Original Article

Efficacy of multi-wave locked system laser therapy on nerve regeneration after crushing in Wister rats

MOHAMED SALAHELDIEN MOHAMED ALAYAT¹*, MOHMAD ABUBAKAR BASALAMAH², WAGIH GAMAL EL-DIN EL-ELGHANY EL-BARRANY³, NASER AHMED MAHMUD EL SAWY⁴, Ehab Mohamed Abdel-Kafy¹

¹) Physical Therapy Department, Faculty of Applied Medical Science, Umm Al-Qura University: 4888 Batha Qurish, Mecca, Mecca 21955, Saudi Arabia
²) Department of Pathology, Faculty of Medicine, Umm Al-Qura University, Saudi Arabia
³) Faculty of Medicine, Umm Al-Qura University, Saudi Arabia
⁴) Faculty of Applied Medical Science, Umm Al-Qura University, Saudi Arabia

Abstract. [Purpose] To investigate the efficacy of the multi-wave locked system laser therapy on the regeneration of peripheral nerve injuries by evaluating the functional, electrophysiological, and morphological changes of the crushed sciatic nerve in Wistar rats. [Materials and Methods] Sixty male Wistar rats (200–250 g) were randomly assigned to control negative, control positive, or laser groups and subjected to no laser therapy or crushing, to crushing without laser therapy, or crushing followed by multi-wave locked system laser therapy five times/week for four weeks (power=1 W, energy density=10 J/cm², total energy=100 J), respectively. Functional, electrophysiological, and morphometric analyses were performed before and 7, 15, 21, and 28 days after crushing. The sciatic functional index, compound motor action potential amplitude, motor nerve conduction velocity, and nerve and myelin sheath diameters were measured. [Results] The sciatic functional index value decreased significantly, while the compound motor action potential amplitude, motor nerve conduction velocity, nerve diameter, and myelin sheath diameter increased significantly in the laser group post-treatment compared to the values in the control groups. [Conclusion] Multi-wave locked system laser therapy was effective in accelerating the regeneration of crushed sciatic nerves in Wistar rats.

Key words: Laser therapy, Sciatic nerve crushing, Peripheral nerve regeneration

(INTRODUCTION)

Rehabilitation after traumatic injuries of the peripheral nerves is challenging. According to the severity of peripheral nerve injury, Wallerian degeneration of the nerve occurs distally¹, followed by retrograde degeneration proximal to the injured site². Despite significant efforts during rehabilitation, these injuries result in residual loss of sensory and motor functions and are often associated with incomplete recovery³. Previous studies have used experimental protocols to induce crushing of the sciatic nerve in rats⁴,⁵ or rabbits to investigate the efficacy of physical modalities, such as photobiomodulation, on the treatment of these injuries⁶.

Low-level laser therapy (LLLT) proved to be a useful modality in promoting the regeneration of peripheral nerves⁷–¹⁰ due to its anti-inflammatory and reparative effects⁶,¹¹. Multi-wave locked system (MLS) laser therapy is a recently developed rehabilitation modality that was proven to reduce pain and inflammation in musculoskeletal conditions due to its unique...
Unlike single-wavelength lasers, an MLS laser involves a synchronized emission of a continuous laser emission of 808 nm and pulsed laser emission of 905 nm. Owing to its two laser waveforms, MLS laser therapy provides better penetrability and anti-inflammatory and reparative effects than those of LLLT. Currently, no study has investigated the effects of MLS laser therapy on the regeneration of the sciatic nerve after crushing. Therefore, the aim of this study was to investigate the efficacy of the MLS laser therapy on the regeneration of peripheral nerve injuries by evaluating the functional, electrophysiological, and morphological changes of the crushed sciatic nerve in Wistar rats.

