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ORIGINAL ARTICLE

Evaluation of Low-Level Laser on Sciatic Nerve Regeneration in White Male Albino Rat: An Experimental Study

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ABSTRACT

Background: Peripheral nerve lesion is frequent and could result in lifetime disability if untreated. Low-level laser therapy (LLLT) could affect connective tissue cells (fibroblasts) and their functions, accelerate their repair functions and act as an anti-inflammatory agent. The present work aimed to assess the value of using LLLT in peripheral nerve regeneration and how it affects it and to evaluate the effect of LLLT on regeneration of injured sciatic nerves of albino rats.

Methods: In an experimental study twenty-four Sprague-Dawley young adult male rats with an average weight of 250-350 grams were subjected to sciatic nerve transected and repaired primarily using 9/0 Polypropylene sutures under a surgical microscope, that were split into groups: Group (1): (Therapeutic Group): LLLT was applied around epineural repair site. Group (2): (Control Group): Primary epineural repair only, Histologic evaluation was performed using light microscopy where the number of myelinated axons proximal and distal to the epineural repair site was counted by professional examiner who wasn't aware of the nature of the experiment.

Results: High statistically significant ($P<0.001$) increased number of myelinated nerve fibers (proximal) in group 1 (mean= 180 ± 58 and ranged from 84 to 276) was found when compared with that of group 2 (mean= 60 ± 1.5 and ranged from 58 to 62). High statistically significant ($P<0.001$) increased number of myelinated nerve fibers (distal) in group 1 (mean= 247 ± 88 ranging from 113 to 374) was found when compared with that of group 2 (mean= 68 ± 1.7 ranging from 65 to 70).

Conclusions: The study demonstrated that low-level laser therapy (LLLT) significantly enhances sciatic nerve regeneration in white male Albino rats. LLLT could be a promising non-invasive therapeutic approach for enhancing peripheral nerve repair.

Keywords: Low Level Laser; Sciatic Nerve; Nerve regeneration.

INTRODUCTION

Nerves are like cables that carry electrical impulses between brain and the rest of the body, Peripheral nerve anatomy composed of nerve fiber & nerve components; nerve fiber proper; may be myelinated or unmyelinated, schwann cells, and connective tissue composed of: Endoneurium, Perineurium, Epineurium, Mesoneurium [1]. Untreated peripheral nerve lesions are a leading cause of permanent disability and affect 2.8% of trauma patients each year. Regenerating nerve fibers are guided into the correct distal endoneurial tubes using numerous repair procedures nowadays. Methods as like autograft bridging and end-to-end healing of nerve stumps are the most common approaches to nerve repair [2].

However, scarring at the healing site is a big issue with nerve restoration. Scar tissue can form on 50% of regenerating axons, even in well-repaired nerves; this can cause local neuromas and restrict axonal regeneration to the injured area. Therefore, restoring nerve function is typically not satisfactory. Increased nerve adhesion—a physical barrier to the nerve—and inhibition of angiogenesis are the main mechanisms by which scar tissue impedes nerve regeneration. Neuroregeneration, or nerve regeneration, is the process of creating new neurons, glia, axons, myeline, or synapses, as well as repairing or regrowing damaged neurons, cells, or cell products [3].

There are different types of nerve repair; Epieneural repair which is: shorter operative time, less traumatic & easier,but may not ensure proper fascicular alignment. Fascicular or "perineural" which is: maximal control over fascicular alignment, but longer operative time & more traumatic [1].

Laser is an acronym for light amplification by stimulated emission of radiation. Laser can be delivered in three modes: Continuous which is constant beam, Pulsed mode which may be single or train of pulses, Q-switched very short pulses at high peak power [4].

For more than half a century low-level laser (LLLs) has been a popular treatment option. A subset of lasers known as LLLs achieve their effects on biological systems without the use of heat. LLLT uses a single wavelength of light and does not involve any penetration of the skin. No sound, vibration, or heat is released by it. The terms photobiology and bio stimulation are interchangeable. LLLs has a number of purported effects, including influencing fibroblast function, speeding up connective tissue healing, and reducing inflammation [5].

Research in underdeveloped nations has shown that adults (63.54%) and children (72.36%) suffer nerve damage most frequently in the upper limbs. In children, the most common causes of nerve injury were obstetric lesions (46.78%), iatrogenic lesions (16.95%), traffic accidents (15.7%), and sharp lacerations (12.8%). In adults, sharp lacerations (27.57%), iatrogenic lesions (25.67%), and traffic accidents (23.77%) were the most common causes of nerve injury [6].

