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RESEARCH ARTICLE

Effectiveness of Low-Level Laser Therapy on Brain Derived Neurotropic Factor and Sciatic Function Index in Experimentally Induced Sciatic Nerve Crush Injury Wistar Rat Model

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ABSTRACT

Background: Photo bio-modulation, or bio-stimulation, is the process by which Low-Level Laser Therapy (LLLT) induces a photochemical reaction within cells. LLLT utilizes radiation with a power range of 1-1000 mW and wavelengths from 632 to 1064 nm to promote biological responses. **Methods:** Eighteen healthy adult male Wistar rats were employed in this study. The rats were randomly divided into two groups of nine. A surgical technique was performed to induce sciatic nerve crush injury. LLLT was applied to assess its effects on the nerve regeneration process. The Sciatic Function Index (SFI) and RT-PCR for Brain-Derived Neurotrophic Factor (BDNF) were utilized to evaluate nerve recovery. **Results:** The expression of BDNF and SFI were assessed on days 2 and 21 post-injury. Statistical analysis was conducted using one-way ANOVA to compare the means ± SD between the two groups. Results indicated that LLLT significantly enhanced both BDNF expression and functional recovery, demonstrating accelerated nerve regeneration. **Conclusions:** Both groups exhibited beneficial effects on nerve regeneration; however, LLLT notably accelerated regeneration in both biological (BDNF levels) and functional (SFI scores) parameters.

Keywords: Low-Level Laser Therapy; RT-PCR; Sciatic Nerve; Sciatic Function Index; Wistar Rat

INTRODUCTION

Photobiomodulation, also known as biostimulation, refers to the process by which Low-Level Laser Therapy (LLLT) induces photochemical reactions within cells. LLLT utilizes radiation within a power range of 1-1000 mW and wavelengths from 632 to 1064 nm to elicit biological responses, making it a promising approach for enhancing tissue repair and regeneration [1,2].

From the spinal segments L4-L6, the rat sciatic nerve emerges. At the trochanter, it is unifascicular; 5-7 mm distally, the nerve divides into two, and then into four fascicles. The tibial portion innervated by both the sural and tibial nerves, whereas the peroneal region is supplied by peroneal nerve and a cutaneous branch that innervates the proximolateral aspect of the calf by perforating the lateral hamstring muscles [3].

A peripheral nerve injury results in subsequent muscular atropy and varying degrees of disability. A mechanism known as Wallerian degeneration allows peripheral nervous system axons to recover and remyelinate after sustaining injury. The effect of Schwann cells on regenerating axons is caused by several growth factors such NGF (Nerve growth factor), CNTF (Ciliary neurotrophic factor), bFGF (Basic fibroblast growth factor) and BDNF [4]. BDNF(Brain-Derived Neurotrophic Factor) can enhance axonal sprouting during axonal regeneration. Research has demonstrated that BDNF has triggering effects. BDNF administration, however, appears to be particularly efficient in reversing the negative effects of chronic axotomy by increasing both axonal regeneration and neural cell repair at low doses and over an extended period of time [5].

One of the most common tools for functional assessment is the sciatic function index. It gives information regarding the recovery of sensory-motor connections and cerebral integration related to gait function and mediated by the sciatic nerve, without requiring terminal assessment. It does this by comparing parameters from the normal and experimental footprints using a mathematical formula[6]. Low-level lasers (wavelength of 904 nm, dose of 4 J/cm2, Gallium Arsenide) were used to treat rats with crush injuries to the sciatic nerve. The results demonstrated that the lasers were successful in promoting nerve regeneration[7]. Rochkind et al. conducted a double-blind randomized study. According to morphological data, the laser-treated group had more myelinated axons overall after the reconnection of the nerve deficit with a PGA neurotube during postoperative 780 nm laser phototherapy[8].

