



Regeneration of Nerve Injuries using Low Power Laser Irradiation

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ABSTRACT

The experimental study of the effect of low power laser irradiation (LPLR) on nerve injury histologically and histochemically had been carried out. Ten dogs were used in this study and divided into two groups, control and experimental. Surgical exposure of their mental nerves was done for all in the same way. In the experimental group, the exposed nerve was subjected to laser irradiation for 5 minutes following the application chart of the device for neural disorders, and then the animals were scarified at 1, 3, 7, 14, 30 days intervals. The control group were followed the same regime but without laser radiation. Biopsies were taken for light microscopy study (H&E and silver impregnation stain), electron microscopy study and histochemical study (OTAN and Gomori's acetyl thiocholine methods) was used. The obtained results were as follow: temporary mild degenerated traumatic changes occurred at the first, third- and seventh day following laser radiation. Regeneration started at 14th day with complete regeneration at 30th day. It can be concluded that laser therapy enhances recovery of injured irradiated nerve without any residual effects.

Keywords: Low Power Laser Irradiation, nerve regeneration

1. Introduction

In a surgical procedure in any specialty of dentistry there is an inherent risk of damage to peripheral. Nerve's injury not only produces very rapid analgesia, but also causes intense pain and a profound numbness which may persists for a few days or several weeks. Sunderland (Sunderland, 1978) classified the nerve injury into five degrees depending on the nature and extends of the damage to the internal anatomy of the nerve trunk, in the first-degree injury; there is interruption of conduction at the site of injury while axonal continuity is preserved. The function is rapidly restored. No Wallerian degeneration. In second-degree injury, there is axonal disintegration, breakdown of myelin sheath and Wallerian degeneration. The third degree- injury is similar to the second, but there is disorganization of the internal structures. Fourth- degree injury, this results from deep trauma extending to the perineurim and Wallerian degeneration. In the fifth- degree injury, there is a complete transection and loss of continuity of the nerve trunk. Another author (Hafttek and Thomas1968) agrees to classify the nerve injury into: neurapraxia, axonotmesis and neurotmesis.

Degeneration after nerve injury is a destructive process, but rather a formative process that is an integral part of repair and regeneration (Anders *et al.*, 1993). In the degeneration process, a retrograde neural reaction occurs in the nerve cell or the cell body of the nerve fiber. It involves changes in the structure, biochemistry, and function. The outcome of this reaction may be complete recovery or the persistence of residual defect which adversely affects function of the cell (Barez *et al.*, 2017). The amount of trauma that a cell can stand is limited. If injury is so close to the cell body, the amount of axon to be replaced is great and the prognosis for survival is poor. The most conspicuous cellular changes ⁽⁶⁾ in the initial stages are, early cellular swelling, peripheral migration of the nucleus,

