melatonin by intraperitoneal route of administration. The histological findings of the study revealed the remyelination process had initiated, and the typical myelin structures could be seen in the aminoguanidin group; it also showed that the myelin debris was uncommon in this group, and there was very limited myelin degeneration; also aminoguanidine showed the most efficient effect of preventing degeneration of myelin and reducing myelin debris aggregation. In this manner, it causes preventing axonal degeneration, and rises in the quantity of collagen fibers are prevented. On the other hand, the group treated with melatonin was more limited axonal degenerative behaviour and shows a rise in collagen fibers that cause slows in neural recovery. The surprising result was that the myelin and axonal degeneration in the group treated with Mps was more significant, and myelin debris accumulated more frequently in this group; despite the fact that this group was effective at preventing the growth of collagen fibers, the propagation of Schwann cells in this group was found to be worse than in the Cont group as well as other groups (Yanilmaz et al., 2015a). Therefore, this study can be summarized by knowing the limited effect of Mps in promoting myelin and axonal regeneration therapeutic effect compared with aminoguanidin and melatonin.

Another study also aimed to evaluate the possible effect of Mps, but this study was conducted on spinal cord injury based on stereological analysis. The study was based on the anti-inflammatory as well as the antioxidant activity of Mps. The Mps was administrated to an experimental group that received spinal cord injury. The stereological analysis was conducted via the optical fractionator by counting the total number of oligodendrocytes in the dorsal funiculus. The results of the study revealed that the number of oligodendrocytes has dramatically increased. However, the number of neurons remained constant. These findings suggest that Mps selectively prevents oligodendrocyte cell death rather than neuronal cell death through a receptor-mediated action, which may explain why it has a minimal protective effect at the level of spinal cord injury (J.M. Lee et al., 2008).

In this context, Mps, according to some literature, has possible effect in promoted nerve regeneration and preserved myelination of the sciatic nerve following injury by preserving and reducing nerve fibers' damage, and the effect extends up to the number of oligodendrocytes in the spinal cord injury; on the other hand, other studies have been proven counterproductive results that Mps were acting negatively or with limited effect to enhance the peripheral nerve regeneration this seem to be broadly compatible to the current study according to stereological findings.

4.2.5. Functional Assessment

The second part of the study involved the functional assessment in evaluating nerve regeneration; histomorphometry and functional measurements are widely used; functional measurements include electrophysiological EMG and Sciatic SFI measures. The use of both techniques results in a precise result. The functional approach alone is insufficient; however, its use is simple, secure, and does not necessitate animal sacrifice. Furthermore, the correlation between histomorphometry and functional assessment is arguable; some researchers have found a correlation between the two approaches, whereas others have found a variance (T. Wang et al., 2018). In the current study, we have used functional assessments EMG and SFI in addition to stereological analysis methods as well as light and electron microscopic analyses.

In the present study, however, the SFI walking tracks were analyzed, and the footprints of the rats' posterior extremities were coated with ink were obtained and analyzed, using the comparison footprint between the right and left posterior extremities for each animal, and the findings are assessed with a special formula. Thus, SFI provides details about the grade of functional rehabilitation or regeneration after sciatic nerve crush injury. Furthermore, the current study statistical analysis revealed significant differences between the mean value of SFI in Inj + Dex and Inj + Mps groups compared to the Inj group, which concluded the positive potential effect on nerve regeneration by restoring the motor activity. At the same time, the Inj + Bet has no significant difference. It is noteworthy according to the results obtained, none of the drugs examined was able to enhance the CMAP's amplitude or latency; this is statistically evidenced by non-significant differences observed. These surprising outcomes might be related to the immaturity of the nerve fibres formed after the crush, as seen by their thin myelin sheath (Fang et al., 2013).

Several studies were conducted to evaluate the effect of Dex in promoting peripheral nerve regeneration based on functional assessments as an evaluation factor; one of these studies mention earlier came in line with the current study and reported that topically administered of Dex through silicon conduit revealed that a group of rats treated with Dex had an SFI mean value -51.3 ± -2.17 while the compare group showed mean value -57.1 ± -4.10; that can be translated based on statistical analysis that the nerve function recovery differs significantly between the Dex treated group, and the other group which means Dex topically promoted the functional recovery of injured sciatic nerve significantly (Mohammadi, Azad-Tirgan, et al., 2013); which is broadly compatible with the current study.