This study was conducted to evaluate the effectiveness of Low-Level Laser Therapy on functional recovery and the acceleration of Brain-Derived Neurotrophic Factor expression following sciatic nerve crush injury. By assessing both functional outcomes through the Sciatic Function Index and the biological response via BDNF levels, we aimed to elucidate the potential of LLLT as a therapeutic intervention for enhancing nerve regeneration. Our findings are expected to contribute valuable insights into the role of photo biomodulation in peripheral nerve injury recovery.

METHODS

Ethics Statement

The ARRIVE Guidelines (Animal Research: Reporting of In Vivo Experiments) was followed throughout the study and the study was approved by the Institutional Animal Ethics Committee (IAEC), Biomedical Research Unit, and Laboratory Animal Centre (BRULAC) in SDCH, Chennai, India. The approval number for the research is BRULAC/SDCH/SIMATS/IAEC/01-2023/11.

Sample Size Estimatio

The G*power 3.0 program was used to determine the sample size, and a power test result of 0.9 was attained.

Animal Model:

We employed eighteen adult male Wistar rats in good condition, weighing 250 g on average. A seven-day period of quarantine was followed. featuring a climate-controlled area with a 12-hour light/dark cycle and a temperature between 180 and 200 C. Under standard laboratory conditions, three animals are assigned to each standard cage with a cushion made of corn cob grains. Rat pellet feed and filtered tap water were provided as part of the daily/nightly diet regimen.

Control Group

Animals are subjected to non-intervention with posterior right sciatic nerve crush injuries.

Experimental Group

9 Animals are subjected to low level laser therapy to stimulate posterior right sciatic nerve crush injuries.

Anesthetic Method

To anesthetize the animals, ketamine hydrochloride (70 mg/kg) and xylazine hydrochloride (10 mg/kg) are given intraperitoneally and intramuscularly, respectively.

Surgical Methods

After the animals were completely unconscious, the rat was placed for a posterior right side sciatic nerve crush injury (right paw). The superficial muscles of the gluteus maximus and femoral biceps were divided with a retractor, and this allow the sciatic nerve to be exposed. A hemostatic tweezers with a force of 54 N was applied on the right sciatic nerve for 30 seconds in order to cause a crush injury (Figure 1). The incision was easily closed with silicone thread once crush was induced[9,10].

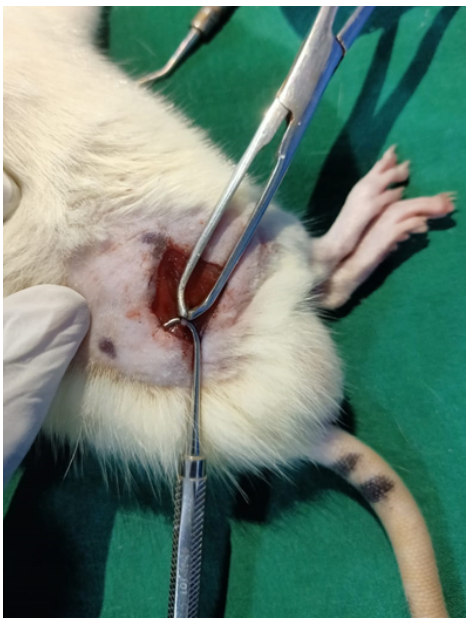


Fig. 1: Surgical procedure of posterior right side sciatic nerve crush injury

Postoperative Care

During the course of two days, a single subcutaneous injection of Meloxicam 1 mg/kg was used as an analgesic to treat post-operative pain. All of the operated rats are kept in

a single cage for the duration of the trial.

Interventions

- **Control Group:** No intervention.
- **Experimental Group:** Low-level laser therapy was applied using the Grid Method, Infrared, 808nm, Gallium Arsenide, Continuous, 3Jsq.cm, and Laser Therapy 302 (TECHNOMED Electronics). The grid method is run for one minute with a 200 mW infrared probe at a wavelength of 808 nm. Laser radiation was started on the second day of operation and continued in alternate consecutive sequences for 21 days (Figure 2).