MATERIALS AND METHODS

The present experimental protocol was approved by the research committee in the Faculty of Applied Medical Science, Umm Al-Qura University, Saudi Arabia with local registration number (12-MED-2958-10). The estimated sample size was calculated using the Gpower 3.1 for windows, with repeated analysis of variance, an effect size of 0.20, an alpha error of 0.05, and a power of 0.95, for three groups and five repeated measures. The total sample size was 60 rats divided into three groups of 20 rats each. Rats with intact sciatic nerves were placed in the control-negative (CT-ve) group. The sciatic nerves of rats in the control positive (CT+ve) group were crushed and received no treatment, while rats in the MLS group received MLS laser therapy at the site of crushing.

The average weight of each rat was 200–250 g. Rats were kept in a plastic cage placed in an animal house with controlled room temperature (23 °C) and received water and food ad libitum throughout the study. The rats were weighed and the anesthetic dose was calculated. After inhalation of ether, an intraperitoneal injection of ketamine and xylazine was administered. Then, the front and hind paws were fixed on the table while the rat was lying prone. The hair on the left thigh was removed, and it cleaned with an iodine-alcohol solution. The left sciatic nerve was then exposed, detached, and crushed for 30 s at the greater sciatic foramen using non-toothed forceps. The nerve was then re-attached, and the incision was closed with nylon sutures (Ethicon). Finally, the rats were returned to the cage with ad libitum access to water. The same surgeon crushed the sciatic nerves of every rat in the study.

The functional, electrophysiological, and morphometric measurements of the nerve were obtained before and 7, 15, 21, and 28 days after crushing. For functional assessment, the sciatic functional index (SFI) value was estimated for all rats. After dipping the rat’s foot in black ink, a wooden passage was lined with paper strips to detect the footprint of the rat by allowing the rat to walk through the passage. The measurements were based on both normal and experimental footprints and included print length (PL, from the heel to the longest toe), toe spread (TS, from the first to the fifth toe), and intermediate toe spread (IT, from the second to the fourth toe). The following formula was used for calculation of the SFI: SFI = −38.3 [PLF] + 109.5 [TSF] + 13.3 [ITF] − 8.8, where PLF is the printed length factor, TSF is the toe spread factor, and ITF is the intermediary toe spread factor. The following formulas were used to calculate PLF, TSF, and ITF: PLF = (EPL - NPL) / NPL, TSF = (ETS - NTS) / NTS, and ITF = (EIT - NIT) / NIT. The amplitude and sciatic nerve conduction velocity were measured using a MYOHANDY™ portable electromyograph (Micromed, Veneto, Italy). Stimulations (200 mV, 0.2 ms) were applied with a 5 mm needle electrode to the sciatic nerve. The cathode was inserted close to the medial gastrocnemius muscle while the anode was in the sole of the paw, and the ground was placed over the thigh area. A suprathreshold stimulus was used to obtain the maximal amplitude of compound motor action potential (CMAP). Motor nerve conduction velocity (MNCV) was calculated by dividing the distance between the stimulation and recording over the time taken to record the CMAP.

Every week, four rats were sacrificed from each group for histological assessment. After sacrifice, the sciatic nerves were soaked in a 25% glutaraldehyde solution in 0.025 M sodium cacodylate (pre-fixed for 2 min and fixed for 12 h in a refrigerator at 6 °C). After fixation, these were treated with 2% osmium tetroxide and 0.2% sodium cacodylate. The samples were then treated with 2% osmium tetroxide and 0.2% sodium cacodylate. The samples were then

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Fig. 1. Schematic drawing of measured parameters in the normal and experimental footprints. PL: print length; TS: toe spread; IT: intermediate toe spread; N: normal; E: experiential.
washed in an isotonic sodium cacodylate buffer and dehydrated in increasing concentrations of ethanol and propylene oxide. The samples were embedded in a plastic resin (EPON 812) for 48 h at 60 °C in an oven. The blocks were cut transversely to the sciatic nerve (2.0 μm in thickness). Then, the nerve fibers in the injured region were imaged using a high-power light microscope (400×). Each section was photographed using the Leica Q-Win program, and the photographs were used for quantitative analysis of nerve fiber myelin sheath diameters.