New, encouraging methods of neurotization that use a small portion of the donor nerve have recently appeared in the scientific literature. It seems that reestablishing deltoid muscle activity via the transfer of the triceps motor branches from the radial nerve to the axillary nerve was a safe and efficient procedure. Due to compensation by the other heads, the functional loss relative to the triceps is minor when a single nerve is moved. [4]. So, we aimed at this study for evaluating the effect of LLLT on regeneration of injured sciatic nerves of Albino rats at the Zagazig University Hand and Microsurgery Center (ZUHMC), Plastic and Reconstructive Surgery Department.

METHODS

This experimental study was conducted on 24 Sprague-Dawley young adult male rats with an average weight of 250-350 grams that were all subjected to the experiment, during the study period between May 2023 to February 2024; the Institutional animal care and Use committee of the Faculty of Medicine, Zagazig University, approved the study (ZU-IACUC/3/F/52/2023). Following the recommendations of the Declarations of Helsinki as well as the European Community's rules for the use of experimental animals, the experiment was carried out.

Methods

All the maneuvers carried on in this experiment concerning the rats were highly ethical and merciful. The subjects of the experiment were 24 Sprague-Dawley young adult white male albino rats with an average weight of 250-350 grams where 19 survived and 5 perished before completion.

All the rats were anesthetized properly and had a sciatic nerve transected and repaired primarily using 9/0 Polypropylene sutures under a surgical microscope then divided into two equal groups: Group (1): (Therapeutic Group): Low level laser therapy (LLLT) was applied around epineural repair site. Group (2): (Control Group): Primary epineural repair only.

Surgical Procedure

Anesthesia was administered via an intra-peritoneal injection of a Ketamine / Xylazine cocktail (Ketamine 25mg + Xylazine 10mg per mL) with a dosage of 0.1 mL/100 gram rat weight then preparing the rats by shaving the hind limbs and abdomen.

In Group (1)

The rats were placed in a prone position with the tail and a 3mL syringe tucked below the limb.

The limb was sterilized using povidine iodine 10% solution and skin incised along a line connecting the knee joint to the ischial tuberosity then skin incised using blade 15 scalpel, the skin was dissected bluntly from the underlying muscles.

After exposing and identifying the plane between the Biceps Femoris and the Gluteus Maximus muscles it was developed using a mix of blunt and sharp dissection revealing the Sciatic Nerve. After placing a contrast material behind it, the sciatic nerve was transected proximal to the splitting site and immediately repaired with an end-to-end epineurial repair using a poly-propylene 9-0 suture then approximating the muscles and suturing the skin using a poly-propylene 5-0 suture and painting the skin with povidone iodine 10% solution. Then 1 week post operatively, those rates were taken to undergo low level laser therapy irradiation sessions around the site of repair, they underwent 4 laser sessions 1 week apart for a duration of 20 minutes of each session.

In Group (2)

The rats were subjected only to the transection and primary repair of the sciatic nerve part of the procedure without application of Low level laser therapy and it was carried out in the same surgical manner as before. All the procedures were performed using surgical microscope and microsurgical tools to enable the proper dissection and repair of the sciatic nerves.

Follow up

The rats were closely monitored during surgery and recovery. Each rat was kept in a separate cage with food and water. They were checked on daily during the first four weeks for feeding, cleaning, antibiotics administration and wound care. Then every three days during the rest of the experiment up to 12 weeks. The antibiotic was administered only for 7 days as following (Tetracycline PO in drinking water 0.8 mg/100g rat weight/24h).

Biopsy preparation and histological evaluation

At the end of the 8th week postoperatively, all the surviving rats (19) were humanly euthanised with an overdose of anaesthesia (triple the surgical dose). The sciatic nerve was exposed and a 15 mm segment (containing the epineurial repair centrally) harvested with the proximal stump marked using a suture knot then fixated with a 20% formalin solution in a sterile sample collection tube.

After 48 hours the samples were washed to remove formalin in distilled water for 30 minutes then embedded in paraffin wax blocks then cut into histologic sections that are 4-5 microns thick and stained with Toluidine Blue and H&E stains separately.

Histologic evaluation was performed using light microscopy where the number of myelinated axons proximal and distal to the epineurial repair site was counted by professional examiner who wasn't made aware of the nature of the experiment. Counting was performed using 400X magnification within a series of adjacent sections and across the long axis of the fascicles, then the average count of the two observers was calculated.