Another study was conducted and suggested the hypothesis of the therapeutic effect of Dex based on SFI assessment in promoting peripheral nerve regeneration. The study aimed to evaluate the impact of different steroid doses. Experimental rats received crush nerve injury and divided into groups depending on the daily dose of Dex (0.5mg/kg), (1.0mg/kg), and (2.0mg/kg), respectively, were injected into the injured site and compared with the Cont group. The SFI was conducted in weekly sequences. The results of 7 days showed a dramatic decrease in SFI values which concluded the total loss of function; also, the findings of the 21 days revealed the insignificant difference between the treated groups compared to the Cont group. However, on day 28, the groups treated with Dex showed the following values to −58.15 ± 3.1, −56.56 ± 1.7, and −56.07 ± 3.5, respectively; these results are considered to be significantly higher than the Cont group. These functional findings indicate that Dex facilitated regeneration in the injured sciatic nerves (Feng and Yuan, 2015).

From the studies conducted as well and come in favour with the current study, a comparative experiment aims to compare the effects of topical artesunate on peripheral nerve regeneration to those of topical tacrolimus and Dex by using several criteria; the focus in this section will be on functional assessment include the SFI and EMG. The study conducted induced a crush injury in the sciatic nerve and treated it with tacrolimus, artesunate, and Dex as separate groups compared with the Cont group. The findings of SFI measurements were assessed among tacrolimus, artesunate, and Dex groups exhibited a statistically significant difference with the Cont group, which concluded the positive potential effect on nerve regeneration. Regarding the electrophysiologic findings, amplitude showed differences in statistics between sham, Artesunate, Tacrolimus, and Dex groups are statistically significant (P=.002,.03,.03, and.009, respectively); however, the latency revealed differences between the sham, Artesunate, Tacrolimus, and Dex groups were statistically significant (P=.002, 0.017, 004, and 0.03, respectively) (Uzun et al., 2019). Finally, it is worth mentioning that no studies using EMG to assess the functional effects of Dex on peripheral nerve regeneration have been identified.

Concerning Bet, a number of studies were performed for the purpose of assessing the therapeutic impact in promoting peripheral nerve regeneration as well, using the same assessment criteria. A recent study was conducted to evaluate the effect of Bet and growth factor NGF in accelerating regeneration after induced a peripheral nerve injury. The criterion for measuring depends on SFI in judging the therapeutic effect will be highlighted. The SFI was kept in weekly intervals as following 7, 14, 21, 28, and 35 days. The study revealed that on the 7th and 14th days, there was no substantial functional recovery among the treated groups. It draws concern that functional recovery started to occur on the 21st day, and the regeneration accelerated seemingly on the 28th and 35th days. Although there was no significant difference between the Cont, NGF, and Bet groups, the NGF+Bet group showed significant functional recovery. On the 28th and 35th days, this recovery became more visible. Although the functional recovery percentages in the Cont and Bet groups were similar to each other on day 35, a more substantial improvement was observed in the NGF group relative to the Cont and Bet groups (Sencar et al., 2020). This study is partially consistent with our results; there was no substantial functional recovery among the group treated by Bet. That is justified by drug duration directly proportional to the SFI improvement, so maybe the short duration reflects a lack of significant difference compared to the Inj group.

Rahim Mohammadi found that in his experimental study, findings are inconsistent with the current study; he reported that Bet works as a stimulus regeneration after peripheral nerve injury. The functional assessment of topical administration of Bet promotes peripheral nerve regeneration by injecting the medication in the vein graft. In the study's findings on the fourth week, the SFI mean value was -52.35 ± -3.78 in the group treated with Bet compared to SFI mean value -92.86 ± -3.44 experimental Cont group. Seemingly on the 8^{th}, 12^{th} weeks, the SFI mean value was significantly more than those of the Cont group. According to statistical analyses, nerve activity regeneration was significantly better in the group that received Bet than in the Cont group (Mohammadi, Amini, et al., 2013).

