DOI: 10.1590/1809-2950/16557525012018

ORIGINAL RESEARCH

Low intensity laser therapy effectiveness in controlling neuropathic pain in mice

Eficácia da terapia a laser *de baixa intensidade no controle da dor neuropática em camundongos*

Eficacia de la terapia láser de baja potencia en el control del dolor neuropático en ratones

Ariane Dutra Sanchez[1], Ana Laura Martins de Andrade[2], Nivaldo Antonio Parizotto[3]

ABSTRACT | Low-level laser therapy (LLLT) has been widely discussed in the literature as an alternative form of treatment for several types of pain, especially neuropathic pain. This kind of therapy stands out for not being invasive, rarely causing side effects and being cost effective. However, for its effectiveness, it is necessary more detailed parameters, which are still very discrepant in the literature. Thus, this study aims to investigate the effect of LLLT, in the infrared range, with 30J/cm^2 fluency, on the control of neuropathic pain in animal models. A total of 24 male Swiss albino mice, weighing 25.30 grams, were divided into three groups: the Control Group (CG), the Laser Group (L30G) and the Sham Group (SG). The induction of neuropathy was held through the model of chronic constriction of the sciatic nerve (CCI), and the LLLT treatment was conducted as follows: The CG was treated with 0 J/cm^2 fluency, whereas the L30G was treated with 30 J/cm^2 fluency, and the SG with simulation of surgery without intervention. The irradiations were performed 3 times a week, for 90 days, at the nerve's point of compression, using the contact technique. For the evaluation, the Hot Plate Test was used for thermal hyperalgesia, and the Randall-Selitto test was used for mechanical hyperalgesia. In the CG's results, we observed no significant improvement in the days after surgery in any of the tests conducted, and, in the GL30, a significant improvement in both tests was observed: from the 30th day of treatment for the Hot Plate Test, and from the 45th for the Randall-Selitto Test, in which the mice showed total restoration of sensitivity. We thus conclude that the use of LLLT with 30 J/cm^2 fluency in the treatment of neuropathic pain in animal models is effective.

Keywords | Low-Level Light Therapy; Sciatica Neuropathy; Pain Measurement.

RESUMO | A terapia a *laser* de baixa intensidade (LLLT) vem sendo amplamente discutida na literatura como forma alternativa de tratamento para diversos tipos de dor, com destaque para a neuropática. Essa terapia sobressai pelo fato de não ser invasiva, raramente causar efeitos colaterais e ser de baixo custo. Em contrapartida, para sua eficácia, é necessário o detalhamento dos parâmetros, que ainda são muito discrepantes na literatura. Assim, este trabalho tem como objetivo investigar o efeito da LLLT, na faixa do infravermelho, com fluência de 30J/cm^2, no controle da dor neuropática em modelo animal. Foram utilizados 24 camundongos da cepa suíço albino, machos, pesando 2530 gramas, divididos em três grupos: Grupo Placebo (GP), Grupo *Laser* (GL30) e Grupo Sham (GS). A indução da neuropatia foi feita através do modelo de constrição crônica do nervo isquiático (CCI), e o tratamento da LLLT realizou-se da seguinte maneira: GP com o *laser* com fluência de 0J/cm^2, GL30 tratado com fluência de 30J/cm^2, e GS com simulação de cirurgia sem intervenção. Executaram-se as irradiações 3 vezes por semana, durante 90 dias, no ponto de compressão do nervo, utilizando-se a técnica de contato. A fins de avaliação, foram utilizados o teste da placa quente, para hiperalgesia térmica, e o Teste de Randall-Selitto para hiperalgesia mecânica. Nos resultados do GP, observamos que não houve melhora

[1]Degree in Physical Therapy at Universidade Federal de São Carlos (UFSCar), São Carlos (SP), Brasil.
[2]Doctoral student from the Department of Physical Therapy at Universidade Federal de São Carlos (UFSCar), São Carlos (SP), Brasil.
[3]Senior professor at the Department of Physical Therapy at Universidade Federal de São Carlos (UFSCar), São Carlos (SP), Brasil.

