

A comparative study of red and blue light-emitting diodes and low-level laser in regeneration of the transected sciatic nerve after an end to end neurorrhaphy in rabbits

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Abstract This study aimed at evaluating the effects of red and blue light-emitting diodes (LED) and low-level laser (LLL) on the regeneration of the transected sciatic nerve after an end-to-end neurorrhaphy in rabbits. Forty healthy mature male New Zealand rabbits were randomly assigned into four experimental groups: control, LLL (680 nm), red LED (650 nm), and blue LED (450 nm). All animals underwent the right sciatic nerve neurotmesis injury under general anesthesia and end-to-end anastomosis. The phototherapy was initiated on the first postoperative day and lasted for 14 consecutive days at the same time of the day. On the 30th day post-surgery, the animals whose sciatic nerves were harvested for histopathological analysis were euthanized. The nerves were analyzed and quantified the following findings: Schwann cells, large myelinic axons, and neurons. In the LLL group, as compared to other groups, an increase in the number of all analyzed aspects was observed with significance level ($P < 0.05$). This finding suggests that postoperative LLL irradiation was able to accelerate and potentialize the peripheral nerve regeneration process in rabbits within 14 days of irradiation.

Keywords Photobiomodulation · Low-level laser · Red LED · Blue LED · Sciatic · Nerve regeneration

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Introduction

An injury to a nerve can result in problems with the muscles or a loss of sensation. Following axonal transection, a sequence of pathologic events occur in the cell body and the axon. The regeneration and repair phase following the nerve injury may last for many months. The human peripheral neuron's capacity to initiate a regenerative response seems to persist for at least 12 months after the injury, and a robust response can be elicited even after repeated injuries [1].

Low-level laser (LLL) therapy has been investigated and used in clinical practices for the last two decades because of clinical benefits obtained from divergences in the encountered results [2]. The initial studies were conducted in Europe by Mester [3, 4] at the beginning of the 1970s. There has been a growing interest in the effects of laser energy and its application on both animals and human beings [5, 6]. The LLL therapy uses low-powered laser light in the range of 1–1000 mW, at wavelengths ranging from 632 to 1064 nm, to stimulate a biological response. These lasers emit no heat, sound, or vibration. Instead of generating a thermal effect, LLL therapy acts by inducing a photochemical reaction in the cell, a process referred to as biostimulation or photobiomodulation. Photobiology works based on the principle that when light hits certain molecules called chromophores, the photon energy causes electrons to get excited and jump from lower-energy orbits to higher-energy orbits. In nature, this stored energy can be used by the system to perform various cellular tasks [3, 5, 7].

Various in vitro experiments have found that cellular respiration was upregulated when mitochondria were exposed to an He–Ne laser or other forms of illumination. LLL irradiation caused an increase in mitochondrial products such as ATP [8], NADH, protein, RNA [9], and a reciprocal augmentation in oxygen consumption. A similar effect is produced when the

