LOW INTENSITY LASER (830 NM) FUNCTIONAL TO RECOVER OF THE SCIATIC NERVE IN RATS

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ABSTRACT

Objective: This study aimed to evaluate the effect of low-intensity laser on functional improvement of the walking of rats after sciatic nerve axonotmesis. Methods: We used 18 rats divided randomly in two groups: control (Sham) and irradiated with an energy density of 40J/cm² for 21 consecutive days, using 830nm laser (AsGaAl). The animals were subjected to right sciatic nerve crushing by a portable device and assessed by the "Sciatic Functional Index" (SFI) at an acrylic platform through video recorded by a digital camera. The footprints were collected preoperatively, and on the 7th, 14th and 21st postoperative days. Results: The results of the SFI were significant when comparing the groups on the 7th and 14th postoperative day ($p<0.05$). On the 21st postoperative day there was no difference between groups. There were intra-group differences detected in each evaluated week ($p<0.01$). The irradiated animals showed improvement in motion pattern, shown by the SFI values in the initial periods, but after 3 weeks, there was a similar recovery. Conclusion: The low-intensity laser has shown to be effective in accelerating regeneration of the sciatic nerve of rats after crushing.

Keywords: Sciatic nerve. Crush syndrome. Laser therapy, Low-level.

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INTRODUCTION

Peripheral nerve injuries present high incidence among traumatic injuries, caused by crushing or sectioning, producing important functional disabilities, which may determine lifelong sequelae. These injuries can be classified as neuropraxia, axonotmesis or neurotmesis.[1] Sunderland[2] divided these lesions into five grades, bearing in mind the affected structures.

Several surveys are being conducted with the objective of determining the parameters and types of stimulation that can accelerate the regeneration and the functional recuperation of the peripheral nerve, aiming to minimize future dysfunctions. Among the physiotherapeutic resources available, low-intensity laser therapy has been used on a large scale in the experimental scope.[3] Studies have demonstrated that this resource promotes modification in the enzymatic reactions, as it interferes by inhibiting prostaglandin synthesis, besides inhibiting the release of autocoids. Low-intensity laser therapy has also been employed in the healing of several tissues, as it promotes a stimulus in microcirculation, through the paralysis of the precapillary sphincters, and consequent vasodilation of arterioles and capillaries. The resulting angiogenesis also leads to an increase of the blood flow in the irradiated area and in the production of cellular ATP, causing the acceleration of cellular mitotic activity.[4]

Medinacelli et al.[5] proposed the Sciatic Functional Index (SFI) used to evaluate the injury grade and the functional recovery of the sciatic nerve. The method uses a platform where the footprints are printed on a strip of paper for analysis of parameters such as print length (PL), toe spread (TS) and intermediate toe spread (IT).

The data obtained through the recording of footprints are included in the following equation proposed by Bain et al.[6]

$$SFI = -38.3 \times \frac{EPL - NPL}{NPL} + 109.5 \times \frac{ETS - NTS}{NTS} + 13.3 \times \frac{EIT - NIT}{NIT} - 8.8$$

Where:

SFI: Sciatic Functional Index
N = Normal
E = Experimental
PL = Print Length
TS = Toe Spread
IT = Intermediate Toe

All the authors declare that there is no potential conflict of interest referring to this article.

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Monte Raso et al.[7] evaluated the method through specific software and concluded that it is a quantitative, reliable and reproducible method of the functional condition of the sciatic nerve of rats.

Gasparini et al.[8] studied the Sciatic Functional Index using an acrylic platform, in the same dimensions as the wooden platform. The prints were recorded by a digital camera and the footprint images evaluated by the computer program *Image J®*, to quantify the parameters predetermined by De Medinacelli et al.[5,9] and modified by Bain et al.[6]

The aim of this study was to investigate the effects of low-intensity laser on the functional recovery of gait of rats after crushing of the sciatic nerve.

MATERIAL AND METHODS

The study subjects were 18 adult, male rats (*Rattus norvergicus: var. albinus, Rodentia, Mammalia*), around three months of age, weighing between 280 and 310 grams, of the Wistar lineage, originating from the Central Bioterium and allocated in the Bioengineering Department of Faculdade de Medicina de Ribeirão Preto - USP. The experimental procedures were carried out in the Bioengineering laboratory where the animals were kept in collective cages, receiving commercial fed and water *ad libitum*. This study was approved by the Committee of Animal Experimentation Ethics of Faculdade de Medicina de Ribeirão Preto - USP, protocol no. 103/2006.