Corresponding address: Ariane Dutra Sanchez – Rodovia Washington Luís – São Carlos (SP), Brazil – Zip Code: 13565-905 – Telephone: +55 (16) 3351-8111 – E-mail: ariane.dutra.sanchez@gmail.com – Financing source: CNPq – Conflict of interest: Nothing to declare – Presentention: June 21st, 2016 – Accepted for publication: Dec. 12nd, 2017 – Approved by the Research Ethics Committee of the Universidade Federal de São Carlos under protocol no. 026/2014.

significativa nos dias após a cirurgia em nenhum dos testes realizados e, no GL30, observou-se uma melhora expressiva em ambos os testes a partir do 30º dia de tratamento para o teste de Placa Quente e a partir do 45º para o Randall-Selitto, em que os camundongos apresentaram restauração total da sensibilidade. Concluímos, pois, que a utilização de LLLT com fluência de 30J/cm^2 no tratamento da dor neuropática em modelo animal é eficaz.

Descritores | Terapia com Luz de Baixa Intensidade; Neuropatia Ciática; Medição da Dor.

RESUMEN | La terapia láser de baja potencia (LLLT) ha sido ampliamente discutida en la literatura como forma alternativa de tratamiento para muchos tipos de dolor, especialmente para el neuropático. Esa terapia se destaca por el hecho de no ser invasiva, raramente causar efectos secundarios y ser de bajo costo. En cambio, para su eficacia, es necesario que se presenten los detalles de los parámetros, que todavía son muy discordantes en la literatura. Así, este trabajo tiene como objetivo investigar el efecto de la LLLT, en el rango del infrarrojo, con fluidez de 30 J/cm^2, en el control del dolor neuropático en modelos animales. Se utilizaron 24 ratones de la cepa suizo-albino, machos, con peso de 2530 gramos, divididos en tres grupos: Grupo Placebo (GP), Grupo Láser (GL30) y Grupo Sham (GS). Se llevó a cabo la inducción de la neuropatía por medio del modelo de constricción crónica del nervio isquiático y se realizó el tratamiento de la LLLT como sigue: GP con el láser con fluidez de 0 J/cm^2, GL30 con fluidez de 30 J/cm^2, y GS con simulación de cirugía sin intervención. Se realizaron las irradiaciones tres veces por semana, durante 90 días, en el punto de compresión del nervio, utilizándose la técnica de contacto. A efectos de evaluación, se utilizaron la prueba de placa caliente para hiperalgesia térmica y la prueba de Randall y Selitto para hiperalgesia mecánica. En los resultados del GP, observamos que no hubo mejora significativa en los días después de la cirugía en ninguna de las pruebas realizadas y, en el GP30, se verificó mejora significativa en ambas pruebas: desde el 30.º día de tratamiento para la prueba de placa caliente y desde el 45.º día para la prueba de Randall y Selitto, en que los ratones presentaron restauración total de la sensibilidad. Concluimos, por lo tanto, que el uso de la LLLT con fluidez de 30 J/cm^2 en el tratamiento del dolor neuropático en modelos animales es efectivo.

Palabras clave | Terapia de Luz de Baja Potencia; Neuropatía Ciática; Medición del Dolor.

INTRODUCTION

Nowadays, pain is considered a complex subjective experience, which does not only involve the transduction of harmful environmental stimuli, but also cognitive and emotional processing by the encephalon[1].

According to the International Association for the Study of Pain (IASP), it can be defined as an unpleasant emotional or sensory experience associated with actual or potential tissue damage[2].

Lesions of the central or peripheral nervous system may lead to a special kind of pain in the absence of nociception, called neuropathic pain[3]. Unlike nociceptive pain, which results from the physiological activation of nociceptors due to actual or potential tissue damage and acts as an alert to the organism, neuropathic pain has no "beneficial" effect and may cause damages to the somatosensory system[4].

Neuropathic pain was recently redefined by the International Association for the Study of Pain (IASP) as "pain caused by an injury or disease of the somatosensory system", and can be classified according to its intrinsic cause or to the site of the nervous injury – central or peripheral[5].