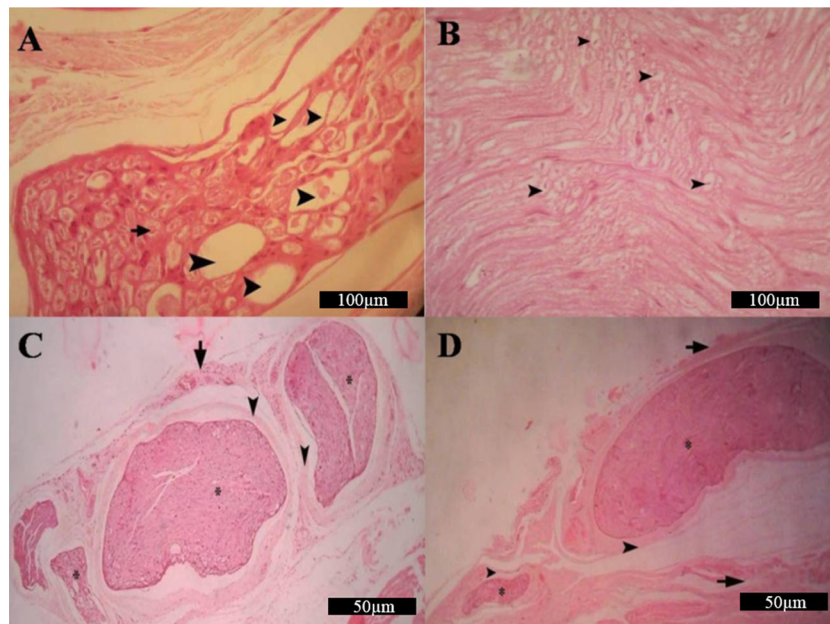


Fig. 1 Representative cross-sections of sciatic nerves. **a** Control group animals were submitted to sciatic lesion and did not receive phototherapy. There was a myelin sheath but with severe degenerative and inflammatory reactions (*arrowhead*). **b** Lesioned group that received low-level laser therapy; the application of laser therapy at 680 nm, 10 J/cm²,

10 mW, spot size 4 mm² for 14 consecutive days. **c** Sciatic nerve lesion submitted to blue LED phototherapy at 450 nm for 14 days. **d** Sciatic nerve crush lesion with red LED 650 nm, 2.4 J/cm² treatment; axonal accumulation (*star*), epineurium (*arrows*), perineurium in figures B, C, and D (*arrowhead*), H&E stain

tissue that contains mitochondria is exposed to LLL radiation. The use of therapeutic resources of such an instrument with a regenerative purpose is as common a practice in physical therapy as to gain the patients' functional status after recovery from peripheral nerve injuries. So far, there are various reports of disagreement over the optioned results [10]. Animal models have been employed to study LLL therapy effects in nerve repair [11, 12]. Byrnes et al. [13] used 1600 J/cm² of 810-

nm diode laser to improve healing and functionality in a T9 dorsal hemisection of the spinal cord in rats. Anders et al. [14] studied LLL therapy for regenerating crushed rat facial nerves; by comparing 361, 457, 514, 633, 720, and 1064 nm, and found the best response with 162.4 J/cm² of 633-nm He-Ne laser. It is understood that laser affects tissues differently according to the wavelength, pulse duration, pulse/energy, energy density, and the delivery system [15]. Wavelength is probably the parameter where there is the most agreement in the LLL therapy community. Wavelengths in the 600–700 nm range are chosen for treating superficial tissues, and wavelengths between 780 and 950 nm are chosen for deeper-seated tissues due to the longer optical penetration distances through tissue. There is of course much more work to be done to define what the optimum wavelength is for the different indications for which LLL therapy is employed [12–15].

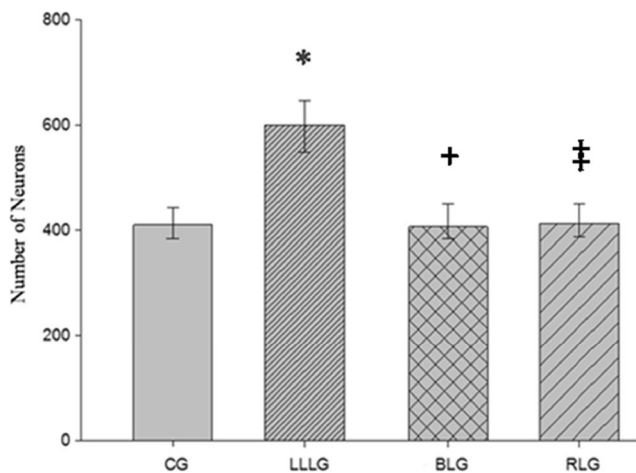


Fig. 2 Neurons in histological findings. Control group (CG), low-level laser group (LLLG), blue LED group (BLG), and red LED group (RLG). *LLLG is significantly different from CG, RLG, and BLG and $P=0.004$ is the same in all groups. †BLG is not significantly different ($P=0.873$) from CG. ‡RLG is not significantly different from CG ($P=0.631$) and BLG ($P=0.873$)

New radiation sources such as light-emitting diodes (LED) have recently attracted attention for their potential to be expanded in applicability to be used in phototherapy [16–20]. Light sources from LED can be employed to produce radiation energy. The effectiveness and applicability of LED arrays for injuries have been partially studied *in vitro* [16] and *in vivo* [17] which suggest that LED radiation has many biological effects similar to those of lasers. Serafim et al. [19] suggested that the use of phototherapy after nerve damage improves the morphofunctional recovery and nerve regeneration.