A portable deadweight device with a load of 5000g applied for 10 minutes was used in this study on the crushing of the sciatic nerve of rats. This device is characterized as being a faster, easier and more reliable crushing process, in relation to the load used. This equipment was produced by Oficina de Precisão da Prefeitura do Campus de Ribeirão Preto da Universidade de São Paulo. The equipment consists of a platform for the support of the animal (A), with a main structure (B) that receives the backup support for the weights gauged (0.5 Kg, 1.0 Kg, 5.0 Kg, 10.0 Kg and 15.0 Kg) with telescopable axis (C), a support base for the nerve (D), pressure application axis (E), a lever to activate the weights and to position the nerve on the support base (F) and a spring to keep the lever balanced (G)[10,11]. (Figure 1)

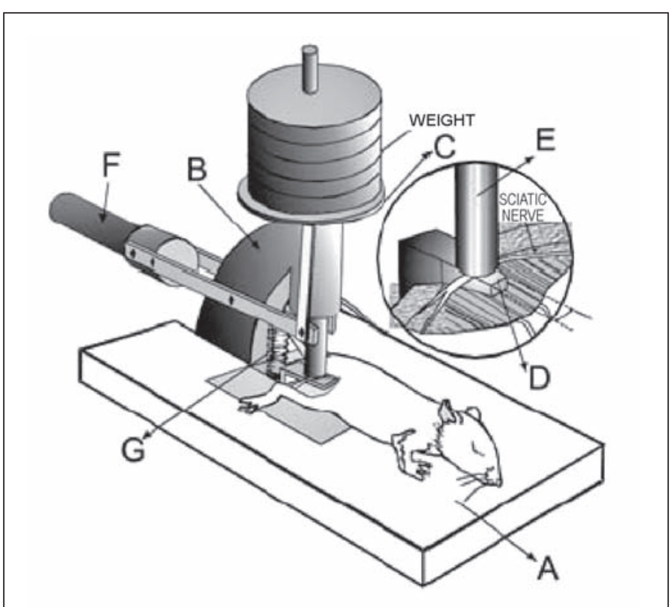

Figure 1 – *Dead Weight Device, Pachioni et al.[11]*

In this study they used Aluminum Gallium Arsenide (AlGaAs) diode laser equipment, which emits a wavelength of 830nm, with power of 30mW, beam area of 0.116cm² and with continuous bean of the *Ibramed®* medical equipment brand.

The rats were weighed and distributed randomly in two groups of nine animals each. All the animals underwent the same surgical procedure for crushing of the right sciatic nerve. Group 1 was the Control Group (sham) and group 2 was irradiated with energy density of 40 J/cm², and emitted energy of 4.64 J.

The laser radiation for the irradiated group was applied at 1 point predetermined in the surgical act on the nerve crushing site, with the pen positioned at 90° in relation to the cutaneous tissue, using the punctual contact technique, immediately after the operation and in the 21 days subsequent thereto, using the 830 nm diode laser. Daily trichotomy was performed before laser application. The rats were sacrificed with an overdose of anesthesia after the 21st postoperative day.

An acrylic platform Gasparini et al.[8] was used for visualization of gait, which was filmed with the use of a SONY® digital camcorder (model DCR-DVD 203), for subsequent analysis of the footprints in the preoperative period, 7th, 14th and 21st postoperative days. After the collection, the footprints were analyzed by the sciatic functional index, with the use of the *Image J®* software for quantification of the SFI parameters. The evaluation using the SFI indicates normal function when the index is close to 0, and when the index approaches -100 is considered a total dysfunction of the studied segment. (Figure 2)