Another study has a contraindicating finding with our study. This study aimed to see whether a biodegradable membrane and Bet could help rats regenerate their transected sciatic nerve. Several evaluation criteria were used in this study; the focus of this section will be on SFI, withdrawal reflex latency WRL, and EMG. Regarding the WRL was achieved by using the hot water bath, the examination was done in weekly intervals as following 2, 4, 6, and 8 weeks; the findings considerably reduced in therapeutic groups, especially those treated with chitosan membrane combined with Bet, as compared to Cont, Bet, and chitosan membrane groups. Regarding the latency and amplitude of the EMG in the group received with chitosan membrane combined with Bet increased relative to the other groups of chitosan membrane and Bet. Seemingly the SFI also statistically demonstrated the significant difference in the group treated with chitosan membrane combined with Bet. The study concludes that the beneficial effect of the chitosan membrane combined with Bet promotes nerve regeneration in the injured sciatic nerve (Moattari et al., 2018). However, it must be taken into consideration that the route of administration plays an important role in SFI mean result as mentioned above.

Likewise, another study that came in discrepancy lines, using the same criteria in the evaluation, yielded the same results that support Bet's therapeutic effect. The study analyzed the effect of Bet and amniotic membrane in repairing the injured sciatic nerve. The study depends on the following criteria: measurements of WRL as well as the electrophysiological results, the SFI via the walking track analysis, and the histological evaluation of the sciatic nerve, which had been already discussed earlier. Regarding the SFI values in the amniotic membrane combined with the Bet, the group declined four weeks after surgery relative to the Cont and sham groups. SFI values in the amniotic membrane combined with the Bet group declined six weeks after surgery compared to the Cont and sham groups. Enhancement in SFI was reported in all animals eight weeks after surgery, particularly in the amniotic membrane combined with the Bet group compared to the Cont and sham groups. At eight weeks after surgery, the WRL findings were statistically substantially lower in the group administered with amniotic membrane coupled with Bet relative to the Cont, sham, amniotic membrane, and Bet groups. Concerning the electrophysiological results, after eight weeks followed the surgery, based on the statistical analysis, the intact group was significantly different from the Cont and sham groups in latency and amplitude. The latency recovery index of the amniotic membrane combined with the Bet group dramatically decreased compared to the Cont and sham groups. However, the amplitude recovery index in the group treated with amniotic membrane paired with Bet was significantly higher than in the Cont and sham groups. In conclusion, in this study, utilising functional assessment found that the amniotic membrane and Bet had a beneficial impact on an injured sciatic nerve's nerve regeneration (Sadraie et al., 2016).

The systemic administration of Bet was considered in the literature. However, it generated contraindicating outcomes; when the SFI was used as an assessment factor in rats with an induced crush sciatic nerve injury. Therefore, the experimental animals received Bet via the subcutaneous route of administration. According to the results obtained from SFI testing, that performed for six weeks, twice weekly. According to the findings, Bet positively impacts the regeneration of the wounded sciatic nerve; this was proven by the statistical results (Al-Bishri et al., 2005).

With regards to the Mps, it is one of the steroids medications that has been studied, and its therapeutic role is assessed in promoting peripheral nerve regeneration utilizing functional assessment as part of the evaluation criteria. The study aimed to evaluate Mps-laden hydrogel's effect in promoting peripheral nerve regeneration after inducing peripheral nerve injury. Study findings that include SFI and electrophysiological values showed rapidly regenerated axons in the administered Mps Group than the CHIT/Hydrogel group is verified; These results began to emerge in the fourth week following the surgical approach. The mean value of SFI in the Mps treated group was -69,5 ± -2,39 in comparison with CHIT/CGP-Hydrogel, equivalent to -91,67 ± –3,22. However, an increase in SFI in the Mps treated group -55.3 ± -2.25 was significantly higher than the CHIT/CGP-Hydrogel treated group -75.2 ± -3.39 were noted eight weeks after surgery. The average SFI value for the CHIT/Mps group was -38,4 ± -3,57 12 weeks following the surgery, while the average SFI values in the CHIT/CGPHydrogel treated groups were -57,1 ± -4,10. At the end of the study, the mean value for CHIT/Mps animals 16 weeks after the operation was -29,5 ± -3,75 SFI while being in the CHIT/CGPHydrogel group; it was observed that the mean value was -46,3 ± -3,43. Eventually, the statistical analysis showed that the regeneration of nerve activity was significantly better in the CHIT/Mps group compared to CHIT/CGP-Hydrogel group (Mehrshad et al., 2017). This endorses our findings since we observed a significant difference between the Inj+Mps group and the Inj group.