According to our knowledge, this is the first comparative study that has evaluated the effects of red and blue LEDs and

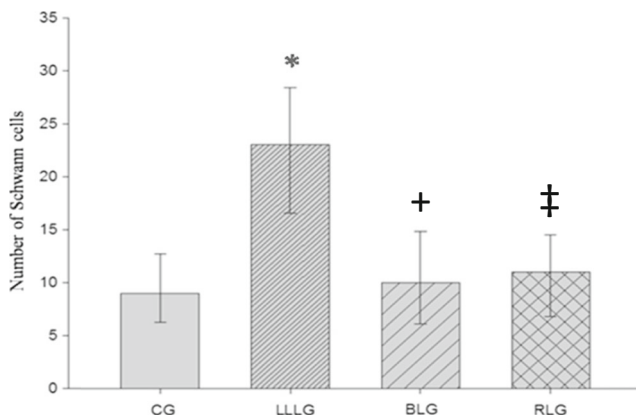


Fig. 3 Presence of Schwann cells in the histological findings. Control group (CG), low-level laser group (LLLG), blue LED group (BLG), and red LED group (RLG). *LLLG is significantly different from CG ($P=0.000$), RLG ($P=0.001$), and BLG ($P=0.001$). ⁺BLG is not significantly different ($P=0.968$) from CG. [‡]RLG is not significantly different from CG ($P=0.980$) and BLG ($P=1.000$)

LLL on the regeneration of the transected sciatic nerve after an end-to-end neurorrhaphy in rabbits.

Materials and methods

All experimental procedures were performed according to the guidelines for the ethical treatment of experimental animals and were approved by the Islamic Azad University, Faculty of Veterinary Sciences, Animal Care and Use Local Ethics Committee.

Animals

Forty healthy mature male New Zealand rabbits (weight 2.50–3 kg) were purchased from the Pasteur Institute of Iran (Tehran, Iran). The rabbits were assigned randomly into four groups, ten rabbits in each: control, LLL, red LED, and blue LED. Animals were kept in individual stainless cages in adequate sanitary conditions, with commercial food and water provided ad libitum for 7 days before surgery.

Anesthesia

The rabbits were weighed and anesthetized using an intramuscular injection of ketamine hydrochloride 10 %, and xylazine hydrochloride 2 % (50 and 10 mg/kg), respectively.

Surgical procedures

Once anesthetized, each animal was positioned on the left lateral recumbency. The site of the surgery was prepared for sterile surgery using local antiseptics with the alcohol-iodized solution on the caudal region of the right thigh from the height

of the greater trochanter to the knee. A 5-cm longitudinal incision was made along the right hind limb, and the sciatic nerve was exposed by longitudinal separation of the muscles which was carried out to allow a thorough vision of the nerve. The sciatic nerve was addressed, and a nerve injury was carried out by a complete transection of the sciatic nerve. After the injury, an epineural anastomosis of the sciatic nerve was done with four simple sutures, using the nylon 6-0. Then, the surgical closure of the region was done with simple sutures, using the nylon 3-0. All the Surgical procedures were performed by a surgeon. The rabbits were returned to their cages and were provided with food and water ad libitum.

Postoperative management

To prevent any post-operation infection, a single dose of oxy-tetracycline (15 mg/kg) was administrated through subcutaneous injection. Tramadol hydrochloride (4.4 mg/kg, Sc) was also administered as an analgesic. The skin sutures were removed 12 days post operation.