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enlargement of the nucleolus, disappearance of Nissl granules and reduction of the nucleoprotein with 24 hours of transection of the axon. While the area of injury shows a densely packed collagen fibrils and basement membrane. The degenerative changes reached a maximum towards the ends of the first week when the myelin sheath had broken into fragments in the proximal stump. On the other hand, at the distal stump, an irregular outgrowth of loss collagen fibrils arises after one week of nerve injury and accumulation of erythrocytes, leukocytes, macrophages, and column of Schwann cells (Andreo *et al.*, 2017). Two or three days after injury, the stumps are composed chiefly of Schwann cells providing the pathways that guide the regenerating axons across the damaged region through the Schwann tubes. If no axonal penetration occurs in a specific time, these connective tissue pathways disintegrate. The regenerative powers of the cell body and proximal stumps are vary greatly depending on the nature of the injury, level of the injury related to the cell body, type and size of the neuron, age, and phylogeny (Bray and Aguayo 1974). The time of regeneration is allocated into four periods. The first is called initial delay includes cell body recover and axonal growth to the injured area. The second period encompasses the time necessary to traverse the scarred area. The third period is the amount of time necessary for the axon to grow a given distance through the endoneural sheath to the organ. The final period is the time necessary for functional recovery (Camara *et al.*, 2011). Laser light has certain properties that make it useful in dermatologic therapy. Monochromaticity allows for emission of a single wavelength of light determined by the medium (gas, liquid, solid) through which the light passes. Monochromaticity permits specific absorption of laser energy by distinct cutaneous targets or chromophores such as melanin, hemoglobin, or tattoo ink. A second property, coherence, occurs as light waves travel in phase with accordance to time and space. Laser light is also collimated: it is emitted in a parallel fashion through an intense, narrow beam, which allows for the creation of a small, focused spot. Collimation permits propagation without divergence or loss of intensity along the optic fiber. It has been stated that low power laser irradiation (LPLI) of peripheral nerves had the possibility of producing analgesia in a manner similar to that obtained by application of transcutaneous nerve stimulation (Takada 1988). What occurs when a laser beam strikes a tissue depends on the wavelength, intensity and duration of the irradiation and types of tissue, its color, composition, and size. Several studies (Shamir *et al.*, 2011) evaluated the therapeutic effect of low-power laser irradiation (LPLI) on peripheral nerve regeneration and suggested that postoperative LPLI with a wavelength of 780 nm enhances the regenerative processes of peripheral nerves after complete transection. Immunohistochemical staining in the laser-treated group showed an increased total number of axons and better quality of the regeneration process, due to an increased number of large-diameter axons compared to the non-irradiated control group. It has been found that low-power laser irradiation had a statistically significant stimulating effect on collagen synthesis in the wound and the rate of wound closure was enhanced significantly between the 3^{ed} and 12th postoperative days. Injury to a mammalian peripheral nerve is accompanied by a restorative process that is manifested after a delay. This process is expressed morphologically by the emergence of new nerve fibers. Restoration of function occurs when the regenerating fibers reconnect with the target organ (Kana *et al.*, 1981). Because of the low rate of fiber elongation, the denervated target is partially degenerated by the time that the regenerating fibers approach it. To prevent such an atrophy, one must find a way to prevent the degeneration of the nerve, to speed up regeneration, or to maintain the target during the period of nerve degeneration (Rochkind *et al.*, 1987)

In the present work, we examined the potential of treatment with low energy laser radiation for improving regeneration or preventing degeneration of mammalian peripheral nerve after injury.

2. Materials and Methods

Ten healthy dogs, with average weight 11kg were used in this study. They were divided into two groups, a control, and an experimental group. Both groups were subdivided into five subgroups according to scarification date after exposure to laser irradiation: after 1, 3, 7, 14, and 30 days. The type of laser used was of pulsed low-power irradiation of semiconductor medium, Gallium Arsenide (Ga As), 904 nm wavelength (infrared) and energy output of 14 mW. Under general anesthesia, a mucoperiosteal flap was carried out in the premolar region to expose the mental nerve. The laser beam was applied in direct contact way at the working area for 5 minutes at frequency position no. 9 according to the application chart of the device for neural disorders then the flap was repositioned and

sutured. In the control group, the mental nerve was exposed surgically without exposure to laser irradiation. The animals in both groups were killed according to scarification date. For histological study, H& E and silver iron impregnation stain were used for neural tissue Electron microscopic preparation for the specimens. For histochemical study, osmium tetroxide naphthylamine (OTAN) method for detection the phospholipids contents of the myelin sheath. Gomori's acetyl thiocholine method for detection the acetyl cholinesterase activity (Drury 1976).

3. Results

The microscopic findings of the present work were compared based on the degree of degeneration and regeneration, which may occur as a result of the laser irradiation.

3.1. Histological results

A) H&E stains

In the control group, the nerve fibers have a snake-like appearance, snake-like manner with scattered thin nuclei. (Fig.1A). While in experimental group; subgroup 1&2 showed some degenerative traumatic changes with loss of the snake-like appearance and fragmentation of the axons and myelin sheath. (Fig.1, A, B). In subgroup 3 shows less degenerative changes where some axons being swollen (Fig.1, D). In subgroup 4&5 the nerves start to show signs of regeneration in the form of completing the continuity the nerve fibers, increase the number of macrophages and restoring the normal architecture (Fig.1, E &F)