A supplementary study comes in the same line as the present study and aims to compare propolis, curcumin, and Mps's effects in promoting nerve regeneration after inducing a crush injury. However, only the functional criteria, specifically Electrophysiological assessments and study of the walking track, will be addressed in this section. The SFI values' findings were tested in all experimental groups (propolis, curcumin, and Mps) and statistically revealed significant variations compared with sham and Cont groups. Concerning the electrophysiological measurements when comparing amplitude values from surgically treated groups, little difference was observed between both the curcumin and propolis groups; however, the experimental, as well as the Cont groups, had statistically significantly increased values. There were no significant differences between the Cont groups. In both the Cont and study groups, latency times were observed to be prolonged in limbs that had surgery. Limbs with and without surgery have shown no differences throughout the sham group. There was no difference among shame groups, Mps, curcumin and propolis when comparing latency times test with experimental groups in the limbs subjected to the operation, which statistically was higher than Cont group. In conclusion, as mentioned previously, traditional medications acting as an antioxidant can be used to supplement routine steroid medication (Mps) in promoting the regeneration of the injured peripheral nerve; this was proven by functional assessment (Yüce et al., 2015). It is worth noting that almost no research employing EMG to evaluate the functional effects of Mps on peripheral nerve regeneration with the same experimental protocol have been revealed.

4.2.6. Microscopic Evaluation

The third part of the study entailed microscopic evaluation; the histological examination is used to determine peripheral nerve regeneration following the crush injury. The histological image gives a simple vision of the components of the tissue. Histological sections show microscopic modifications. However, the histological examination of nerve tissue reveals the microscopic configurations of degenerated in addition to regenerated that occurs in the peripheral nerves. Peripheral nervous injuries lead to numerous histologic changes, such as degenerative alterations in Schwann cells, including organelles accumulation of the cytoplasm, myelinated nerve axons, vacuoles; besides, a loss of myelin triggered by poly-axonal Schwann cells is defined as a nerve injury and increase in macrophage count (Arikan et al., 2016). The axon and myelin sheath degenerated after peripheral nerve injury, and the myelin thickness was declined after WD. The axons in the distal segment are more significant than the number of axons in the next segment, so axonal sprouting from the existent axons is generated so that some would achieve their destination. The presence of macrophages is an indication of nerve degeneration, and WD exists distal to the injury site. Schwann cells and macrophages destroy tissue waste, and therefore the presence of macrophages is evidence of nerve degeneration. Following injury, many alterations arise in the neuronal cell body, including cell swelling and chromatolysis, structural changes. Furthermore, the nucleus moves to the cell's periphery, the size of the nucleus and nucleolus expands, and the dendrites diminish (Navarro et al., 2007).

Concerning the evaluation by electron or light microscopy of peripheral nerve tissue is dependent on peripheral nerve structures that are visible and analyzed in the tissue. The consistency of the myelin sheath and the diameter of the myelinated sheath of axons visible in the peripheral nerve histological section are the most significant configurations for microscopic examination of the nerve. In the peripheral nerve section, the type of cells that can be found in various histological sections sites, along the lines of macrophages, Schwann cells and mast cells. In evaluating the peripheral nerve condition, it is necessary to observe the connective tissue, which can be seen particularly in epineurium. However, blood vessels and blood cells are also taken into consideration when assessing the peripheral nerve. The present study used light microscopy and electron microscopy of the sciatic nerves to assess structural changes in degenerated and regenerated nerve fibers.

4.2.7. Light Microscopic Findings

In this circumstance, the sciatic nerve structure appears to be ordinarily apparent within the Cont group. Myelinated nerve axons and the myelin sheath that wrapped myelinated axons were both found to be normal. The epineurium surrounding the nerve has been shown to be pretty healthy, too. In addition, the Cont group had regular blood cells besides blood vessel structures.