After an automated tissue processor had processed specimens from the sciatic nerve of rats preserved in a 20% fomol-saline solution for 48 hours, the samples were ready for analysis. Submerging the tissue in 10% buffered formalin for 48 hours was part of the fixation and dehydration process. For dehydration, we used a graduated alcohol series, and then we cleared the samples in Xylene solutions.

We looked for degeneration, necrosis, apoptosis, metaplasia, fibroplasia, inflammation, and granuloma after we treated the samples with Xylene, impregnated them with paraffin wax, embedded and blocked them. We stained them with hematoxylin, eosin, and toluidine blue.

Statistical analysis

We used SPSS version 28 (IBM Co., Armonk, NY, USA) to complete the statistical analysis. We used the terms mean, standard deviation, and range to display quantitative parametric data. A statistically significant result was defined as a P-value less than 0.05, and non-parametric data was evaluated using the Chi-square test, while abnormally distributed data was examined using the Mann Whitney U test. Categorical variables were shown as percentages and frequencies.

RESULTS

No statistically significant (P= 0.78) difference between studied groups as regard age; in group 1, the mean was (1.6 ± 0.39) months and ranged from 1 to 2 months. While in group 2, the mean was (1.7 ± 0.37) months and ranged from 1 to 2 months. As regard gender, all rats (100%) in both studied groups were males. No statistically significant (P= 0.63) difference between studied groups as regard weight; in group 1, the mean was (213 ± 16) grams and ranged from 180 to 230 grams. While in group 2, the mean was (216 ± 23) grams and ranged from 180 to 250 grams **(Table 1).**

Table (2) shows description of laser data of group 1; as regard energy, it was 2 J, with power 180 mW, with duration 20 S with method of Contact (on the skin of injury area) in all studied rats.

High statistically significant (P<0.001) increased number of myelinated nerve fibers (**proximal**) in group 1 (mean= 180 ± 58 and ranged from 84 to 276) was found when compared with that of group 2 (mean= 60 ± 1.5 and ranged from 58 to 62) in all

studied rats. High statistically significant (P<0.001) increased number of myelinated nerve fibers (**distal**) in group 1 (mean= 247 ± 88 and ranged from 113 to 374) was found when compared with that of group 2 (mean= 68 ± 1.7 and ranged from 65 to 70) in all studied rats **(Table 3).**

Histopathological Findings

Examined sections from Proximal and distal anastomosed sciatic nerve segments of control untreated rats showed mild to moderate perineural edema, congestion, focal hemorrhage and neuronal degeneration. Actual neuronal counting was computably estimated and recorded as 61, 73, 69, 119 nerve cell /HPF for the distal and proximal segments respectively (Figs.1-3).

Proximal and distal segments of eight other cases were computably estimated and recorded as follow;67,70,86,59,81,78,65,77 for the distal segments and 89,94,99,102,123,117,107, 126 for the proximal segments.

Proximal and distal anastomosed sciatic nerve segments of laser treated rats showed highly reactive angiogenesis and capillarization of the peri-neural tissue with reactive immune lymphoid cells infiltration. Normal organization of the myelinated and unmyelinated nerve fibers with interstitial new capillarization was seen in the proximal segments, neurons were apparently heathy with prominent nuclei. Branches of the distal sciatic segment showed orderly organized myelinated and unmyelinated neurons with prominent nuclei. Actual neuronal counting was computably estimated and recorded as 61, 84, 125 and 113, 136 nerve cell /HPF for the distal and proximal segments respectively.

Proximal sciatic nerve segments of laser treated rats of groups 6, 7, 8 showed clear angiogenetic changes with marked interneuronal capilirization, normal organization of the myelinated and un-myelinated nerve fibers, proliferative and hypertrophic changes and a few infiltrated immune lympho-plasmacytic cells. Neurons were apparently healthy with prominent nuclei. Branches of the distal sciatic segment showed orderly organized myelinated and un-myelinated neurons with prominent nuclei with focal neuronal proliferation. Actual neuronal counting was computably estimated and recorded as 116, 199, 267 and 135, 213, 294 nerve cell /HPF for the distal and proximal segments respectively. Sections from sciatic nerve of laser treated groups (9-15) revealed neurons with apparently healthy and prominent nuclei. Branches of the distal and proximal sciatic segments showed orderly organized myelinated and un-myelinated neurons with prominent nuclei and focal neuronal proliferation. Actual neuronal counting was computably estimated and recorded as 137, 181, 226, 196, 226,216 and 250, 339, 309, 253, 374, 297 nerve cell /HPF for the distal and proximal segments respectively.

Table (1): Comparison of all studied groups as regard (Age – gender – weight) in all studied rats.