Irradiation procedures

A GaAlAs laser device with the following parameters was used: pulsed radiation, wavelength of 680 nm, power of 10 mW, spot area of 4 mm², and an energy density at the point of entry of 10 J/cm². The equipment was calibrated using a power meter Ophir Nova (Ophir) and an IR laser detection card. Radiation was applied transcutaneously over the site of the surgery. Treatments were applied via the contact technique, directly above the site of the injury (across the surgical scar) at three points within a time of 200 s per point. The red LED irradiation had a 650 nm wavelength with delivering energy intensity of 2.4 J/cm². The 450 nm wavelength with

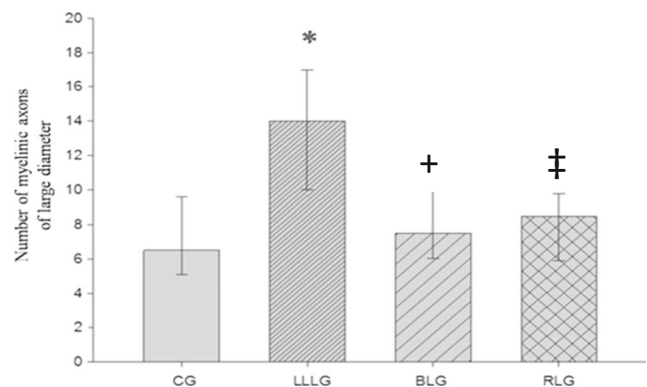


Fig. 4 Presence of myelinic axons of large diameter in the histological findings. Control group (CG), low-level laser group (LLLG), blue LED group (BLG), and red LED group (RLG). *LLLG is significantly different from CG, RLG, and BLG and $P=0.013$ is the same in all groups. ⁺BLG is not significantly different ($P=0.684$) from CG. [‡]RLG is not significantly different from CG ($P=0.371$) and BLG ($P=1.000$)

delivering energy intensity of 2.4 J/cm^2 of blue LED was used for the blue LED group. The LED irradiation had a 20 nm bandwidth and a beam area of 1.5 cm^2 . The radiation source was attached to a support and kept perpendicular at 1 cm above the skin surface. A single point in the middle of the surgical incision was irradiated (transcutaneous method) for 10 min. The light source employed in this study included a single commercial LED and was specially developed for the experiment by the Optical Laboratory of the Department of Physics of the Islamic Azad University. The optical output power of the light source was verified prior to experimental procedures using a broadband power and energy meter (Standard Photodiode Sensor PD 300; Ophir Optonics). The phototherapy was initiated on the first postoperative day, for 14 consecutive days at the same time of the day.

Euthanasia

Thirty days after surgery, all rabbits were euthanized with an anesthetic overdoses and the repair site was harvested from each sciatic nerve.

Histopathological analysis

Immediately after the material collection, the samples were prepared for histopathological analysis, realized in the Laboratory of Pathology. All nerves were fixed in 10 % neutral formalin, and a routine tissue processing for light microscopy was performed. Tissues were embedded in paraffin. Sections ($7 \mu\text{m}$) were cut by microtome and stained with hematoxylin-eosin. For the qualitative analysis, a descriptive qualitative histopathological evaluation of the injured nerve area from each animal was carried out under a light microscope (Olympus, Optical Co. Ltd, Tokyo, Japan). Ten images were captured from each animal using a Moticam imaging device (Motic group, Xiamen, China) and were analyzed via Motic Image Plus 2.0 software (Motic, Xiamen, China). In each image, Schwann cells, myelinic axons with large diameter, and neurons were measured. Histopathological analyses were carried out by a pathologist who was blinded to the experimental groups.

Statistical analysis

Statistical analyses were carried out using SPSS statistical software (Version 16). Results were expressed as the mean \pm standard deviation (SD). Data were analyzed by Kolmogorov–Smirnov test to ensure more statistical precision. The Mann–Whitney U test and the ANOVA test were employed to analyze the four groups consecutively. A value of $P < 0.05$ was considered as statistically significant.