Rats in the MLS group were treated with MLS laser five times a week for four weeks. An MLS laser (M6-ASA; Arcugnano, Italy) with dual laser beams at 808 nm (CW peak power of 1,000 mW, mean power of 500 mW, and spot area of 3.14 cm²) and 905 nm (PW 1,500 Hz, peak power of 25 W, and mean power of 54 mW) was used. The MLS handpiece was placed perpendicular to and not in contact with the target area (10 cm²) and its output was placed perpendicular to and not in contact with the target area (10 cm²) and its output was 1.5 cm away, with the intervals of 48 h. The level of significance was set at p<0.05.

The present study showed a significant decrease in SFI value and an increase in values of the electrophysiological and morphological measures in the MLS group compared to the corresponding values in the control group. This result was consistent with those of previous studies that found that laser to be effective in accelerating the regeneration of crushed sciatic nerves in Wistar rats4, 5, 11, 16, 20–29. Laser therapy increases adenosine triphosphate production, nerve metabolism, and myelin production, and enhances axonal sprouting, thereby increasing the proliferation of the nerves after crushing6, 7). Additionally, the nerve fiber diameter and myelin thickness showed no significant change in the CT-ve group and CT+ve group. In the CT+ve group, there were no significant differences in the mean values of the SFI at any measurement interval. Comparison of pre-treatment values between groups showed no significant changes, but a significant decrease in the mean SFI values was seen after 21 and 28 days in the MLS group compared to those values in the CT+ve and CT-ve groups (Table 1).

Moreover, the amplitude of CMAP showed no significant changes in the CT-ve and CT+ve groups, but a significant increase was seen in the MLS group. Comparison of the results between groups showed a significantly higher CMAP amplitudes at 15, 21, and 28 days after crushing in the MLS group compared to the values in the CT-ve and CT+ve groups. Similarly, MNCV was significantly increased in the MLS group compared to those in the CT-ve and CT+ve groups (Table 2).

Furthermore, the nerve fiber diameter and myelin thickness showed no significant change in the CT-ve group and CT+ve groups, but a significant increase in the nerve fiber diameter and myelin thickness were observed in the MLS group. Between the groups, MLS showed a more significant increase in the nerve fiber diameter and myelin thickness compared to the CT-ve and CT+ve groups (Table 3).

### RESULTS

Post-treatment, the SFI value showed a significant decrease in the MLS group and a non-significant decrease in the CT+ve group. In the CT-ve group, there were no significant differences in the mean values of the SFI at any measurement interval. Comparison of pre-treatment values between groups showed no significant changes, but a significant decrease in the mean SFI values was seen after 21 and 28 days in the MLS group compared to those values in the CT+ve and CT-ve groups (Table 1).

Moreover, the amplitude of CMAP showed no significant changes in the CT-ve and CT+ve groups, but a significant increase was seen in the MLS group. Comparison of the results between groups showed a significantly higher CMAP amplitudes at 15, 21, and 28 days after crushing in the MLS group compared to the values in the CT-ve and CT+ve groups. Similarly, MNCV was significantly increased in the MLS group compared to those in the CT-ve and CT+ve groups (Table 2).

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### DISCUSSION

The present study showed a significant decrease in SFI value and an increase in values of the electrophysiological and morphological measures in the MLS group compared to the corresponding values in the control group. This result was consistent with those of previous studies that found that laser to be effective in accelerating the regeneration of crushed sciatic nerves in Wistar rats4, 5, 11, 16, 20–29. Laser therapy increases adenosine triphosphate production, nerve metabolism, and myelin production, and enhances axonal sprouting, thereby increasing the proliferation of the nerves after crushing6, 7). Additionally,

### Table 1. Changes in sciatic functional index value among treatment groups

|                      | Pretreatment | After 7 days | After 15 days | After 21 days | After 28 days |
|----------------------|--------------|--------------|---------------|---------------|---------------|
| CT-ve                | −8.4 ± 2.6   | −8.8 ± 2.5   | −8.5 ± 2.7    | −8.6 ± 1.8†   | −8.9 ± 3.5†   |
| CT+ve                | −7.9 ± 2.4   | −8.8 ± 13.5  | −8.0 ± 12.8   | −67 ± 11.7†   | −59 ± 12.4†   |
| MLS                  | −9.5 ± 1.9†  | −90.2 ± 4.2† | −75.2 ± 2.4†  | −40.12 ± 5.3† | −29.34 ± 4.6† |

CT-ve: Control negative group, CT+ve: Control positive group, MLS: laser group. §: Significant changes among treatment groups (one-way analysis of variance (ANOVA)). †: Significant changes between measurement intervals (repeated measure ANOVA).