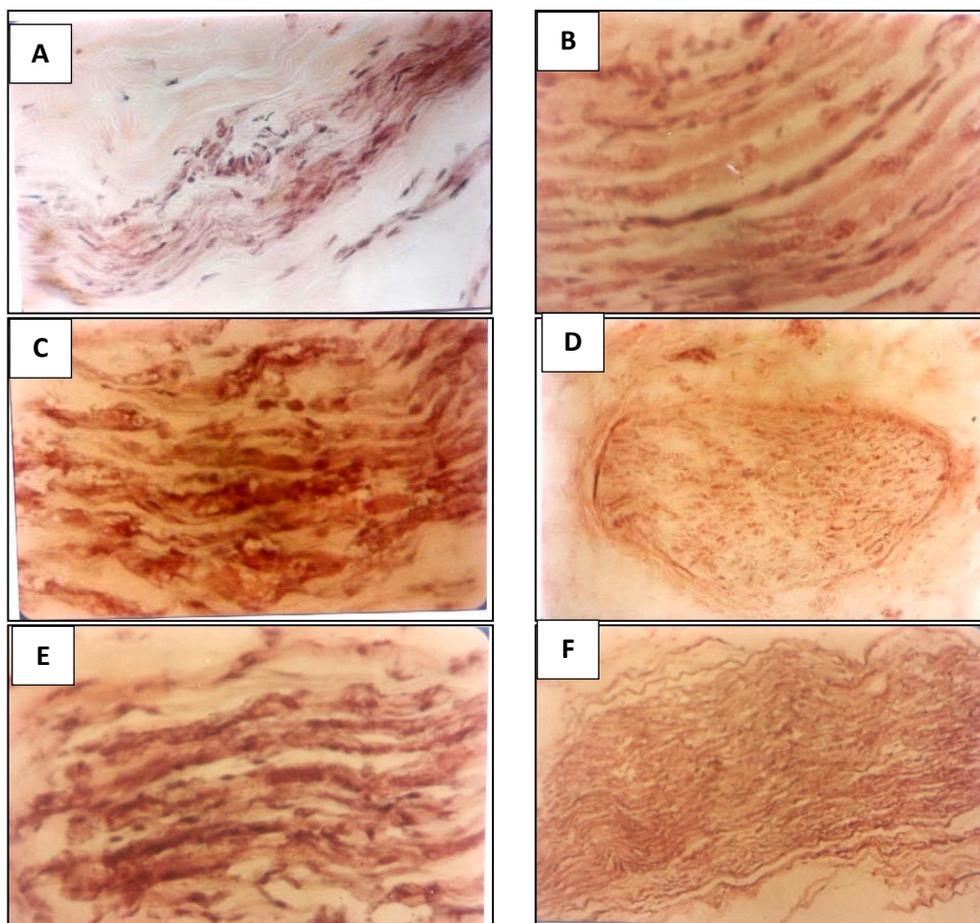


Fig.1: **A.** Normal snake like appearance of the mental nerve with abundance of Schwann cells at the periphery. H&E (X 100). **B.** Nerve at the 1st day of laser irradiation showing degenerative traumatic changes with fragmentation of the axons and myelin sheath. **C.** The nerve at the 3rd day after laser application still showing degenerative changes. **D.** At 7th day after irradiation, most of the axons being swollen with degenerative changes. Other axons appear normal. **E.** At 14th day after irradiation, the nerve starts to show regeneration with accumulation of macrophages. **F.** The nerve at 30th day after irradiation shows complete healing and restoration of the normal snake-like appearance H&E (X 400).

B) Silver impregnation method

In the experimental group, subgroup 1 &2 showed axonal degeneration with discontinuity of myelin sheath (Fig. 2, A). In subgroup 3, the neurofibrils started their continuation in most of the nerve fibers (Fig. 2, B). In subgroup 4 &5 axonal regeneration was present with normal corrugated appearance (Fig.2, C).