Variable		**Group 1 (n= 11)**		**Group 2 (n= 8)**		**Stat. test**	**P-value**
Age (months)	**Mean ± SD**	1.6 ± 0.39		1.7 ± 0.37		T= -0.29	0.78 NS
	Min – Max	1 – 2		1 – 2			
Gender	**Males**	11	100%	8	100%	-------	--------
	Females	0	0%	0	0%		
Weight (gm)	**Mean ± SD**	213 ± 16		216 ± 23		T= -0.49	0.63 NS
	Min – Max	180 – 230		180 – 250			

T= Independent sample t test. **NS: P>0.05 is considered non-significant.**

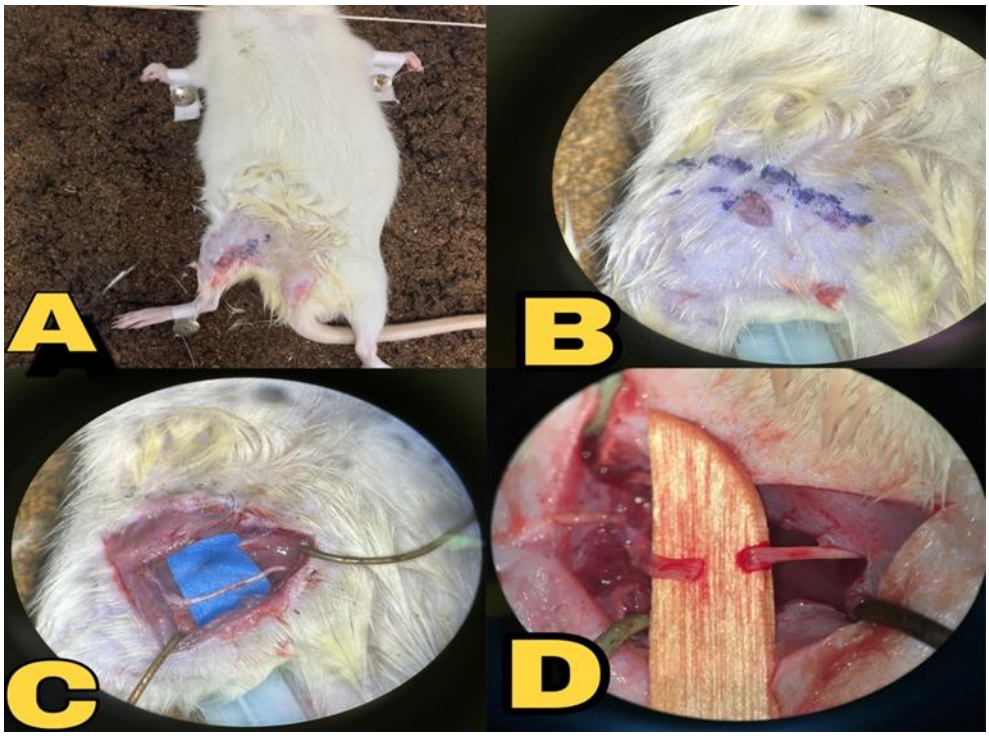
Table (2): Description of laser data in group1

Laser data	
Energy (J)	**2**
Power (mW)	**180**
Time (S)	**20**
Laser irradiation Method	**Contact (on the skin of injury area)**

Table (3): Comparison of all studied groups as regard Numbers of myelinated nerve fibers (proximal and distal) in all studied rats.

Numbers of myelinated nerve fibers		**Group 1 (n= 11)**	**Group 2 (n= 8)**	**T**	**P-value**
Proximal	**Mean ± SD**	180 ± 58	60 ± 1.5	5.8	**<0.001 HS**
	Min – Max	84 – 276	58 – 62		
Distal	**Mean ± SD**	247 ± 88	68 ± 1.7	5.7	**<0.001 HS**
	Min – Max	113 – 374	65 – 70		

T= independent sample t test. HS: P<0.001 is considered highly significant.