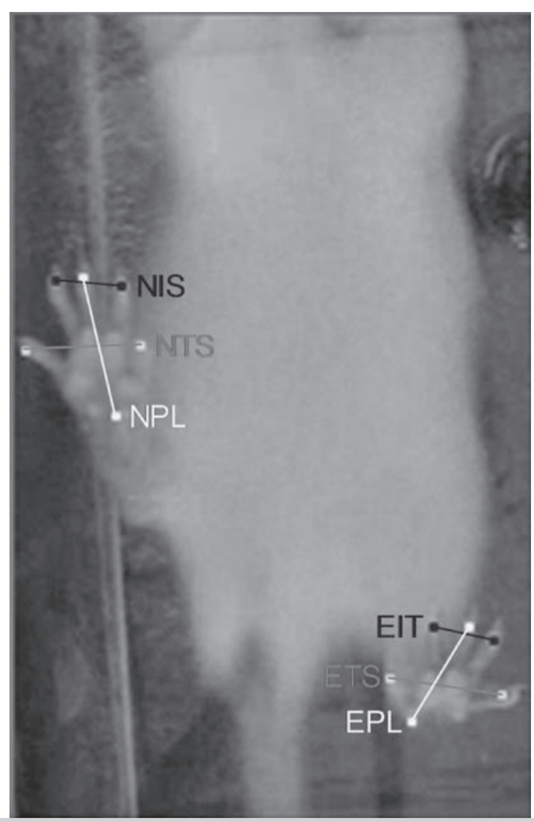

Figure 2 – *Footprint image captured by the SONY® camcorder, kindly lent by the Bioengineering laboratory of FMRP-USP, and showing delineation of SFI parameters.*

The statistical analysis of the values obtained by the SFI, in the treated and control groups, was conducted by the linear fixed effects model. This model considers the individuals as random effect and, as fixed effects, the groups, times and interaction between these, calculating: the mean, standard deviation, and coefficient of variation. The confidence interval was 95%, with significance index of $p<0.05$.[12]

RESULTS

In the initial phase of the experiment 3 animals died after the neural segment crushing surgery, probably due to an overdose or reaction to the anesthetic. Nonetheless, there was no sign of infection and/or suture dehiscence in these animals. During the experiment there was suture dehiscence in 1 rat from the control group.

The surgical procedure and the laser application were tolerated by all the other animals.

The recording of footprints was performed in the different periods, preoperative, 7th, 14th and 21st postoperative day, totalizing 72 footprints. The footprint images were evaluated by the formula of the sciatic functional index proposed by Bain et al.[6] The results of the SFI of the 2 groups are described in Table 1.

The results obtained with the SFI of the two groups on average were: in the preoperative period, control -6,10 and the irradiated group -10,42. The mean values obtained on the 7th, 14th and 21st PO days, were, respectively: control group – 92.94, - 83.70 and 32.58 and for the irradiated group -82.85, -64.36 and -29.38 (Figure 3).

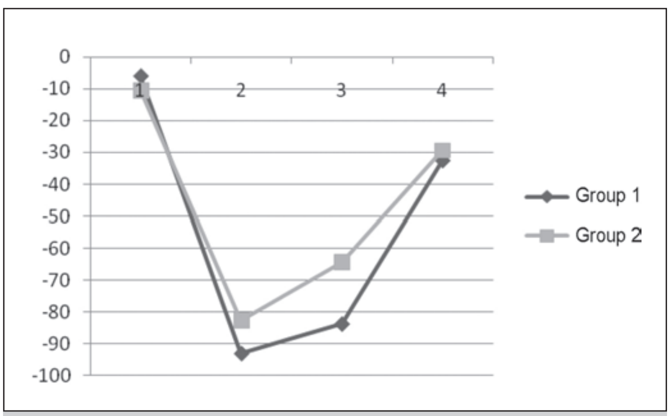

Figure 3 – *Mean values of SFI obtained for the groups from preoperative period up to 21 postoperative days.*

The comparison of times analyzed by the sciatic functional index in each group was significant in all the periods. (Table 2)

Table 2 – *Intra-group comparison between times, with confidence interval of 95% and p<0.05.*

Group	Time	Period	Dif Adjust	Lower CI 95%	Upper CI 95%	p-value
Control	Pre	7dd	86.84	77.86	95.82	< 0.01
	Pre	14dd	77.60	68.62	86.58	< 0.01
	Pre	21dd	26.48	17.50	35.46	< 0.01
	7dd	14dd	-9.24	-18.22	-0.26	0.04
	7dd	21dd	-60.36	-69.34	-51.38	< 0.01
	14dd	21dd	-51.12	-60.10	-42.14	< 0.01
40 J/cm	Pre	7dd	72.43	63.45	81.41	< 0.01
	Pre	14dd	53.95	44.97	62.93	< 0.01
	Pre	21dd	18.97	9.99	27.95	< 0.01
	7dd	14dd	-18.49	-27.47	-9.51	< 0.01
	7dd	21dd	-53.47	-62.45	-44.49	< 0.01
	14dd	21dd	-34.98	-43.96	-26.00	< 0.01

In the analysis of the sciatic functional index in the preoperative times, 7th, 14th and 21st PO day, in the intra-group comparison, with confidence index of 95% and $p<0.05$, on the 7th and 14th PO day there was statistically significant difference between the control group and the group that was submitted to low-intensity laser irradiation.