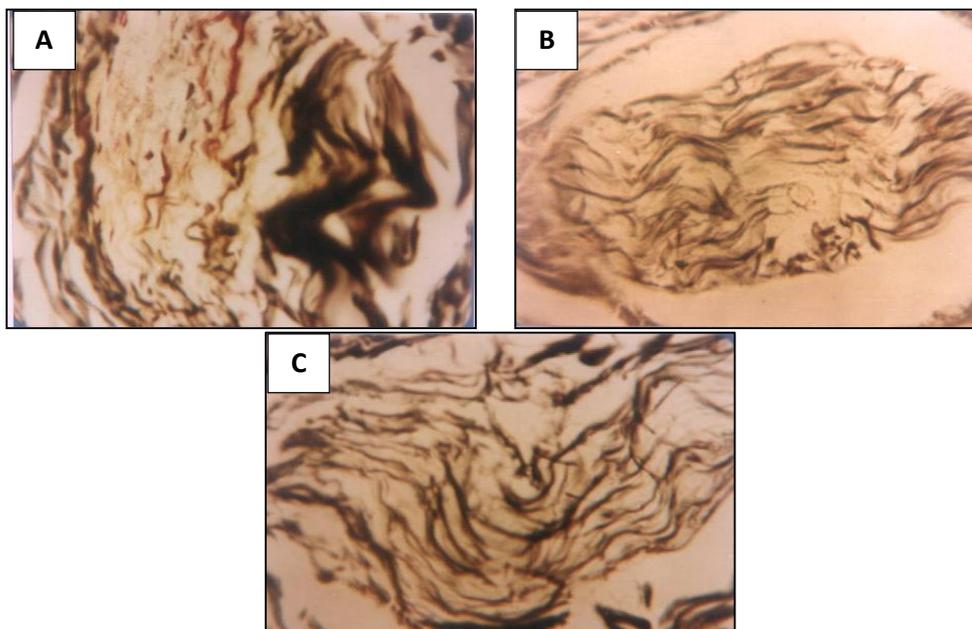


Fig. 2: **A.** Subgroup 1&2 showing discontinuity of the neurofibrils of the axons and some axons are completely disappear. **B.** Subgroup 3, the neurofibrils started their continuation in most of the nerve fibers. **C.** 30th day of nerve irradiation, the neurofibrils appeared normal with the corrugated snaky appearance Silver stain (X 400).

3.2. Electron microscope results

At the first and third day of laser exposure to the mental nerve, there were axonal disintegration, break down of the myelin sheath and disorganization of the internal structure of the nerve (Fig. 3 A&B). Later, at the 14th and 30th day after laser irradiation, there were neural regeneration and remyelination (Fig.3 C& D).

3.3. Histochemical results

A) Osmium tetroxide naphthylamide (OTAN) method

In the control group, the phospholipids contents of the nerve showed normal continuity around the snaky outline of the nerve fibers (Fig.4, A). In the experimental group, subgroup 1 &2 showed massive degeneration of the myelin sheath and fragmented phospholipids (Fig.4.B). In subgroups 3, there was some swollen myelin sheath, while others showed some degree of myelin continuity (Fig.4, C). In subgroups 4 & 5 where the continuity of the myelin sheath reached its complete normal thickness revealing the process of regeneration (Fig. 4 D&E).

B) Gomori's acetyl thiocholine method

In the control group, there was a normal positive reaction for choline-esterase enzyme. In the experimental group, subgroup 1 presented negative cholinesterase activity which indicated a severe degenerative stage (Fig. 5 A). Subgroup 2 &3 exhibited gradual cholinesterase activity (Fig.5 B&C), while subgroup 4 &5 showed strong positive reaction for cholinesterase enzyme and restoration of the normal snaky appearance of the nerve fibers (Fig.5, D).