The pathophysiology of neuropathic pain has not been completely clarified; however, changes such as awareness of peripheral receptors and of central projection cells[6], cross-excitation between neurons as a result of damages to the myelin[7], as well as abnormal activity of the central processing units of the sensitive afferent[8] have been described as important mechanisms involved in the genesis of this kind of pain.

Morphological and electrophysiological changes resulting from peripheral neuropathies are usually investigated through animal models, such as Spinal Nerve Ligation (SNL), Partial Sciatic Nerve Ligation (PSL), Spared Nerve Injury (SNI) and those involving chronic constriction. Among the models mentioned, the method of chronic constriction of the sciatic nerve (CCI), first described by Bennet and Xie[9], has been widely investigated due to its high reliability, for being easily reproduced and for featuring the development of thermal and mechanical hyperalgesia and allodynia, these symptoms being similar to those that occur in humans suffering from neuropathies[9-11].

Recently, low-level laser therapy (LLLT) have been mentioned in the literature, in the fields of Medicine and Dentistry – it has a biomodulating effect and is indicated in cases of pain and tissue repair[12].

LLLT is based on light, which penetrates the skin and reaches specific receptors, stimulating a response for each type of injury and/or symptoms. This therapy has as main purpose promoting the release of histamine, serotonin, bradykinin and prostaglandin, in addition to modifications in enzymatic action, favoring tissue regeneration and pain reduction. LLLT leads to the emission of photons that reach the mitochondria and the cell membranes of fibroblasts, keratinocytes and endothelial cells, allowing the absorption of light energy by cell chromophores and converting it into chemical kinetic energy inside the cell[13,14].

The discussions on LLLT for the control of various types of pain has been gaining prominence in the literature, with great emphasis on neuropathic pain[15,16]. A significant amount of studies using this therapy is observed; however, the parameters used are quite disparate, hindering the establishment of appropriate protocols to ensure the effectiveness of LLLT[17].

A study conducted by Andrade et al.[17] highlight the use of LLLT in the control of neuropathic pain from a systematic review, in which the authors report the use of lasers in the infrared range as the most used for the control of this type of pain, in addition to emphasizing that very low fluencies may not promote significant analgesia, reinforcing the idea that there is a lack of detailing of the parameters used in the studies.

Thus, this study sought to identify whether the use of LLLT on the 808nm wavelength, with 30J/cm^2 fluency, can be effective to control neuropathic pain in an experimental model.

METHODOLOGY

This study was approved by the Research Ethics Committee of Universidade Federal de São Carlos under protocol no. 026/2014.

A total of 24 male Swiss albino mice, weighing 25.30 grams and from the vivarium of Universidade Federal de São Carlos (UFSCar) were used. The animals were grouped in collective cages, kept under controlled conditions of temperature (24+/ 1ºC), humidity (55 +/- 5%), light (light/dark 12/12 hours cycle, lights on at 7 a.m. and turned off at 7 p.m.) and free access to food and water, except during the brief test sessions. All experiments followed the rules of ethics established for animal experimentation, recommended by the International Association for the Study of Pain (IASP)[18].

Induction of neuropathy through chronic constriction of the sciatic nerve (CCI)

The method of Bennett and Xie[9] was used for the reproduction of the model of neuropathic pain due to the great reliability of the studies that have used this model, in addition to it reproducing very closely the symptoms developed in humans. The surgery started with the application of anesthesia with Ketamine and Xilasina (90 mg/Kg, IP), followed by the sectioning of the fascia between the gluteus and the femoral biceps and the exposition of the right sciatic nerve, next to its trifurcation. The tissue around the nerve was carefully cut at a distance of approximately 8 mm and then the compression of the nerve was carried out using four ligatures with sterile non-inflammatory monofilament nylon thread 5.0.