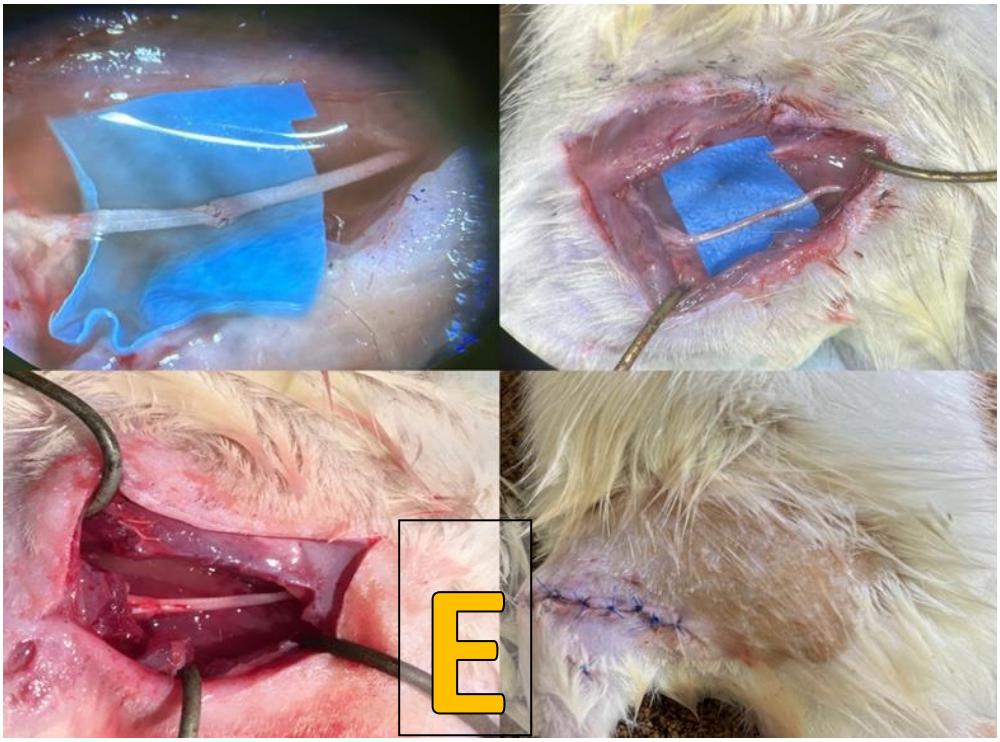


Figure (1): (A) Positioning of the rat, (B) Incision marking, (C,D) Muscle dissection, (E) Sciatic nerve repair done.