However, there was a significant decay in the general structure of the sciatic nerve in the Inj group. When comparing the Cont versus Inj groups, striking variations were observed due to the induced crush injury. The Inj group showed in this regard that the architecture of the sciatic nerve had a deterioration; morphological alterations were shown to be abnormal: the thickness of the myelin layer had been reduced; the myelin layer was dissociated, and the epineurium had been destroyed. The amount of connective tissue and adipose cells were observed to be increased in the amounts in the Inj group. Furthermore, macrophages were seen clumped together at the site of nerve injury (gitter cell), and their cytoplasm was distended attributable to myelin debris ingestion. The existence of a substantial percentage of macrophages, an increase in connective tissue density, and a significant number of Schwann cells are all indicators of nerve degeneration. The crush damage was thought to be causing these degenerative alterations, which were due to ischemia triggered by microvascular obstruction.

Based on the stereological analysis of the Inj + Dex group, it was noticed that Dex had a beneficial impact on the regeneration of the sciatic nerve. As appear also in the stereological evidence. However, the microscopic examination of the Inj+Dex sciatic nerve sections demonstrated a promising outcome for sciatic nerve regeneration. The myelinated nerve axons, as well as the myelin sheath, were well maintained in this context. Regarding the connective tissue, epineurium remained seen preserved around the nerve. A significant number of nuclei and macrophages are present at the regeneration site and indicate the ongoing phase of nerve repair and regeneration. Regarding the blood cells were shown to be regular, suggesting a normal blood supply within the nerve. Although the majority of myelinated nerve axons were shown to be intact, others were found to be damaged. The regeneration of the myelinated nerve axon site and myelin density was observed, which might be contributed to Dex's beneficial impact on myelin sheath repair and axon regeneration initiation. Some previous studies have developed an explanation for this therapeutic effect by knowing that Dex blocks arachidonic acid metabolism and all subsequent metabolites through cyclooxygenase and lipoxygenase tracts; Dex has also been documented in laboratory studies on rat gliomas (brain and spinal cord tumors) to decrease tumor-alpha necrosis factor control of subcutaneous tissue and to reduce transcapillary permeability (Mohammadi, Azad-Tirgan, et al., 2013). Also, it had been reported that Dex contributes to a positive influence on afferent and efferent conduction and the enhancement of the brain-derived neurotrophic factor countenance, which may lead to regeneration of myelinated nerve fibers and the growth of Schwann cells (Sun et al., 2012). Previous studies have also proven by the immunohistochemical examination that administration of Dex reduces the number of CD3-positive cells, which is an indicator for T-cell activation and thus contributes to its immunosuppressive activity; It has also been proven that Dex induces increased expression of GAP-43, which considered as a factor that associated with nervous system progression and plasticity by promoting the neurite outgrowth and hence lead to sciatic nerve regeneration (Feng and Yuan, 2015).

It was observed that depending on the stereological analysis of the Inj + Bet group. Bet negatively influences the nerve recovery of the injured sciatic nerve. This group of animals had a fragmented nerve trunk, a less intact epineurium, and a greater distance between myelinated axons. Increased connective tissue and the presence of small blood vessels in the endoneurium indicate this. Macrophages and mast cells were also recruited in large numbers, in addition to Schwann cells. Axons with varying diameters of myelinated axons showed separations and discontinuities in myelin architecture. Myelinated axons revealed a reduction in the thickness of the myelin coating and separation of the myelin sheath, and degenerated axons and myelin debris were seen throughout the section. A number of previous studies have a conflict with this therapeutic effect by knowing that the benefit of the effects on the regeneration of the nerve of moderate perioperative Bet dosage is expressed in macrophage recruitment but in the term, p75 to only a limited degree, which would lead to recovery of the nerve (Al-Bishri et al., 2008). Our findings disagree with the study results conducted by *Mehrnaz et al.* The Bet administration causes a distal segment to be prepared for regenerating axon sprouts through this well-coordinated series of macrophage responses. The macrophage reaction winds down when pro-inflammatory cytokines are typically produced, and antiinflammatory cytokines are significantly increased. Steroid therapy has been shown to reduce inflammation and lipid peroxidation; this suppression reduces functional impairment after peripheral nerve injury and accelerated nerve regeneration (Moattari et al., 2018). Correspondingly, it has been reported that Bet can inhibit the phospholipase A2 activity, which is an enzyme that can participate in the degradation process in WD of the peripheral nerves subsequently lead to accelerating the peripheral nerves regeneration (Sadraie et al., 2016; H.-M. Lee et al., 1998). It has been reported that prescription of steroid medication, namely Bet plays an essential role after surgical procedures in the sciatic nerve in order to eliminate postoperative edema and thus suppress the inflammatory response; hence, macrophage engagement occurs (Al-Bishri et al., 2005) as occur in the current situation. In conclusion, the therapeutic efficacy of Bet in promoting peripheral nerve regeneration is the subject of a debate between research among both proponents and detractors.