After the surgery, the animals were kept in the vivarium of the Department of Physical Therapy where they were randomly allocated into two groups, 8 animals each:

- Control Intervention Group (CIG): the animals were induced to neuropathy through CCI and treated with LLLT at a 0 J/cm^2 fluency.
- 30 J/cm^2 Laser Intervention Group (L30IG): the animals were induced to neuropathy through CCI and treated with LLLT at a 0 J/cm^2 fluency.
- Control Placebo Group (CPG): the exposure of the sciatic nerve was conducted without compressing the sciatic nerve, in order to simulate the CCI surgery.

Laser application

Three days after the constriction of the sciatic nerve, the animals were subjected to irradiation with infrared aluminum gallium arsenide (AsALGa) laser with 808 NM continuous wavelength, 30 mW power and 0.028 cm^2 transverse beam area.

The animals, subdivided into three groups, received the application of LLLT according to the parameters described in Table 1:

Table 1. Detailed LLLT used in the study

Group	Fluency	Time	Energy
GP	0 J/cm²	28 sec	0J
GL30	30 J/cm²	28 sec	0,84 J
GS	–	–	–

The irradiation was carried out three times a week for 60 days, that being the total period of the experiment, with the site of compression of the nerve having been determined at one point, using the contact technique.

Functional evaluations

It is known that during prolonged nociceptive processes, the exacerbation of the perception of harmful stimuli occurs, due to peripheral and central sensitization, causing the appearance of thermal and mechanical hyperalgesia[19]. For the evaluation of this state, the hot plate test was carried out to evaluate thermal hyperalgesia, and the Randall-Selitto Test was used to assess mechanical hyperalgesia.

The evaluations were started the day before the pre-surgical period, so the data would serve as baseline for the study. After 48 hours of surgery, the functional evaluations were carried out again to demonstrate the appearance of symptoms, such as spontaneous pain and hyperalgesia, so the treatment with LLLT could be subsequently initiated.

Thermal hyperalgesia

For the evaluation of thermal hyperalgesia, the time for withdrawal of the rear paw was recorded in seconds, after applying heat at 52°C (51.8–52.4 °C) as the threshold of maximum pain tolerance, through the hot plate test[20]. The maximum time of permanence of the animal on the plate was 25 seconds to avoid possible lesions[21].

Mechanical hyperalgesia

Hyperalgesia to mechanical stimuli was evaluated through the Randall-Selitto Test[22]. This test consists of evaluation parameters of maximum load supported on the injured rear paw, in grams, and the latency for the response of withdrawal with the maximum load, in seconds. After the beginning of the treatment, the evaluations were conducted every 15 days for a period of 60 days (end of the experiment).

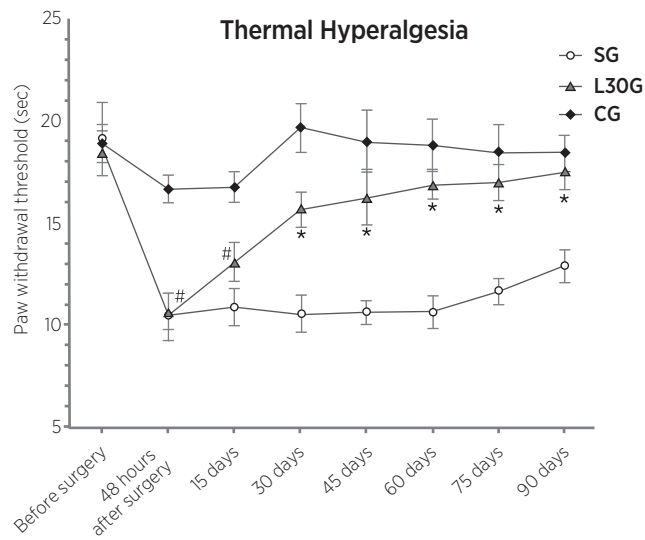
Statistical analysis

The results were expressed as mean ± standard deviation of the mean (E.P.M.). Two-way analysis of variance (ANOVA) was used. The *post-hoc* comparisons were performed using Bonferroni correction. The level of significance was $p<0.05$. The GraphPadPrisma 5.0 program was used for obtaining data and making graphs.