On the other hand, there was no statistical difference in the comparison between the control group and the laser irradiated group, when evaluated on the 21st PO day. (Table 3)

DISCUSSION

Peripheral nerve injuries cause important dysfunctions, and can give rise to lifelong sequelae, depending on the nerve injury grade. Several experimental and clinical studies have aimed to analyze the regeneration process and functional recovery of the nerve with the assistance of therapeutic resources, such as: electrical stimulation,[13] therapeutic ultrasound,[14] and low-intensity laser therapy.[3,15,16]

Table 1 – *Mean, standard deviation (SD) and coefficient of variation (CV) of the Sciatic Functional Index (SFI), by group and postoperative period (PO). Q1 and Q3= quartile 1 and 3.*

Variable	Group	Time	n	Mean	SD	C.V.	Min	Q1	Median	Q3	Max
SFI	Control	Pre	9	-6.10	3.82	-62.71	-12.46	-7.83	-6.56	-3.29	-0.86
		7dd	9	-92.94	10.59	-11.39	-105.18	-100.64	-90.32	-88.74	-75.15
		14dd	9	-83.70	8.04	-9.61	-97.62	-88.12	-85.75	-76.39	-73.95
		21dd	9	-32.58	18.63	-57.17	-63.57	-39.87	-31.82	-14.09	-11.39
	40 J/cm	Pre	9	-10.42	4.19	-40.21	-19.93	-10.43	-10.00	-8.17	-4.49
		7dd	9	-82.85	6.15	-7.42	-92.67	-86.87	-81.50	-77.78	-74.75
		14dd	9	-64.36	6.60	-10.26	-71.75	-68.90	-68.02	-60.71	-53.13
		21dd	9	-29.38	5.86	-19.95	-35.46	-33.32	-31.03	-26.91	-17.25

Table 3 – *Comparisons between the groups at each time (confidence interval of 95% and p<0.05).*

Time	Group	Group	Dif Adjust	Lower CI 95%	Upper CI 95%	p-value
Pre	Control	40 J/cm	4.32	-5.65	14.29	0.39
7dd	Control	40 J/cm	-10.09	-20.06	-0.12	0.04
14 dd	Control	40 J/cm	-19.34	-29.31	-9.37	< 0.01
21dd	Control	40 J/cm	-3.20	-13.17	6.77	0.53

The rat was used as the experimentation animal in this study, due to the greater ease of obtainment, laboratory handling, low cost and also as there are a great number of studies with this animal model in literature, enabling comparisons among the results obtained.[17]

Previous studies used some nerve tissue crushing methods, such as the universal testing machine and the jeweler's forceps, which require constant adjustments in the load to be applied. Mazzer et al.[10] reported that with the viscoelasticity of the nerve tissue, this equipment is susceptible to an accommodation of the load applied. Now the portable deadweight device that was used for crushing of the rats' sciatic nerve is characterized as being a quick, easier and more reliable crushing process in terms of the load used. The crushing load used in this experiment was that of 5000g applied for 10 minutes, since, according to the survey by Mazzer et al.[10] it promotes an axonotmesis type injury.

Low-intensity laser is one of the types of biostimulants used most often in rehabilitation at the present time, which has contributed to a better understanding of its principles and range of applicability.[4] According to Enwemeka[18] low-intensity laser therapy is being widely used in situations of healing processes, aiming to obtain faster tissue healing. Its success is suggested by the particularities of responses induced in the tissues, such as decrease of the inflammatory process, edema reduction, increase of phagocytosis, of collagen synthesis and of epithelization. Low-intensity laser irradiation has also been indicated due to its ability to accelerate the formation of new vessels after tissue injury.[19] However, scientific and clinical evidence is still limited and usually contradictory, a fact that justifies the need for performance of further research involving this therapeutic resource.