Results

In this study, an investigation was done to evaluate regenerative capacity of nerve repair by the end-to-end method of neurorrhaphy after being exposed to LLL and red and blue LED irradiation for 14 days, with different wavelength and penetration power.

No operative and postoperative complications were encountered. All of the rabbits tolerated surgery and phototherapy well and survived until the final experimental time. No wound openings or infections were observed.

The gross examination of nerves showed that, although they have all recovered their original width, the injury site could still be identified because of its appearance starting from the point where blue epineural suture (which could be easily viewed) had passed.

The histological aspect was essentially normal on proximal segments of the right sciatic nerves in all experimental groups, with a regular distribution of narrow and wide nervous fibers. In the intermediate segments (injury site), blood vessels were more prevalent and thicker in the LLL group than the other groups. Fibers with very thin myelin sheaths were prevalent in the intermediate segment in all groups. Schwann cells with reactive appearance nuclei, characteristic of synthesis activity, as well as typical images of axonal sprouting were more prevalent in the LLL group, while Wallerian degeneration was more evident in the control and the blue LED groups. Small-gauge fibers and thin myelin sheaths were prevalent although thick fibers and thick myelin sheaths were also frequent on the LLL group next to a large number of Schwann cells. In this segment, Wallerian degeneration was not so evident for the LLL group (Fig. 1). The LLL group showed an increased total number of neurons, myelinated axons, and Schwann cells compared to the other groups. Histopathological findings are summarized in Figs. 2, 3, and 4.

Discussion

The phototherapy is utilized for treating a variety of pathological conditions of the system of the skeletal muscle, including the peripheral nerves. The irradiation with laser somehow interferes in the nerve function, according to some demonstrations that have found evidence that there is a reduction of latency time and an increase in the speed of the nerve conduction [21]. There is some experimental evidence that lasers have positive effects on the regeneration of injured nerves. The use of AlGaAs laser (660 nm) provided significant changes to the morphometrically assessed area of the myelin sheath [22]. In the present study, the investigators observed a significant difference among the groups for the proliferation of neurons and

Schwann cells over 14 days of treatment through LLL therapy, making the treatment efficiency through this resource, even in a short period of time, statistically proven. Shamir et al. [23] reported that the postoperative treatment of traumatized peripheral nerves enhances the regenerative processes after a complete transaction and anastomosis. The fact that laser therapy is not invasive in the ability of irradiating injured nerves without surgical interventions has been useful [24]. Mohammed et al. [7] showed a significant variation between control and treatment animal groups when examined histopathologically. The animals in the treatment group showed better results regarding the two factors studied in the experiment: larger diameter of nerve fibers and longer internodal distance. These findings suggest a better nerve function, an acceleration of the regenerative process, less inflammatory response (less debris and macrophages accumulation), and less swelling of paranodal areas after the LLL therapy. Therefore, Dos Reis et al.'s [22] study highlighted the effectiveness of AsGa laser for the treatment of peripheral nerve lesions, using the histological analysis for proving such effect. The use of AlGaAs laser (660 nm) provided significant changes in the morphometrically assessed area of the myelin sheath, but it did not culminate in positive results for functional recovery in the sciatic nerve of the rats after injury through neurotmesis. Wavelength is probably the parameter in which there is the most agreement in the LLL therapy community. Wavelengths in the 600–700 nm range are chosen for treating superficial tissues, and wavelengths between 780 and 950 nm are chosen for deeper-seated tissues due to longer optical penetration distances through the tissue. Wavelengths between 700 and 770 nm are not considered to have much activity. There is of course much more work to be done to define the optimum wavelength for the different indications for which LLL therapy is employed.