RESULTS

Thermal hyperalgesia

Graph 1 represents the results related to the Hot Plate test in all groups, considering the paw withdrawal threshold (in seconds) and the days of evaluation. For the CG (0 J/cm ²) it was possible to observe that after undergoing the CCI surgery, the animals obtained a significant decrease of the paw withdrawal threshold, which remained low throughout the treatment period, not returning to its pre-surgical conditions, without significant improvements. In the L30G group, significant improvements started being observed from the 30th day of treatment, and kept increasing until it was over; consequently, the animals became less vulnerable to thermal hyperalgesia induced by neuropathy, configuring the restoration of sensitivity, which became similar to that of the pre-surgical period.

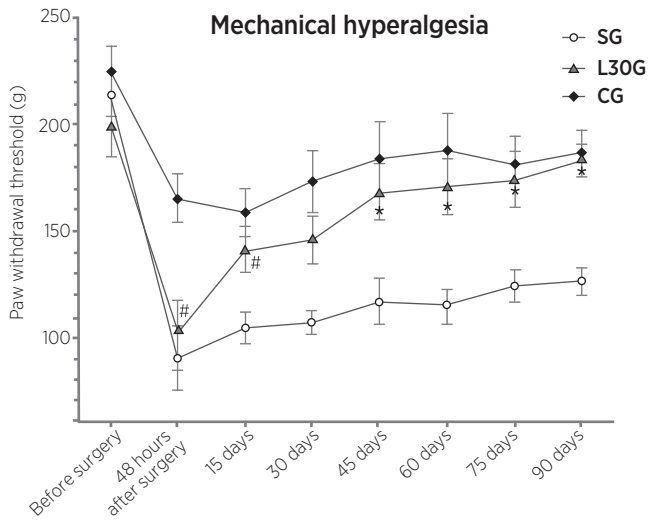


Graph 1. Thermal hyperalgesia evaluated through the hot plate test
* Significant difference of the L30G in comparison with CG. # Significant difference between L30G and SG.

Mechanical hyperalgesia

The Randall-Selitto test was also held in all groups. It was observed that after the CCI surgery, there was a significant decrease of the paw withdrawal threshold in the CG, which remained low during the entire experiment – the animals did not return to the mechanical tolerance conditions of the pre-surgical period. For the L30G group, a gradual improvement from the 45th day of treatment until the end of the experiment was observed, in which the pressure tolerated by the animal's paw was similar to the pressure tolerated in the pre-surgical period, with it consequently becoming less vulnerable to mechanical hyperalgesia and maintaining this improvement at the end of the treatment (Graph 2).

When comparing the groups, we observed the animals from the CG group did not return to the pre-surgical state, when evaluated through the Hot Plate and Randall-Selitto tests, whereas the animals that received 30 J/cm² fluency managed to return to values similar to those found in the pre-surgery period. Thus, there was significant difference between the groups from the 30th day of treatment in relation to thermal hyperalgesia and from the 45th day of treatment for mechanical hyperalgesia (Graph 1 and 2).



Graph 2. Thermal hyperalgesia evaluated through the hot plate test

* Significant difference of the L30G in comparison with CG. # Significant difference between L30G and SG.

It is worth noting that SG showed significant reduction of values throughout the entire experimental period – it is even possible to report that only the groups that underwent CCI surgery presented exacerbated response to the stimuli. At the end of the experiment, it was possible to observe, through the functional evaluations, that the L30G was not significantly different from the SG, thus demonstrating the effectiveness of treatment with LLLT.

DISCUSSION

Low-level laser is one of the most used biostimulation rehabilitation modalities at present, which has contributed to a better understanding of its principles and applicabilities[14,23]. LLLT is a non-invasive method that demonstrates being clinically effective in reducing sensitivity to pain, as demonstrated in this study, in which the group that received laser irradiation with 30 J/cm² fluency was proved to be less vulnerable to thermal and mechanical hyperalgesia when compared to the CG.