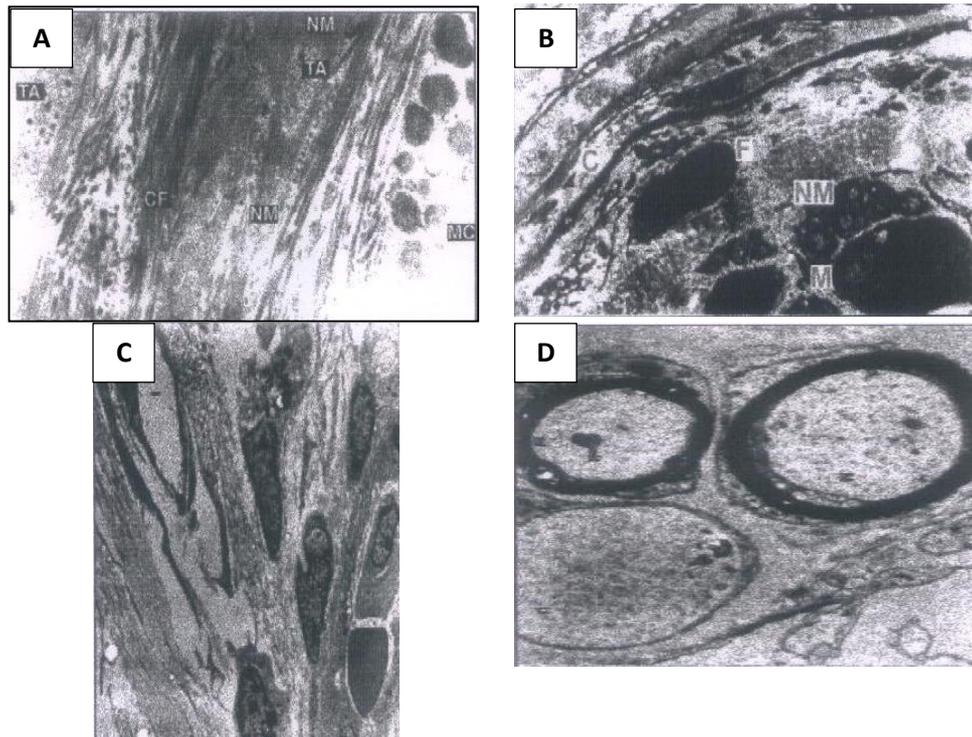


Fig. 3: A. Ultrastructure of the degenerated nerve fibers after one day of laser irradiation showing disorganization of the internal structures and absence of the myelin sheath B. Degenerated nerve fibers at the 3rd day of laser irradiation. Note the presence of swollen axons. C. Regeneration of the nerve fibers after two weeks of irradiation. D. Complete neural reorganization of the internal structures and regeneration

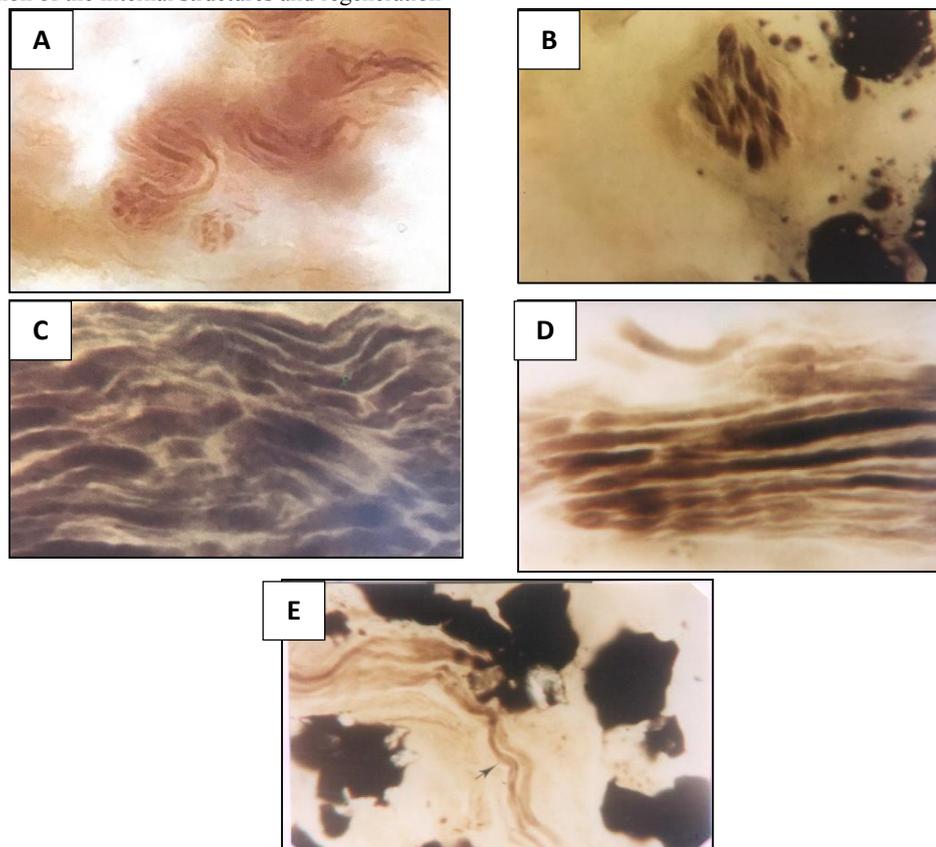


Fig.4: A. Normal nerve exhibiting its phospholipids coating with reddish brown color. B. The nerve at the first day of laser irradiation, a massive degeneration of the myelin sheath is shown with the appearance of swollen brownish particles. C. The nerve at 7th day after laser irradiation, still swollen myelin sheaths are observed D. At the 14th day after irradiation, the process of regeneration started, and the nerve exhibited its normal thickness and continuity. E. 30th day after irradiation, normal snaky appearance revealing remyelination of the nerve. Otan method (X 400).