Rochkind[20] described some laser mechanisms in nerve recuperation such as: immediate effect of protection and increase of functional activity, maintenance of functional activity in the nerve injury in excess time, influence of scar tissue formation at the site of the injury, prevention or decrease of degeneration in the corresponding motor neuron in the spinal cord and influence of axonal growth and of myelin sheath.

Various preliminary studies, both clinical and experimental, demonstrated that low-intensity laser therapy has positive effects on the regeneration of peripheral nerve injuries.[3,15,16,21,22] Bagis et al.[23] did not observe beneficial effects of the use of low-intensity laser on nerve injuries.

Several parameters: wavelengths, energy density, pulse mode and power of the laser, are being used to stimulate regeneration and to accelerate functional recuperation of the peripheral nerve.[3,21,22] This study used low-intensity laser with wavelength of 830nm, 30mW and beam area of 0.116cm^2.

The footprint images were analyzed by the sciatic functional index, in which some authors evaluated it as being a quantitative, reliable and reproducible method.[7] The acrylic platform was used to obtain the footprint images in this experiment, in the same dimensions as the wooden platform developed by Gasparini et al.[8]

The video filming allows the real-time capture of footprints without problems such as the rat's slipping on the paper that leads to loss of print, as these intercurrences deform the footprint, particularly of the injured foot, hindering the viewing of the SFI parameters. The video filming method was used in this study to record the footprint images, which were evaluated by the *Image J®* computer program to quantify the parameters predetermined by De Medinacelli et al.,[5,9] and modified by Bain et al.[6]

In this study the animals were irradiated for 21 days, based on previous findings. According to studies[3] such as that of Monte-Raso et al.[7] after the 21st postoperative day the functional recovery of the rats after peripheral nerve crushing, treated or not treated, is close to normal, which is consistent with this experiment, the indexes of which were similar at the end of 21 days, regardless of the stimulus applied.

In the study by Oliveira et al.[24], a high degree of correlation was observed between functional recovery and morphologic and morphometric regeneration of peripheral nerve tissue injuries. Indeed, the SFI has proven a highly reliable tool to evaluate the peripheral nerve regeneration process, providing function with a numerical value and allowing a statistical analysis of results.

In this study, like in that of Mendonça et al.[13] the evaluation of the SFI in the preoperative period did not reach the value 0 (zero), as was expected, but rather an oscillation close to -10.

The SFI results obtained in this survey showed that functional recovery reached a greater difference between groups on the 14th day after crushing, indicating that the AlGaAs laser (830 nm) was effective in stimulating acceleration of functional recovery in the animals studied. These data corroborate the data of other studies.[3,22]

Of the results obtained on the 7th day, there was no difference between the groups except when comparing group 1 and group 2, while on the 21st day there was no difference between the SFI results. When the intra-group analysis was conducted statistical differences were observed among all the periods of all the groups studied with a p-value inferior to 0.01.

The statistical analysis of the values obtained by the SFI, through the linear mixed effects model, with confidence interval of 95%, showed that the true value of the mean difference between the control group and the irradiated group on the 14th PO day, was between -29.31 and -9.37 with $p > 0.01$. As value 0 (zero) was not present in the interval the signs point to a difference between the groups. It is also possible to indicate that both on the 7th and on the 14th PO day, the group that underwent low-intensity laser presented better SFI values and consequent functional recovery of gait of the animals.

Therefore, comparing the results obtained, the irradiated group, which used emitted energy of 4.64 J, proved more effective when compared with the control group.

In literature there is a considerable number of studies with low-intensity laser, yet there is no standardization of the parameters employed and the shortage of data in the studies found hindered the comparison of results and the understanding of some mechanisms involved. Accordingly, new surveys are necessary to verify the importance and the dependence between each one of the laser parameters, as well as the possible influences exercised on the biological responses, in order to thus improve the specificity of laser therapy as well as the preparation of protocols with safer and more effective treatments.

CONCLUSION

This study, considering the parameters analyzed, suggested that the use of low-intensity laser (830nm) was effective in the acceleration of gait recovery in the first 2 weeks, of rats after crushing of the sciatic nerve.