Gonçalves et al.[24] highlighted in their findings that the LLLT's action contributed to reduce inflammation by decreasing the degeneration of the myelin sheath and inflammatory infiltrates, concluding that LLLT can reduce the inflammatory process of lesions in the sciatic nerve of rats, and thus the painful stimuli, as did Andrade et al.[10] who, based on their results, concluded that LLLT achieved beneficial effects in the regeneration of nerve tissue, and in eliminating inflammatory processes and promoting analgesia.

The analgesic effects induced by LLLT can be thus justified through the modulation of the chemical mediators of inflammation, in addition to the stimuli to the synthesis of beta endorphin. These factors tend to limit the reduction in the threshold of excitability of painful receptors and to eliminate allogenic substances[25,26].

One of the main characteristics of pathological pain is hypersensitization, i.e., changes in the plasticity of the nervous system, which occurs peripherally through reduction of the threshold for the activation of nociceptors, or centrally through the increased responsiveness of the spinal cord to sensorial stimuli[16,27]. A study conducted by Andrade et al.[10] evaluated the effect of LLLT regarding hyperalgesia and spontaneous nociception in animals subjected to CCI surgery; after 12 weeks of treatment, reduction in hyperalgesia and promotion of analgesia were observed, and the animals returned to the pre-surgical state. It is believed that the light emitted by LLLT, when applied on the injured

nerve, normalizes the speed of transmission of the nervous impulse[13,14].

Coradini et al.[16] conducted an experimental study comparing LLLT with therapeutic ultrasound in the treatment of neuropathic pain. LLLT was held with an infrared laser with 830nm wavelength and 8J/cm^2 fluency. The study showed better results for the group treated with LLLT, when compared to the ultrasound group and the control group. The results corroborate our study, since LLLT with infrared laser was also used here in the treatment of neuropathic pain, significant improvements of the L30G having been obtained when compared with the CG (0J/cm^2).

In a study by Yan et al.[27], treatment with LLLT was used in the control of neuropathic pain in mice induced to neuropathy through the CCI surgery, which was also used in our study. The protocols used were LLLT with 650nm infrared laser, 808nm infrared laser and a control group. Significant reductions were observed in hyperalgesia for the group that used LLLT with 808nm, when compared to the other groups, thus resembling our study, which used the same type of laser, obtaining positive results concerning thermal and mechanical hyperalgesia.

Hsieh et al.[28] investigated the effects of LLLT in rats after they had undergone CCCI surgery, the mechanical paw withdrawal threshold in the control of neuropathic pain having been evaluated. The results showed a significant decrease of mechanical hyperalgesia, demonstrating that LLLT can reduce nervous hyperexcitation, thus promoting the reduction of painful procedures, as found in our study, in which the results of the Randall-Selitto test demonstrated a significant reduction in mechanical hyperalgesia and consequently control of neuropathic pain.

Recently, a review published by Andrade et al.[17] highlighted the difficulty in establishing effective protocols for using LLLT in neuropathic pain control, highlighting the great discrepancy between the parameters used in the studies in addition to the lack of their detailing. In this review, the authors highlight the existence of a possible "therapeutic window" that can generate an effective photobiostimulation of LLLT in the treatment of neuropathic pain, since the wavelength in the infrared range has been showing better results for the promotion of analgesia, like in our study in which the wavelength used is within this range. Regarding the power used, this review showed that the 30mW-70mW range is the most widely used in the literature, promoting positive results regarding the control of neuropathic pain, in addition to the fluency employed, with the studies that used fluencies near 15J/cm^2 having suggested the effectiveness of LLLT in the promotion of analgesia. The data presented by Andrade et al.[17] reinforce the results of our study, which obtained significant analgesia using 30mW power and 30J/cm^2 fluency.