According to the stereological investigation of the Mps+Inj group, Mps was discovered to have a negative impact on the nerve regeneration of the injured sciatic nerve. Microscopic examination of sciatic nerve sections in the Mps+Inj group revealed an increased influence on sciatic nerve regeneration. Individual fascicles were visible with their connective tissue, epineurium surrounding the nerve, and perineurium intact. There were also several new blood vessels formed. In addition, axons with a thin myelin sheath were seen. The appearance of high numbers of macrophages with expanded cytoplasm suggested that they play a role in engulfing degenerated axons and eliminating myelin debris, which helps avoid scar formation and promote axon regeneration. Over the injury areas of the section, the nucleus of the Schwann cell was seen in association with degenerated axons and myelin debris. Although some myelinated axons were impaired, and myelin thickness was reduced, Mps had a beneficial influence on the development of minifascicles made up of axonal sprouts, as well as a high number of active Schwann cells generating myelin sheath surrounding them. However, such regeneration processes were unable to achieve statistically significant levels.

These findings are in the same line with another study conducted by *Karlidag et al.* and proposed the same findings of the present study, that there is no therapeutic effect as well as a rise in the amounts of myelin debris when using Mps in the injured facial nerve; on the other hand, the study revealed that Mps treated group shows enhance in nerve growth according to the results of immunohistochemistry (Karlidag et al., 2012). Furthermore, another study showed that Mps therapy increases motor neuron activation in vitro, suggesting that it can serve as a neurotrophic factor this would lead to enhancing peripheral nerve regeneration (Mehrshad et al., 2017). Additionally, *Yüce et al.* demonstrated how Mps had a positive impact in promoting peripheral nerve regeneration; Mps is classified as a therapeutic agent due to its anti-inflammatory and antioxidant properties, which also aids in the maintenance of blood flow and the elimination of lactate levels. However, Mps reduces the generation of free radicals following a traumatic event and assists in the recovery of neurological function. Within the tissue, it reduces malondialdehyde concentrations, prevents the GSH-Px enzyme, and inhibits the superoxide dismutase enzyme (Yüce et al., 2015).

In general, it is worth noting that glucocorticoids are anti-inflammatory agents which commonly used to treat tissue oedema and traumatic inflammation by inhibiting the release of pro-inflammatory factors like tumor necrosis factor-α also the interleukin-1ß (Li et al., 2016). These factors might enhance the expression of triggered nitric oxide formed in the injured tissue, resulting in increased nitric oxide synthesis and leading to cellular apoptosis (Regina et al., 2009). In the central and peripheral nervous systems, glucocorticoids can achieve anti-inflammatory actions through a number of mechanisms. First, however, glucocorticoids can make inhibition of CD3, which reduces nerve inflammation. This follows by the invasion of positive inflammatory cells into local tissue. In terms of anti-inflammatory impact, phospholipase A2 inhibition as well as the changes that take place in the intercellular structures such as suppression of macrophages migration and the stabilization of lysosomal membrane. All these events would be helpful for treating the injured nerve (A. Mekaj and Mekaj, 2017; Feng and Yuan, 2015).

Regarding the Cont group that received Dex (Cont + Dex) with the same treatment protocol, it showed no significant variation compared to the Cont group. That is affirmed via light and electron microscopic examinations, which showed an ordinary thickness of the myelin sheath. However, the myelinated axons and the myelin thickness are highly shielded and protected. Moreover, in myelinated nerve fibres, epineurium is well preserved, and the presence of Schwann cell nuclei besides the macrophages outweighs axons health. Concerning blood cells, it was clearly observed that ensuring adequate access of blood supply to the nerve.

According to the Cont + Bet group results, it was identical to the previous one; it was most similar to the Cont group in terms of histological composition. That indicates the protective role of Bet. The microscopic findings demonstrated that the thickness of the myelin sheath was found to be normal. Myelinated axons and myelin thickness, on the other hand, are well shielded and secured. Furthermore, epineurium is well maintained in myelinated nerve fibres, and the presence of Schwann cell nuclei in addition to macrophages make up a guarantee of axon protection. Where it comes to blood cells, it is evident that providing sufficient blood flow to the nerve tissue.