According to previous studies, an LED source can reach the same level of biomodulatory effects on tissues as a laser source [18, 20], which suggests that LED phototherapy is an alternative treatment. The main differences between LED and laser technology are that an LED light source emits non-coherent rather than coherent radiation, without generating significant amounts of heat. A coherent beam does not seem to be essential for modulating biological phenomena influenced by phototherapy, since luminous radiation loses its coherence upon contact with living tissue [25]. Ishiguro et al. [26] have suggested that irradiation with LEDs is advantageous to nerve regeneration. They found that the number of myelinated axons in the 1 mm sections was much larger for the LED group than for the control group. In addition, the endoneurial area in the smallest section of the LED group (7 mm section) was significantly larger than that observed in

the control group. The efficacy of 940-nm LED phototherapy on nerve regeneration has already been demonstrated by Serafim et al. [19]. However, in the present study, the investigators have not come across similar findings when using 450-nm and 650-nm LED irradiations. Some factors such as the treatment time, the wavelength, and the energy of the LED phototherapy could have influenced the absence of any effect of these LED irradiations on nerve regeneration.

One of the most topical and widely discussed issues in the LLL therapy clinical community is whether the coherence and monochromatic nature of laser radiation has additional benefits, as compared with a more broadband light from a conventional light source or LED with the same center wavelength and intensity. Two aspects of this problem must be distinguished: the coherence of light itself and the coherence of the interaction of light with matter (biomolecules and tissues). The latter interaction produces the phenomenon known as laser speckle, which has been postulated to play a role in the photobiomodulation interaction with cells and subcellular organelles. It is difficult to design an experiment to directly compare coherent laser light with non-coherent non-laser light for the following reason. Laser light is almost always monochromatic with a bandwidth of 1 nm or less, and it is very difficult to generate light from any other source (even an LED) that has a bandwidth narrower than 10–20 nm; therefore, it will be uncertain if observed differences are due to coherent versus non-coherent light, or due to monochromatic versus narrow-bandwidth light [16–20]. This study shows that postoperative 680-nm LLL irradiation was able to accelerate and potentialize the peripheral nerve regeneration process in rabbits within 14 days of irradiation.

Conclusions

Studies investigating the effects of phototherapy on nerve repair are important to determine the efficiency and the safety of irradiation, and the treatment paradigms required for optimal stimulation. The present study examines the effects of different phototherapy systems on nerve injury repair. The investigators demonstrate that different phototherapy systems produce different responses in the nerve tissue, and show a positive effect of 680-nm laser irradiation upon nerve repair in rabbits. Further investigation is required to elucidate the mechanisms underlying the variable outcomes obtained using LED therapy. Future studies will lead to a better understanding of the efficacy of LED therapy.

Compliance with ethical standards All experimental procedures were performed according to the guidelines for the ethical treatment of experimental animals and were approved by the Islamic Azad University, Faculty of Veterinary Sciences, Animal Care and Use Local Ethics Committee.

Competing interests The authors declare that they have no competing interests.