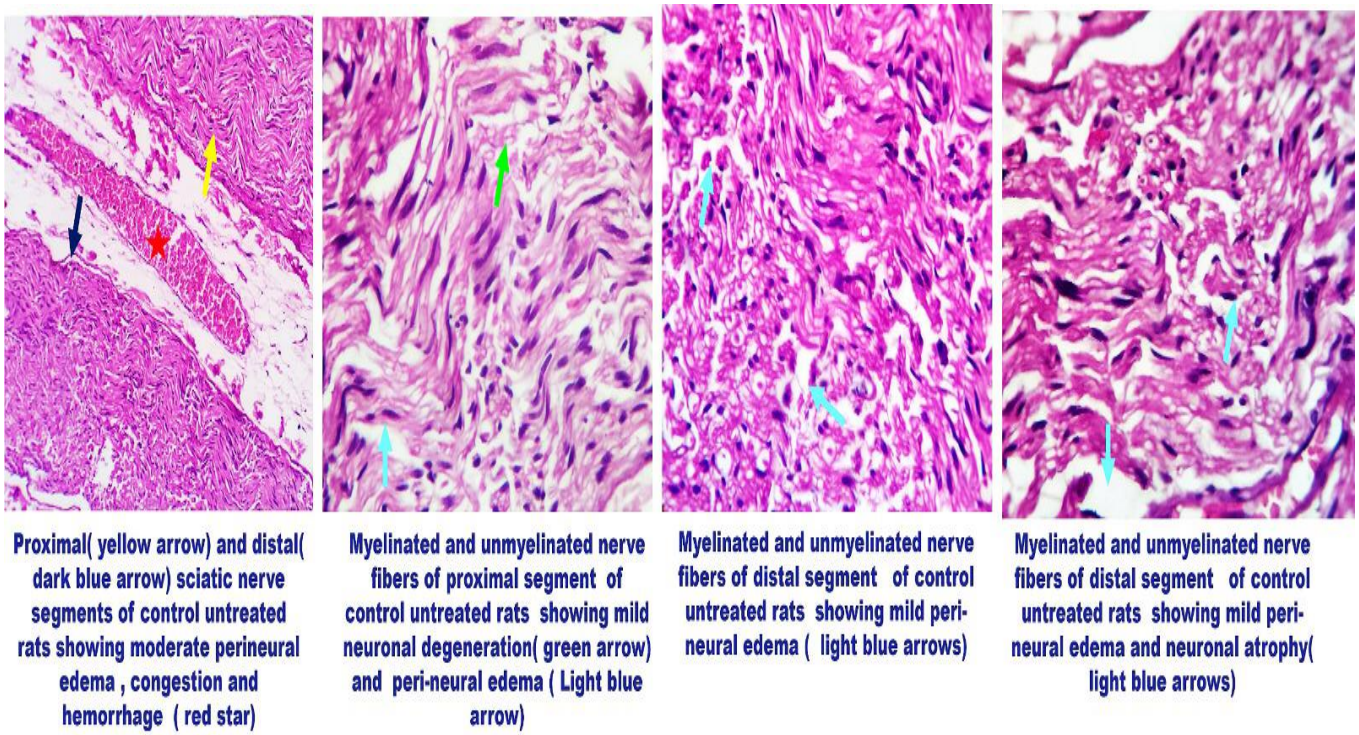


Figure (2): Showing the recorded tissue changes in Proximal and distal anastomosed sciatic nerve segments of control untreated rats.

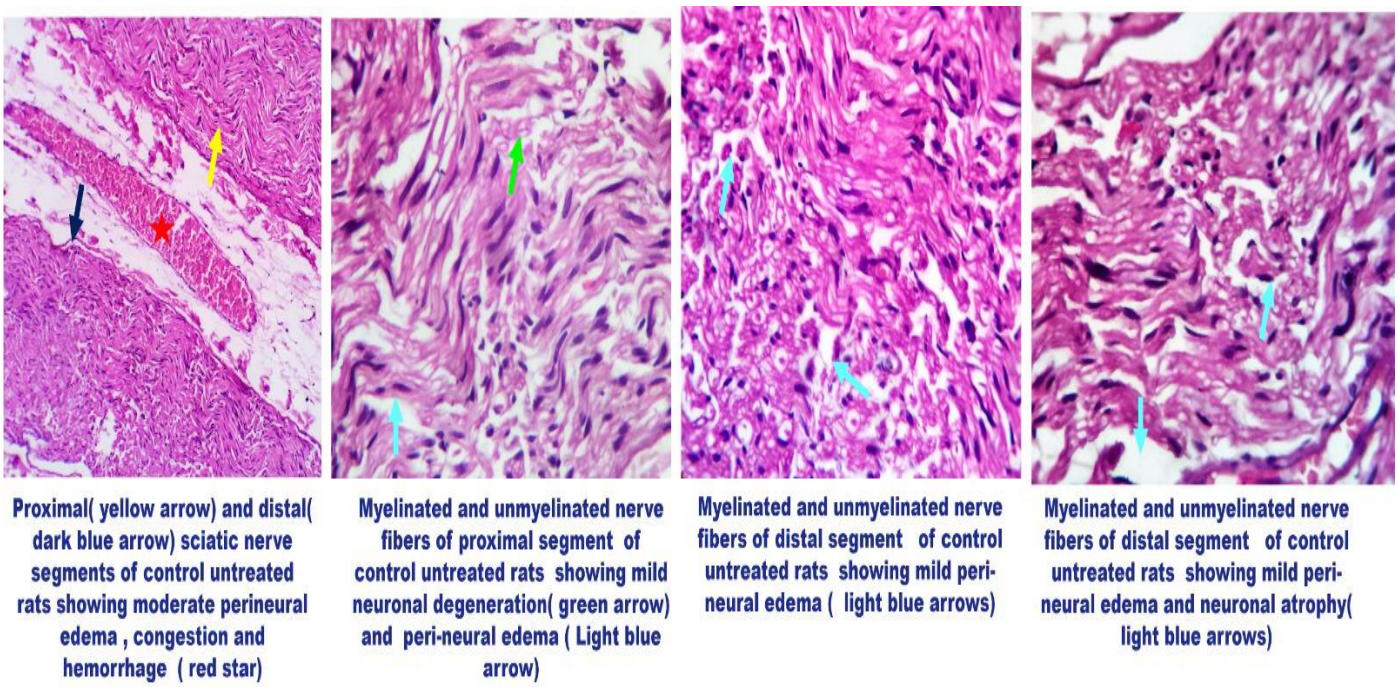


Figure (3): Showing the recorded computably estimated counting in Proximal and distal anastomosed sciatic nerve segments of control untreated rats

DISCUSSION

Nerves carry electrical impulses from the brain to the body, much like cables do. Schwann cells, nerve fibers, components, and connective tissue make up peripheral nerves. 2.8% of trauma patients experience common and serious injuries each year, which, if left untreated, can cause permanent impairment [9].