### Table 2. Change in the electrophysiology assessment measures among treatment groups

|                      | Compound motor action potential amplitude (mV) | Motor nerve conduction velocity (m/s) |
|----------------------|-----------------------------------------------|--------------------------------------|
|                      | Pre-treatment After 7 days After 15 days After 21 days | Pre-treatment After 7 days After 15 days After 21 days |
| CT-ve                | 18.1 ± 6.2 18.01 ± 3.4 17.91 ± 2.6† | 17.95 ± 2.1† 18 ± 3.2† 15.5 ± 3.8 | 55.2 ± 4.12 55.5 ± 3.8 55.2 ± 2.9† |
| CT+ve                | 17.82 ± 3.4 5.4 ± 3.5 5.8 ± 1.4† | 6.8 ± 1.4† 8.5 ± 2.6† 55.12 ± 3.4 24.5 ± 2.19 26.8 ± 2.6† 30.24 ± 2.5† 33.5 ± 3.2† |
| MLS                  | 17.95 ± 2.9 | 6.9 ± 1.9† 8.86 ± 1.6† | 10.6 ± 1.8† 13.4 ± 1.6† 55.28 ± 3.5 28.3 ± 2.67 35.4 ± 3.9† 39.4 ± 3.2† 43.7 ± 3.1† |

CT-ve: Control negative group, CT+ve: Control positive group, MLS: laser group. §: Significant changes among treatment groups (one way analysis of variance (ANOVA)). †: Significant changes between measurement intervals (repeated measure ANOVA).
laser energy decreases the retrograde degeneration of peripheral nerves and limits chromatolysis and neuronal atrophy after crushing. The anti-inflammatory effect of laser decreases the period of acute inflammation by altering the levels of pro-inflammatory cytokines. Furthermore, lasers increase vasodilation and vascular angiogenesis.

In contrast, some studies failed to find a positive effect of lasers and reported no laser effect. The lack of agreement between these studies and our study can be explained by the difference in parameters (wavelengths, power, and energy densities) of the lasers used. Laser effect is known to be dependent on laser type, dose, power, and wavelength. Moreover, the present study was the first to use an MLS laser, which has dual wavelengths, powers, and intensities. These unique parameters with two laser waveforms provide enhanced penetrability and effect. The immediate application of MLS laser therapy after nerve crushing (2nd day after crushing) and its daily use (five sessions per week) may have additional benefits and resulted in better outcomes. The present study’s result introduces a new modality to the rehabilitation of the peripheral nerves, although this study was performed on animals. As MLS laser is considered a new modality with no evidence about its efficacy on peripheral nerves, it can not be used on human nerves so, the experimental protocol starts as a preclinical study on animals with induced nerve lesions. The recommendation from the current study and other future studies may provide a piece of evidence to consider the MLS laser a treating modality for traumatic nerve lesions and/or other types of nerve pathology such as neuropathy. Although the MLS laser showed positive results on nerve regeneration in experimental protocol, the present study was limited by the single dose (10 J/cm²). Comparing different doses may provide more evidence and strengthen the conclusion. Also, comparing the MLS laser as a class IV laser with a LLLT in the same study conditions may show clear differential effects between these two types of laser. Moreover, a single study -like the present study- cannot confirm the effect of such modality on the human nerves as the human body’s response may not be the same as in rats. The improvement in the crushed sciatic nerve after laser application suggests the possibility of using MLS laser therapy in the rehabilitation of patients after traumatic nerve injuries, although further studies are needed to prove the applicability of MLS laser in a clinical setting.

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Conflict of interest
None.

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