References

- Burnett MG, Zager EL (2004) Pathophysiology of peripheral nerve injury: a brief review. *Neurosurg Focus* 16(5):1–7
- Fukuda TY, Malfatti CA (2008) Analysis of low-level laser therapy doses in Brazilian equipment. *Rev Bras Fisioter* 12(1):70–74
- Kitchen SS, Partridge CJ (1991) A review of low level laser therapy. *Physiother* 77(3):161–168
- Gam AN, Thorsen H, Lonnberg F (1993) The effect of low level laser therapy on musculoskeletal pain: a meta-analysis. *Pain* 52:63–66
- Karu TI (1998) Molecular mechanism of low-power laser therapy. *Lasers Life Sci* 2:53–74
- Matera JM, Dagli MLZ, Pereira DB (1994) Efeitos da radiação soft-laser (diodo) sobre o processo de cicatrização cutânea em felinos. *Braz J Vet Res Anim Sci* 31(1):43–48
- Mohammed IF, Al-Mustawfi N, Kaka LN (2007) Promotion of regenerative processes in injured peripheral nerve induced by low-level laser therapy. *Photomed Laser Surg* 25(2):107–111
- Karu TI (1999) Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *J Photochem Photobiol* 49:1–17
- Passarella S, Casamassima E, Molinari S et al (1984) Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium–neon laser. *FEBS Lett* 175:95–99
- Basford JR (1995) Low intensity laser therapy: still not an established clinical tool. *Lasers Surg Med* 16(4):331–342
- Gigo-Benato D, Geuna S, Rochkind S (2005) Phototherapy for enhancing peripheral nerve repair: a review of the literature. *Muscle Nerve* 31:694–701
- Anders JJ, Geuna S, Rochkind S (2004) Phototherapy promotes regeneration and functional recovery of injured peripheral nerve. *Neurol Res* 26:233–239
- Byrnes KR, Waynant RW, Ilev IK, Wu X, Barna L, Smith K, Heckert R, Gerst H, Anders JJ (2005) Light promotes regeneration and functional recovery and alters the immune response after spinal cord injury. *Lasers Surg Med* 36:171–185
- Anders JJ, Borke RC, Woolery SK, Van de Merwe VP (1993) Low power laser irradiation alters the rate of regeneration of the rat facial nerve. *Lasers Surg Med* 13:72–82
- Hirata K, Kawabuchi M (2002) Myelin phagocytosis by macrophages and nonmacrophages during Wallerian degeneration. *Microsc Res Tech* 57:541–547
- Vinck E, Cagnie B, Cornelissen M, Declercq H, Cambier D (2005) Green light-emitting diode irradiation enhances fibroblast growth impaired by high glucose level. *Photo Med Laser Surg* 23(2):167–171
- Marques CRS, Martin AA, Lima CJ, Conrado LAL, Silveira FL, Carvalho MV (2004) The use of hyperbaric oxygen therapy and LED therapy in diabetic foot. *Lasers Surg Med* 53(12):47–53
- Dall Agnol MA, Nicolau RA, de Lima CJ, Munin E (2009) Comparative analysis of coherent light action (laser) versus non-coherent light (light-emitting diode) for tissue repair in diabetic rats. *Lasers Med Sci* 24(6):909–916
- Serafim KG, Ramos Sde P, de Lima FM, Carandina M, Ferrari O, Dias IF, Toginho Filho Dde O, Siqueira CP (2012) Effects of 940-nm light-emitting diode (LED) on sciatic nerve regeneration in rats. *Lasers Med Sci* 27(1):113–119
- Takhtfooladi MA, Shahzamani M, Takhtfooladi HA, Moayer F, Allahverdi A (2015) Effects of light-emitting diode (LED) therapy on skeletal muscle ischemia reperfusion in rats. *Lasers Med Sci* 30(1):311–316
- Takhtfooladi MA, Jahanbakhsh F, Takhtfooladi HA, Yousefi K, Allahverdi A (2015) Effect of low-level laser therapy (685 nm, 3 J/cm²) on functional recovery of the sciatic nerve in rats following crushing lesion. *Lasers Med Sci* 30(3):1047–1052
- Dos Reis FA, Belchior AC, de Carvalho PT et al (2009) Effect of laser therapy (660 nm) on recovery of the sciatic nerve in rats after injury through neurotmesis followed by epineural anastomosis. *Lasers Med Sci* 24(5):741–747
- Shamir MH, Rochkind S, Sandbank J, Alon M (2001) Double-blind randomized study evaluating regeneration of the rat transected sciatic nerve after suturing and postoperative low-power laser treatment. *J Reconstr Microsurg* 17(2):133–137
- Carvalho PTC, Mazzer N, Dos Reis FA, Belchior ACG, Silva IS (2006) Analysis of the influence of low-power He–Ne laser on the healing of skin wounds in diabetic and non-diabetic rats. *Acta Cir Bras* 21(3):177–183
- Karu TI, Kolyakov SF (2005) Exact action spectra for cellular responses relevant to phototherapy. *Photomed Laser Surg* 23(4):335
- Ishiguro M, Ikeda K, Tomita K (2010) Effect of near-infrared light-emitting diodes on nerve regeneration. *J Orthop Sci* 15(2):233–239