Auto-graft bridging and end-to-end nerve stump healing are two techniques used to direct growing nerve fibers into distant endoneurial tubes. The primary problem, though, is the creation of scars at the healing site, which can cause local neuromas and obstruct axonal regrowth, resulting in generally inadequate nerve regeneration [10].

Scar tissue hinders nerve regeneration by increasing adhesion and inhibiting angiogenesis, a process that involves the regrowth or repair of nervous tissues, cells, or cell products, resulting in the generation of new neurons or synapses [11].

There are several forms of nerve repair, such as perineural repair, which offers maximum control over fascicular alignment but has longer operational periods and is more stressful, and epieneural repair, which is shorter, less painful, and simpler but may not assure adequate fascicular alignment. Three modes of delivery are available for lasers, which stand for light amplification: Q-switched, pulsed, and continuous [12].

Low level laser therapy (LLLT), discovered in 1967 by Endre Mester at Semmelweis Medical University, is a non-invasive treatment that affects biologic systems through non-thermal means. It affects connective tissue cell function, accelerates tissue repair, and acts as an anti-inflammatory agent. LLLT uses red near-infrared light with a power output of 0.001-0.1 Watts [13].

The aim of that study was to assess the value of using LLLT in peripheral nerve regeneration & how it affects it.

No statistically significant (P= 0.78) difference between studied groups as regard age; in group 1, the mean was (1.6 ± 0.39) months and ranged from 1 to 2 months. While in group 2, the mean was (1.7 ± 0.37) months and ranged from 1 to 2 months. As regard gender, all rats (100%) in both studied groups were males. No statistically significant (P= 0.63) difference between studied groups as regard weight; in group 1, the mean was (213 ± 16) grams and ranged from 180 to 230 grams. While in group 2, the mean was (216 ± 23) grams and ranged from 180 to 250 grams.

A similar study by FAYEZ et al. [14] reported that the sex distribution of the study group revealed that

there were 11 (73%) females and 4 (27%) males. The sex distribution in the control group revealed that there were 10 (70%) females and 5 (30%) males. There was no significant difference in sex distribution between both groups (p=0.69).

On the other hand, Malanotte et al. [15] showed that a statistically significant difference (P = 0.03) was found between the studied groups regarding age. In Group 1, the mean age was 1.2 ± 0.25 months, ranging from 0.9 to 1.5 months, while in Group 2, the mean age was 2.1 ± 0.50 months, with a range of 1.5 to 3 months. Gender: In Group 1, 70% of the rats were males, and 30% were females, whereas in Group 2, 60% were males and 40% were females. A statistically significant difference (P = 0.04) was observed between the groups with respect to weight. In Group 1, the mean weight was 190 ± 12 grams, ranging from 170 to 205 grams, while in Group 2, the mean weight was 250 ± 28 grams, with a range from 210 to 280 grams.

In agreement with our study Zedan et al. [10] illustrated that no statistically significant difference (P = 0.85) was found between the studied groups with respect to age. In Group 1, the mean age was 1.8 ± 0.42 months, ranging from 1 to 2.5 months, while in Group 2, the mean age was 1.9 ± 0.45 months, also ranging from 1 to 2.5 months. As for gender, all rats (100%) in both groups were males. No statistically significant difference (P = 0.59) was observed between the groups regarding weight. In Group 1, the mean weight was 210 ± 18 grams, ranging from 175 to 235 grams, whereas in Group 2, the mean weight was 220 ± 22 grams, ranging from 180 to 245 grams. Description of laser data of group 1; as regard energy, it was 2 J, with power 180 mW, with duration 20 S with method of Contact (on the skin of injury area) in all studied rats.

A similar study by Fayez et al. [14] reported that the energy used was 3 J, with a power of 150 mW, and a duration of 15 seconds. The method of delivery was Contact (on the skin of the injury area) in all studied rats.

In agreement with our study Al-Shammari et al. [16] stated that the energy applied was 1.5 J, with a power of 100 mW, and a duration of 30 seconds. The method used was also Contact (applied directly to the skin over the injury site) in all the studied rats. The energy was reduced to 1.5 J compared to 2 J in Group 1. Lower energy doses are sometimes used to test whether reduced stimulation can still yield significant healing effects without overstimulating the tissue. The power was set at 100 mW, which is lower than in Group 1 (180 mW). Lower power settings are often used in studies focusing on minimizing the potential for tissue damage while maintaining therapeutic benefits. The treatment duration was extended to 30 seconds compared to 20 seconds in Group 1. Increased exposure time compensates for the lower power output, ensuring sufficient energy delivery over time while reducing the risk of thermal damage.