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REFERENCES

1. Seddon HJ. Three types of nerve injury. Brain. 1943;66:237-88.
2. Sunderland SA. Classification of peripheral nerves injuries producing loss of function. Brain. 1951;74:491-516.
3. Endo C, Barbieri CH, Mazzer N, Fazan VS. A laserterapia de baixa intensidade acelera a regeneração de nervos periféricos. Acta Ortop Bras. 2008;16:305-10.
4. Schindl A, Schindl M, Pernerstorfer-Schön H, Schindl L. Low-intensity laser therapy: a review. J Investig Med. 2000;48:312-26.
5. De Medinaceli L, DeRenzo E, Wyatt RJ. Rat sciatic functional index data management system with digitized input. Comput Biomed Res. 1984;17:185-92.
6. Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg.1989;83:129-38.
7. Monte-Raso VV, Barbieri CH, Mazzer N, Yamasita AC, Barbieri G. Is the Sciatic Functional Index always reliable and reproducible? J Neurosci Methods. 2008;170:255-61.
8. Gasparine ALP, Barbieri CH, Mazzer N. A laserterapia de baixa intensidade acelera a regeneração de nervos periféricos. Acta Ortop Bras. 2007;15:285-9.
9. De Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol. 1982;77:634-43.
10. Mazzer PYCN, Barbieri CH, Mazzer N, Fazan VPS. Avaliação qualitativa e quantitativa das lesões agudas por esmagamento do nervo isquiático do rato. Acta Ortop Bras. 2006;14:220-5.
11. Pachioni CAS, Mazzer N, Barbieri CH, Fazan VPS, Moro CA, Silva CAA. Lesão por esmagamento do nervo isquiático de ratos: estudo da vascularização. Acta Ortop Bras. 2006;14:203-7.
12. Mclean RA, Sanders WL, Stroup WW. A unified approach to mixed linear models. Am Statistician. 1991;45:54-64.
13. Mendonça AC, Barbieri CH, Mazzer N. Directly applied low intensity direct electric current enhances peripheral nerve regeneration in rats. J Neurosci Methods. 2003;129:183-90.
14. Monte-Raso VV, Barbieri CH, Mazzer N, Fasan VS. Can therapeutic ultrasound influence the regeneration of peripheral nerves? J Neurosci Methods. 2005;142:185-92.
15. Rochkind S, Drory V, Alon M, Nissan M, Ouaknine GE. Laser phototherapy (780nm), a new modality in treatment of long-term incomplete peripheral nerve injury:a randomized double-blind placebo-controlled study. Photomed Laser Surg. 2007;25:436-42.
16. Rochkind S, Leider-Trejo L, Nissan M, Shamir MH, Kharenko O, Alon M. Efficacy of 780-nm laser phototherapy on peripheral nerve regeneration after neurotube reconstruction procedure (double-blind randomized study). Photomed Laser Surg. 2007;25:137-43.
17. Rodrígues FJ, Valero-Cabré A, Navarro X. Regeneration and functional recovery following peripheral nerve injury. Drug Discov Today Dis Models. 2004;1:177-85.
18. Enwemeka CS, Parker JC, Dowdy DS, Harkness EE, Sanford LE, Woodruff LD. The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. Photomed Laser Surg. 2004;22:323-9.
19. Agaiby AD, Ghali LR, Wilson R, Dyson M. Laser modulation of angiogenic factor production by T-lymphocytes. Lasers Surg Med. 2000;26:357-63.
20. Rochkind S. Photoengineering of neural tissue repair processes in peripheral nerves and the spinal cord: research development with clinical applications. Photomed Laser Surg. 2006;24:151-7.
21. Reis FA, Belchior AC, de Carvalho Pde T, da Silva BA, Pereira DM, Silva IS, Nicolau RA. 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. 2009;24:741-7.
22. Belchior AC, dos Reis FA, Nicolau RA, Silva IS, Perreira DM, de Carvalho Pde T. Influence of laser (660 nm) on functional recovery of the sciatic nerve in rats following crushing lesion. Lasers Med Sci. 2009;24:893-9.
23. Bagis S, Comelekoglu U, Coskun B, Milcan A, Buyukakilli B, Sahin G, Ozisik S et al. No effect of GA-AS (904 nm) laser irradiation on the intact skin of the injured rat sciatic nerve. Lasers Med Sci. 2003;18:83-8.
24. Oliveira EF, Mazzer N, Barbieri CH, Selli M. Correlation between functional index and morphometry to evaluate recovery of the rat sciatic nerve following crush injury: experimental study. J Reconstr Microsurg. 2001;17:69-75.