Even in the case of the Cont + Mps, the histological composition was similar to that of the Cont group by means of microscopic analysis, which showed the following: The myelin sheath thickness was found to be regular. On the other side, myelinated axons and myelin thickness are well protected. In addition, epineurium is well preserved in myelinated nerve fibres, and the inclusion of Schwann cell nuclei and macrophages ensures axon preservation. Where it comes to blood cells, it is identity the appropriate blood supply to nerve tissue. From the foregoing, it can be asserted that Mps has a protective effect in protecting the peripheral nerve.

We can generally say that the majority of the nerve components were observed normally in the Cont group. However, the majority of the nerve structures of the Inj groups were disturbed, including the myelin sheath and the axonal area. On the other hand, the histological architecture of the nerve was, to a large degree, appeared healthy in the positive Cont groups that treated by Dex or Bet, or Mps. In addition, the injured groups treated by Dex and Mps were shown to be protected, and the emergence of clear nerve regeneration milestones despite the existence of some damaged nerve configurations in certain areas of the nerve.

4.2.8. Electron Microscopic Findings

TEM provided high-resolution pictures of the nerve repair and regeneration processes, allowing researchers to better understand the processes. Treatment with

Dex accelerated the regeneration process in the present investigation, according to TEM imaging results.

In general, we found a normal appearance and healthy components of the sciatic nerve in the Cont group or treated groups that were not subjected to crush injury, Dex, Bet, or Mps groups, with some myelin degradation in the Dex, Bet, or Mps groups. This might be due to poor tissue fixation or other unknown causes. Most nerve structures were observed to be impaired in the Inj groups, including the myelin sheath, axonal area, and connective tissue scaffold. Even though the current study reveals that Bet and Mps have no impact on the crush-model of the sciatic nerve based on stereological analysis. The Inj groups treated with Dex, Bet and Mps had favourable outcomes based on axon number and microscopic assessment. The findings of functional evaluations, particularly the SFI, confirmed these findings in the case of Inj groups treated with Dex and Mps.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

- According to a stereological study, Bet and Mps medications had no impact on nerve regeneration in the crushed sciatic nerve as measured by the number of myelinated axons, axonal area, or myelin thickness.
- According to a stereological study, Dex medication has a favourable effect on the mean number of myelinated axons in the crushed sciatic nerve.
- When the Inj+Dex and Inj+Mps groups were compared to the Inj group, LM and TEM examination revealed a beneficial outcome for Dex and Mps on peripheral nerve regeneration in sciatic nerve section images.
- Based on SFI, walking track analysis reveals functional improvement in the Inj+Dex and Inj+Mps groups compared to the Inj group.
- Electrophysiological analysis of the Dex, Bet, and Mps muscle compound action potentials showed no enhancement in amplitude or latency.

5.2. Recommendation

- Further studies utilising unmyelinated axons and Schwann cells are required to determine Dex, Bet, and Mps impact on peripheral nerve regeneration following crush nerve injury.
- We recommend conducting more studies with different parameters, such as immunohistochemistry.
- Conduct more similar studies using other glucocorticoids medication.
- Perform additional studies with different dosages and durations.
- Conduct additional research using other routes of administration.

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APPENDICES

Ethical Committee Approval Certificate

T.C.
ONDOKUZ MAYIS ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu

Sayı : 68489742-604.01.03-E.6026 12/03/2019
Konu : Hadyek izini hk.

DOÇ.DR.MEHMET EMİN ÖNGER

Laboratuvar Hayvanlar üzerinde Araştırma amaçlı çalışma yapmak üzere başvuran Doç. Dr. Mehmet Emin ÖNGER'in 2018/45 Kabul nolu **"Sıçanlarda periferik sinir rejenerasyonu üzerine deksametazonun muhtemel koruyucu etkilerinin değerlendirilmesi"** 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.

e-imzalıdır
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Evrak teyidi https://ebysorgu.omu.edu.tr adresinden 03VJ-DYVN-0EZ7 kodu ile yapılabilir.

CURRICULUM VITAE

Mohammed Hamid 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. He speaks English and Arabic (mother language) at a good level. He is married and has one boy and two girls.

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