According to the review published by Andreo et al.[29], LLLT is a viable phototherapy mode to treat peripheral nerve injury, demonstrating positive effects on the neuromuscular repair process and improvements in the functional indexes. The study suggests the use of an infrared laser may significantly reduce thermal and mechanical hyperalgesia in groups subjected to nervous constriction and treated with laser, corroborating our study, which obtained similar results using the same type of laser. The authors argue that the results of these studies can be clarified through changes of morphological aspects, for which an increase in the concentration of myelin by cross-section area, increase in the number of blood vessels and of the diameter of nerve fibers, in addition to the expression of cytokines and growth factors are observed. The review also emphasizes that the determination of the parameters is important for the standardization of a LLLT therapeutic protocol and highlights the need for investigating the mechanisms of action of different parameters to confirm the signaling channels.

Given the above, we can verify that LLLT is an effective alternative for the promotion of analgesia and control of neuropathic pain. However, there is great difficulty in establishing protocols, since many studies do not present the parameters used in detail; thus, further research is required to confirm the importance and the dependency between each of the laser's parameters, as well as the possible influences exerted in the biological responses, to improve the specificity of LLLT and also the elaboration of protocols with safer and more effective treatments.

CONCLUSION

We thus conclude that the use of LLLT with 30J/cm^2 fluency, continuous 808 nanometer wavelength, and with 30 mW power in the treatment of neuropathic pain in mice, is effective according to the results obtained in this study, in which the animals irradiated returned to the

pre-surgical state on the 30th day of treatment, this result having been maintained until the end of the experiment.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from CNPQ.