Fig. 2: Low Level Laser Therapy on posterior right side sciatic nerve crush injury

Outcomes

1. Biological Measure: Brain-derived neurotrophic factor

- **Blood Sampling:** On the second and twenty-first days in each group, a retro-orbital blood collection technique is performed for the RT-PCR procedure.
- **RT-qPCR:** Takara's PrimeScript RT Reagent Kit with gDNA Eraser was utilized to synthesize cDNA after the RNA was extracted using RNAzol (Takara). RT-qPCR was performed using Takara's TB Green Premix Ex Taq. After 10 minutes of pre-incubation at 95 °C, 40 cycles of denaturation at 95 °C for 10 seconds, annealing at 60 °C for 10 seconds, and extension at 72 °C for 10 seconds were carried out. Host gene expression was measured using the 2-ΔΔCt method and normalized to beta actin expression[11].

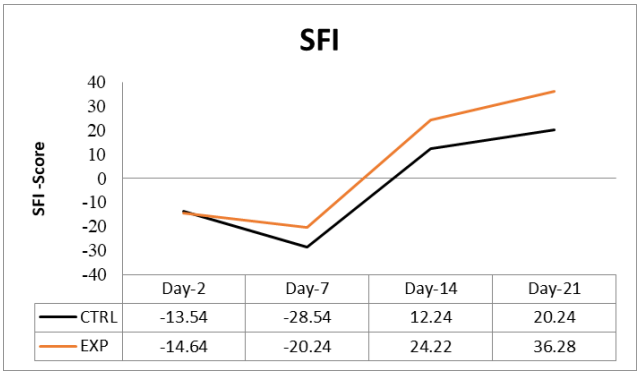
2. Functional Measure

Sciatic Function Index: Measurements were made on millimeter paper, including size, print length (PL), toe spread (TS), distance between fingers one and five, and intermediate TS, or the distance between the second and fourth fingers. The normal side leg (N) and the treated/experimental side leg (E) were used to take the three measures. The formula to compute SFI is SFI=−38.3 (EPL-NPL/NPL) + 109.5 (ETS-NTS/NTS) + 13.3 (EITS-NITS/NITS) −8.8.

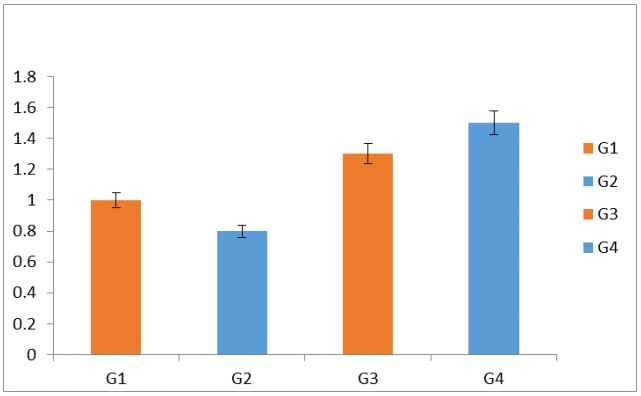
An SFI value of -100 denotes complete malfunction, whereas a normal score is somewhere around zero[12].

Statistical analysis

The data were provided as the means ± standard deviation of two different research groups' work. The statistical analysis was conducted using one-way ANOVA(RT-PCR), and Paired t- test (SFI) a result was deemed statistically significant if * = p<0.05, ** = p<0.01, and *** = p<0.001.



Graph 1: **SFI from Day-2 to Day-21**



Graph 2: **The x-axis displays various samples on the initial and final phases, while the y-axis displays the fold of changes in BDNF Neurotropin [Initial Phase (G1, G2) G1: control, G2: Experimental & Final Phase (G3, G4) G3: control, G4: Experimental-1]**

RESULTS

Biological effect of low level laser therapy

A therapeutic-dependent approach was used to evaluate the BDNF mRNA expression levels based on the expression of the initial (G1 and G2) and final (G3 and G4) genotype in the control and experimental groups. The experimental group created a larger acceleration of genotype expression. According to this research, there was a substantial ($p<0.001$) shift in nerve regeneration using low intensity laser therapy (Graph 2).