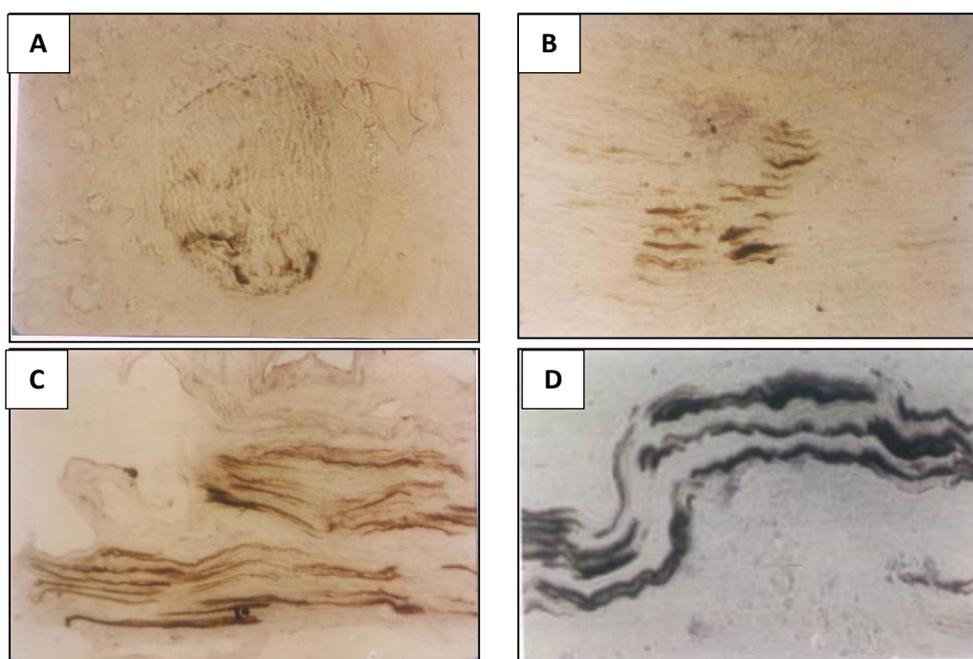


Fig. 5: **A.** First day after laser irradiation, a negative cholinesterase in most of the nerve fibers. **B.** At the third day, there is initial activity of the cholinesterase, while other axons show negative reaction. **C.** At 7th day, moderate positive reaction to cholinesterase with continuity of some axons. **D.** At 30th day, strong positive reaction for cholinesterase and complete regeneration of the nerve fibers. Gomori's acetyl thiocholine method X 100.

4. Discussion

The wound-healing process is divided into 3 stages: inflammation, granulation tissue formation, and matrix remodeling. The initial stage is defined by a structured sequence involving inflammatory cells. This cascade is orchestrated by neutrophils. Subsequently, macrophages elaborate a variety of cytokines, which create an environment amicable to granulation tissue formation. Finally, fibroblasts migrate into the area, proliferate, and recapitulate ontogeny by depositing new collagen, first type III and later type I. simultaneously; angiogenic factors released into the wound environment stimulate formation of new capillaries (Medalha *et al.*, 2012). The histological changes which occurred in the mental nerve after laser irradiation was those of a second degree of injury as described by (Sunderland 1978 and Bradly 1985) found that if the anatomic continuity of the nerve is destroyed, the regeneration is likely to be poor or absent. This observation was supported by the gross examination of sectioned nerves (Mohammed *et al.*, 2007). The histological study shows early edematous and degenerative changes and the neurofibrils lost its normal snaky appearance and continuity with the first 7 days after irradiation, revealing the degenerative actions of the nerve, but they were reversible. After two weeks of irradiation, healing started, and the nerves begin to regain its normal snaky appearance and their continuity. Complete regeneration occurred after 30th days of the laser irradiation. The same results were obtained through the histochemical study for the changes occurred in the phospholipids of the myelin sheath with the OTAN method.

In agreement with the current results, (Rounds *et al.*, 1980) suggested that LPLR He-Ne laser promoted the activity of the irradiated cells due to increase of their metabolic energy. Furthermore, increased collagen synthesis by fibroblasts and increased phagocytic activity of leukocytes, after irradiation of LPIR have been demonstrated by (Bosatral *et al.*, 1984) The direct He-Ne laser irradiation 3.6J/cm² caused a significant amount of sprouting of cellular processes outgrowth in aggregates, compared to small amounts produced by non-irradiated controls. This observation suggests that low power laser irradiation applied to the area of an experimentally injured nerve may induce axonal processes sprouting, thereby improving nerve tissue recovery. The mechanism of low power laser on nerve tissue is not completely understood, but some studies partially explain the photochemical effect of laser irradiation on the biological system. Cytochromes are affected, thereby stimulating redox activity in the cellular respiratory chain, thereby causing increases in ATP