On the other hand, Yin et al. [11] found a non-contact laser method, applying 10 J of energy with a power of 500 mW and a duration of 10 seconds. This was significantly higher than the 2 J used in the previous group, which was used for more aggressive tissue stimulation and repair in severe injuries or for rapid healing. The power setting was also elevated to 500 mW, which is commonly used for faster tissue penetration or deeper tissues. The duration was shortened to just 10 seconds, ensuring the tissue is not overexposed to heat or other side effects while delivering a strong dose of energy in a short time. This method is often chosen to reduce infection risk in open wounds, prevent skin burns, or when the injury site is too sensitive for direct application.

High statistically significant (P<0.001) increased number of myelinated nerve fibers (proximal) in group 1 (mean= 180 ± 58 and ranged from 84 to 276) when compared with that of group 2 (mean= 60 ± 1.5 and ranged from 58 to 62) in all studied rats. High statistically significant (P<0.001) increased number of myelinated nerve fibers (distal) in group 1 (mean= 247 ± 88 and ranged from 113 to 374) when compared with that of group 2 (mean= 68 ± 1.7 and ranged from 65 to 70) in all studied rats.

In agreement with our study Takhtfooladi et al. [17] found a significant increase in the number of myelinated nerve fibers in both proximal and distal regions in rats. Group 1 showed a higher number of myelinated fibers, suggesting enhanced nerve regeneration or maintenance, possibly due to an experimental intervention like laser therapy, electrical stimulation, or pharmacological treatment. Group 2, likely serving as a control, showed a much lower count of myelinated fibers, suggesting that the intervention in Group 1 effectively promotes nerve repair. The substantial difference in the number of myelinated fibers between the groups provides strong evidence that the treatment applied to Group 1 has a significant impact on nerve regeneration, as evidenced by the low P-values, indicating a statistically significant outcome.

Lee et al. [18] revealed a significant increase in the number of myelinated nerve fibers in both proximal and distal regions of rats in Group 1 compared to

Group 2 (mean = 65 ± 2, ranging from 63 to 68). This suggests successful nerve regeneration or preservation. The significant difference between Group 1 and Group 2 suggests that the intervention applied to Group 1 has a strong effect on promoting the growth or repair of nerve fibers. Group 2 shows a consistently lower count of myelinated fibers, likely serving as a control or receiving a less effective treatment. The consistent high statistical significance ($P < 0.001$) reinforces the robustness of the intervention's effect on nerve fiber growth in Group 1.

On the other hand, Yin et al. [11] reported that no significant difference in the number of myelinated nerve fibers between Group 1 and Group 2 in both proximal and distal areas in all rats. This lack of difference could suggest that the intervention in Group 1 did not have a significant impact on nerve regeneration compared to Group 2, or that both groups experienced similar levels of nerve repair. The P-values indicate that the variation between the groups is not statistically meaningful. This could imply that either the treatment is ineffective or both groups received interventions that promote nerve regeneration to the same degree

FAYEZ et al. [14] found that laser therapy could be an effective therapeutic modality in the treatment of painful neuropathy for its ability to modify pain, foot skin microcirculation and some electrophysiological parameters of peripheral nerve function. low-level laser therapy can be an effective treatment for painful neuropathy. The reduction in pain scores suggests that LLLT effectively modulates pain perception. The improvements in microcirculation indicate that LLLT may enhance local blood flow, which is crucial for nerve repair and function. Enhanced electrophysiological parameters reflect the beneficial impact of LLLT on nerve health and function.

Future studies should include Histological examination under microscope to assess stenosis of the anastomosis site, reendothelialization, intimal hyperplasia, vascular structure, and aneurysm formation in Multiple-U technique. Follow up patency rate by taking x-ray images using contrast medium for more detailed image post anastomosis. After having a significant result about Multiple-U anastomosis we suggest performing it on human being to detect its efficacy.

Conclusions

The study demonstrated that low-level laser therapy significantly enhances sciatic nerve regeneration in white male Albino rats. The treated group showed a marked increase in the number of myelinated nerve fibers, both proximally and distally, when compared to the control group, indicating improved nerve repair. Additionally, the functional recovery in the laser-treated group, reflected by improved motor and sensory function, further supports the effectiveness of LLLT in promoting nerve regeneration. The findings suggest that LLLT could be a promising non-invasive therapeutic approach for enhancing peripheral nerve repair. However, further research is recommended to optimize the laser parameters (energy, power, duration).

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