Functional recovery of low level laser therapy

SFI was evaluated from day 2 to day 21. The control group's SFI score on day two was -13.54, indicating a larger gait disruption; on day 21, it was determined to be 20.24, indicating a functional recovery. In comparison to the experimental group, the day two SFI score was -14.64, and on day 21, it was 36.28, indicating a higher degree of functional recovery (Graph 1).

DISCUSSION

According to the study, peripheral nervous system axons may recover and remyelinate over an extended length of time following substantial injury through a process known as Wallerian degeneration. In addition, our research demonstrates that low-level laser therapy can accelerate nerve regeneration in a short duration of time. In 2021, Xellen et al., conclude that some evidence of positive effects has been attained (LLLT enhances peripheral axon regeneration, enhancing motor function) in studies using a variety of animal models, the majority of which were conducted on rats with sciatic nerve injuries. Despite the lack of research that explain the mechanisms of action and assess safety, the results from animal models are so positive[13]. In 2008, Filipe. et. al., came to the conclusion that although the aluminium gallium arsenide LLLT (660 nm) significantly altered the myelin sheath areas that had been measured morphometrically, it had no positive effects on the rats' functional recovery of the sciatic nerve following neurotmesis injury[14]. In 2005 E. Vögelin et. al., came to the conclusion that BDNF treatment of nerve abnormalities resulted in statistically significantly faster axonal development than the untreated controls up to 10 weeks, alleviating neuropathic pain. To enable subsequent clinical implementation of the BDNF's demonstrated favourable benefits, more study is required to create a viable continuous growth factor administration method[15]. In 2001, Xellen Cunha et.al, suggest that numerous studies using animal models, the majority of which involved rats with sciatic nerve injuries, have looked at the effectiveness of LLLT. Some evidence of positive effects has been found (LLLT optimises peripheral axon regrowth, improving motor function). Despite the lack of research that explain the mechanisms of action and assess safety, the results from animal models are so positive. Clinical trials with good methodological integrity are strongly advised in order to demonstrate the scientific validity and efficacy of various electro stimulation parameters. The large discrepancy of parameters identified in the literature and the still contested clinical efficacy of LLLT can then be overcome. The applicability of the published clinical trials can be evaluated by systematic reviews and meta-analyses, which can also identify the parameters that produce reliable and repeatable favourable outcomes[13]. In a study by Bohan Li et al., the effects of LLLT on facial nerve regeneration following crush injury in rats showed that the laser + crush group's recovery of Vibrissa movement was much higher than the crush group's and comparable to values in the sham group. Furthermore, compared to the crush group, the myelin sheaths, and regenerated axons of the laser + crush treated mice were thicker. Repairing the crushed facial nerve damage was effective using LLLT[16]. A study indicated that LLLT was a successful treatment for radial nerve palsy[17]. Lessandra et al. conducted a study to determine whether LLLT is effective in treating neurotrophic factors like BDNF, NT-3 and Induced Nitric Oxide, Nerve Growth Factor, Synthase Expressions. The results showed a positive impact on the intervention grouping rat model[16]. In clinical practise, LLLT has been widely employed to facilitate nerve regeneration. Studies have shown that Schwann cells, a kind of primary glial cell on the peripheral nervous system, release compounds called neurotrophic factors that promote peripheral nerve regeneration. Schwann cells can proliferate when exposed to light in vitro[18–23].

CONCLUSION

Both groups demonstrated beneficial effects on nerve regeneration; however, Low-Level Laser Therapy (LLLT) significantly accelerated nerve regeneration in terms of both biological outcomes, such as increased Brain-Derived Neurotrophic Factor (BDNF) levels, and functional recovery, as assessed by the Sciatic Function Index (SFI). These findings highlight the potential of LLLT as an effective therapeutic intervention for enhancing recovery following peripheral nerve injuries, suggesting its valuable role in clinical applications.

Conflict of interest

No Conflict of interest throughout the duration of the study.

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