production which activates Na⁺, K⁺ -ATPase and other ion carriers, thereby increasing cell activation (Gigo-Benato. *et al.*, 2010) Histological studies supported the electrophysiological findings: low power laser irradiation was found to prevent or decrease scar tissue formation in the injured area. Laser irradiation enhanced axonal sprouting in the crush-injured sciatic nerve, thus accelerating recovery of the severely injured peripheral nerve. In addition, a beneficial effect of low power laser irradiation was found not only in the laser-treated nerve, but in the corresponding segments of the spinal cord as well. Such laser treatment has been found to significantly decrease the degenerative changes in the corresponding neurons of the spinal cord and induce proliferation of neuroglia, both in astrocytes and oligodendrocytes. This suggests a higher metabolism in neurons and a better ability to produce myelin under the influence of laser treatment. Also, low power laser irradiation exerts pronounced systemic effects on severely injured peripheral nerves and corresponding regions of the spinal cord (Nichols and Lillehe 1988). Immuno-histochemical staining in the laser-treated group showed more intensive axonal growth and better quality of the regenerative process due to an increased number of large and medium diameter axons. Chow *et al.* 2009 observed in the irradiated injured sciatic nerve, the organization seems preserved, nerve fascicles surrounded by strands of connective tissue were seen, most axons were sheathed with heavy myelin and a very few infiltrating macrophages were observed while the non-irradiated injured nerve, there was no organization, nerve fibers seen to be smaller and mostly non-myelinated and numerous macrophages and phagocytes were seen. It was demonstrated that light therapy has an anti-inflammatory effect on the injured spinal cord, and may reduce secondary injury, thus providing a possible mechanism by which light therapy may result in axonal regeneration (Ziago *et al.*, 2017). It has been found that the neurofibrils were structurally regenerated 4 months postoperatively without any treatment. In contrast to this current study, laser will promote the regeneration action of the nerve as it was found that the nerve was structurally regenerated one month postoperatively. Acetyl cholinesterase and acetylcholine are believed to play an essential role in the transmission of the neural impulses. It was found that within the first 7 days, the enzyme activity was negative in most axons indicating termination of neural activity, but at the 14th day after laser irradiation there was a positive reaction for the enzyme indicating that the action potential of the nerve started. At the 30th day, all axons revealed positive reaction for the enzyme, denoting return to the normal activity of the nerve. Therefore, it can be said that low-power irradiation laser brings the action potential of the nerve to its normal condition, but this should be manifested electro physiologically. (Rochkind *et al.*, 1988) in agreement with the previous result and added that the effect of low-power irradiation laser lasted for more than 8 months. It can be speculated that the early temporary damage of the irradiated nerve is directly related to the therapeutic effect of the laser therapy. A pain relief can be determined after the successive applications. The repeated irradiation with He-Ne laser produced relief in subjects with chronic pain. Therefore, we can say that laser is beneficial in the treatment of trigeminal neuralgia. Laser therapy is a low-cost, non-invasive method and will be recognized as standard additional treatment for improving the functional recovery of patients with peripheral nerve and brachial plexus injuries. The main advantages of laser therapy are the enhancement and acceleration of the recovery of injured nerve tissue. (Al-Shammari *et al.*, 2019).

5. Summery and conclusion

The experimental study of the effect of low power laser irradiation (LPLR) on nerve injury histologically and histochemically had been carried out. Ten dogs were used in this study and divided into two groups, control and experimental. Surgical exposure of their mental nerves was done for all in the same way. In the experimental group, the exposed nerve was subjected to laser irradiation for 5 minutes following the application chart of the device for neural disorders, and then the animals were scarified at 1, 3, 7, 14, 30 days intervals. The control group were followed the same regime but without laser radiation. Biopsies were taken for light microscopy study (H&E and silver impregnation stain), electron microscopy study and histochemical study (OTAN and Gomori's acetyl thiocholine methods). The obtained results were as follow: Temporary mild degenerated traumatic changes occurred at the first, third- and seventh day following laser radiation. Regeneration started at 14th day with complete regeneration at 30th day. It can be concluded that laser therapy enhances recovery of injured irradiated nerve without any residual effects.

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