REFERENCES

1. Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001;413:203-10. doi: 10.1038/35093019
2. Merskey, H, Albe-Fessard DG, Bonica JJ, et al. Pain terms: a list with definition and notes on usage. Recommended by the IASP Subcommittee on Taxonomy. Pain. 1979;6(3):249-252. PMID: 460932
3. Dickenson A, Suzuki R. Targets in pain and analgesia. In: Hunt S, Koltzenburg. The neurobiology of pain: molecular and cellular neurobiology. Cambridge: Oxford University Press; 2005. Chapter 6.
4. Woolf CJ, Costigan M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. Proc Natl Acad Sci. 1999;96(14):7723-30. doi: 10.1073/pnas.96.14.7723
5. Treede, MD, Jensen TS, Campbell N, Cruccu G, Dostrovsky O, Griffin W, et al. Neuropathic pain: redefinition and a grading system for clinical and research purposes. Neurology. 2008;70(18):1630-5. doi: 10.1212/01.wnl.0000282763.29778.59
6. Pogatzki EM, Gebhart GF, Brennan TJ. Characterization of A-ð and C-fibers innervating the plantar rat hindpaw one day after an incision. J Neurophysiol. 2002;87:721-31. doi: 10.1152/jn.00208.2001
7. Lisney SJW. Functional aspects of the regeneration of unmyelinated axons in the rat saphenous nerve. J Neurol Sci. 1987;80(2-3):289-98. doi: 10.1016/0022-510X(87)90163-8
8. Birklein F, Rowbotham MC. Does pain change the brain? Neurology. 2005;65(5):666-7. doi: 10.1212/01.wnl.0000179148.80687.f3
9. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988;33(1):87-107. doi: 10.1016/0304-3959(88)90209-6
10. Andrade ALM, Bossini PS, Canto-de-Souza ALM, Sanchez AD, Parizotto NA. Effect of photobiomodulation therapy (808 nm) in the control of neuropathic pain in mice. Lasers Med Sci. 2017; 2017;32(4):865-72. doi: 10.1007/s10103-017-2186-X
11. Colleoni M, Sacerdote P. Murine models of human neuropathic pain. Biochim Biophys Acta. 2010;1802(10):924-33. doi: 10.1016/j.bbadis.2009.10.012
12. Enwemeka CS, Parker JC, Harkness EE, Harkness LE, Woodruff LD. Efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. Photomed Laser Surg. 2004;22(4):323-9. doi: 10.1089/pho.2004.22.323
13. Hawkins D, Abrahamse H. Changes in cell viability of wounded fibroblasts following laser irradiation in broad-spectrum or infrared light. Laser Chemistry. 2007;2007:[10 p]. doi: 10.1155/2007/71039
14. Chow RT; Lopes-Martins R., Johnson M., Bjordal J.M. Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised, placebo and active treatment controlled trials. Lancet. 2009;374(9705):1897-908. doi: 10.1016/S0140-6736(09)61522-1
15. Masoumipoor M, Jameie SB, Janzadeh A, Nasirinezhad F, Soleimani M, Kerdary M. Effects of 660- and 980-nm low-level laser therapy on neuropathic pain relief following chronic constriction injury in rat sciatic nerve. Lasers Med Sci. 2014;29(5):1593-8. doi: 10.1007/s10103-014-1552-1
16. Coradini JG, Mattjie TF, Bernardino GR, Peretti AL, Kakihata CMM, Errero TK, et al. Comparação entre o laser de baixa potência, ultrassom terapêutico e associação, na dor articular em ratos Wistar. Rev Bras Reumatol. 2014;54(1):7-12. doi: 10.1016/j.rbr.2014.01.001
17. Andrade ALM, Bossini OS, Parizotto NA. Use of low level laser therapy to control neuropathic pain: a systematic review. J Photochem Photobiol B. 2016;164:36-42. doi: 10.1016/j.jphotobiol.2016.08.025
18. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983;16(2):109-10. doi: 10.1016/0304-3959(83)90201-4
19. Loeser JD, Treede RD. The Kyoto protocol of IASP basic pain terminology. Pain. 2008;137(3):473-7. doi: 10.1016/j.pain.2008.04.025
20. Liu NJ, Gizycki H, Gintzler AR. Phospholipase Cβ1 modulates pain sensitivity, opioid antinociception and opioid tolerance formation. Brain Research. 2006;1069(1):47-53. doi: 10.1016/j.brainres.2005.09.069
21. Kuraishi Y, Harada Y, Aratani S, Satoh M, Takagi H. Separate involvement of the spinal noradrenergic and serotonergic systems in morphine analgesia: the differences in mechanical and thermal algesic tests. Brain Research. 1983;273(2):245-52. doi: 10.1016/0006-8993(83)90849-1
22. Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther. 1957;111(4):409-19. PMID: 13471093
23. Schindl A, Schindl M. Pernerstorfer-Schön H, Schindl L. Low-intensity laser therapy: a review. J Investig Med. 2000;48(5):312-26. PMID: 10979236
24. Gonçalves RB, Marques JC, Monte-Raso V, Zamarioli A, Carvalho LC, Fazan VPS. Efeito da aplicação do laser de baixa potência na regeneração do nervo isquiático de ratos. Fisioter e Pesqui. 2010;17(1):34-9. doi: 10.1590/S1809-29502010000100007
25. Campana EA, Moya M, Gavotto A, Soriano F, Juri HO, Spitale LS, et al. The relative effects of He-Ne laser and meloxicam on experimentally induced inflammation. Laser Therapy. 1999;11(1):36-41. doi: 10.5978/islsm.11.36
26. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J Photochem Photobiol B. 1999;49(1):1-17. doi: 10.1016/S1011-1344(98)00219-X
27. Yan W, Chow R, Armati PJ. Inhibitory effects of visible 650-nm and infrared 808-nm laser irradiation on somatosensory

and compound muscle action potentials in rat sciatic nerve: implications for laser-induced analgesia. J Peripher Nerv Syst. 2011;16(2):130-5. doi: 10.1111/j.1529-8027.2011.00337.x

28. Hsieh YL, Chou LW, Chang PL, Yang CC, Kao MJ, Hong CZ. Low-level laser therapy alleviates neuropathic pain and promotes function recovery in rats with chronic constriction injury: possible involvements in hypoxia-inducible factor 1α (HIF-1α). J Comp Neurol. 2012;520(13):2903-16. doi: 10.1002/cne.23072
29. Andreo L, Soldera CB, Ribeiro BG, de Matos PRV, Bussadori SK, Fernandes KPS, et al. Effects of photobiomodulation on experimental models of peripheral nerve injury. Lasers Med Sci. 2017;32(9):2155-65. doi: 10.1007/s10103-017-2359-7