Effectiveness of Low-Level Laser Therapy and Low Intensity Pulsed Ultrasound in Sensory Recovery in Experimentally Induced Peripheral Nerve Injury Rat Model

Hepsibha C R¹, Prathap Suganthirababu S², Lavanya Prathap³, Mydhili Govindarasu⁴, Kumaresan. A⁵, Vignesh Srinivasan⁶, Jagatheesan Alagesan⁷

¹Post graduate, ²,⁵,⁷Professor, ⁶Assistant Professor, Saveetha College of Physiotherapy, Saveetha Institute of Medical and Technical sciences, Chennai, Tamil Nadu, India, ³Associate Professor, Department of Anatomy, Saveetha Dental College, Saveetha Institute of Medical and Technical sciences, Chennai, Tamil Nadu, India, ⁴Assistant Professor, BRULAC, Saveetha Institute of Medical and Technical sciences, Chennai, Tamil Nadu, India.

How to cite this article: Hepsibha C R, Prathap Suganthirababu S, Lavanya Prathap et. al. Effectiveness of Low-Level Laser Therapy and Low Intensity Pulsed Ultrasound in Sensory Recovery in Experimentally Induced Peripheral Nerve Injury Rat Model. Indian Journal of Physiotherapy and Occupational Therapy / Volume 18 Special Issue 2024;

Abstract

Background: Peripheral nerve injuries are known to cause significant functional impairment and diminished sensory recovery, necessitating the exploration of effective therapeutic interventions.

Purpose: The purpose of this research is to find the effectiveness of low-level laser therapy (LLLT) and low intensity pulsed ultrasound (LIPUS) sensory recovery in an experimentally induced peripheral nerve injury rat model.

Materials and Methods: In this study, 18 adult male wistar rats which are divided into LLLT (n = 6), LIPUS (n = 6), and control (n = 6) groups. All rats underwent a standardized procedure to induce peripheral nerve injury, while the control group received sham procedures. Hot-Plate test and Cold-Plate Tests were conducted for pre- and post-operative evaluation of sensory recovery at POD 7, 14, 21 days.

Results: The study’s findings revealed that LLLT exhibited significantly improved sensory recovery compared to LIPUS and control groups on POD 14 and 21, indicating its potential as a promising non-invasive intervention for managing peripheral nerve injuries (P <0.001).

Conclusion: The study recommends that LLLT is more effective when compared with LIPUS in promoting sensory recovery and enhancing in a rat model of peripheral nerve injury. Positive outcomes indicate LLLT’s potential as a promising intervention for managing peripheral nerve injuries.

Keywords: Low level laser therapy, Sensory recovery, Peripheral nerve injuries.

Introduction

The term “peripheral neuropathy” describes structural damage to the nerves in the peripheral nervous system as well as abnormalities in normal nerve physiology¹. Peripheral nerve injuries can lead to substantial functional impairment and
reduced sensory recovery, prompting the need for effective therapeutic interventions. This study sought to compare the effectiveness of two non-invasive modalities, LLLT and LIPUS in promoting sensory recovery in a rat model of experimentally induced peripheral nerve injury. The findings hold the potential to shed light on the most suitable intervention to facilitate nerve regeneration and enhance sensory recovery in individuals dealing with peripheral nerve injuries. It is important to note that 1-2% of individuals treated in emergency rooms are affected by this type of injury, highlighting its widespread occurrence and clinical significance. Therefore, the investigation of effective therapeutic interventions for PNI becomes imperative to enhance the overall well-being.

The Wallerian degeneration process and the intact endoneurial tube structure make it possible to study peripheral nerve regeneration after crush injury by facilitating morphological and functional recovery.

A complicated chain of actions takes place during axonal regeneration. Fibres first begin to sprout in the proximal nerve stump, marking the beginning of regrowth. In an effort to reestablish connections with their respective target organs, these sprouting fibres then expand through the distal endoneurial tube, which is the protective sheath encircling the nerve.

The shape and gene expression of the neuronal cell bodies alter noticeably during this regeneration phase. Chromatolysis is a condition where changes are seen in the cellular makeup of neurons. This alteration in morphology reflects the reorganisation and adaptation taking place within the neuronal cells as they get ready for reinnervation and development.

Axonal regeneration involves gene expression alteration, enabling effective axon regrowth and restoration of functional connections with target organs. Axonal regeneration involves cellular and molecular alterations, enabling axons to connect with target organs and promote nervous system function.

Research evaluates LLLT and LIPUS therapies for peripheral nerve injury regeneration, focusing on cellular activity, blood flow, and tissue healing.

On the other hand, LIPUS Therapy improves healing by using low-intensity pulsed ultrasound waves. Ultrasound waves can pass through the tissues and stimulate cellular activity as well as the production of growth factors that aid in nerve regeneration. Hot-Plate test and Cold-Plate Tests were conducted for pre- and post-operative evaluation of sensory recovery at POD 7, 14, 21 days. The hot plate test and cold plate test are behavioral tests commonly used in preclinical research to evaluate sensory function in rat models of nerve injury. These tests assess the animal’s response to thermal stimuli and provide valuable information about pain perception and sensory recovery following nerve injury.

The findings of this study could influence future preclinical and clinical approaches to treating peripheral nerve injuries and increase our understanding of the therapeutic benefits of LIPUS and LLLT in peripheral nerve repair.

**Aim**

The main goal of this research is to evaluate the therapeutic potential of LLLT and LIPUS in promoting nerve regrowth with repairing of nerve tissues and sensory recovery following experimentally induced peripheral nerve injury in rats.

**Materials and Methods**

**Study design**: Experimental design

**Study Period**: This study carried out between February 2023 and May 2023.

**Subject and Sample size**: 18 male Wistar rats from Mass Biotech (Chengalpattu) were used to conduct the study.

**Sampling technique**: Random sampling technique.

**Inclusion Criteria**

- Adult male wistar rats were included.
- Rats of average body weight of 250 gms were included
- Rats free from pre-existing neurological disorders or systemic illnesses that may confound the study results.
Exclusion Criteria:

- Rats exhibiting signs of infection or wound complications at the site of injury to prevent potential treatment interference.
- Physically deformed rats
- Aggressive rats and previous experimental exposed rats.

Outcome Measure

Hot Plate Test:

The hot plate test involves a supraspinally organized response, requiring sophisticated mental processing. Rats are introduced into a cylindrical chamber with a metallic floor heated by a thermode, with heat as the primary stimulus. The plate is heated at a constant temperature between 50 and 55 degrees Celsius, and two behavioral elements, paw licking and jumping, are generated. Response latency and baseline latency are measured. Rats should be removed if they don’t respond within 30 seconds to minimize tissue damage. The test will be administered on Days 7, 14, and 21 to gauge response latency in each group21.

Cold Plate Test

The cold plate test assesses the behavioral responses of mice and rats to low temperatures, similar to the hot plate test. It measures the animals’ reaction to a temperature range of 5°C to 15°C, and measures reaction latency, which is the time it takes for the mouse to display pain behavior. The plate is cooled before placing the rodent on it, and the procedure is repeated for further statistical analysis21.

Study Procedure

Animals:

The study involved 18 Wistar rats in controlled laboratory conditions, divided into experimental and control groups, and received standard care.

Grouping:

Six animals with posterior right sciatic nerve crush injury were randomly assigned to control and treated with LLLT and LIPUS therapy.

Anaesthesia procedure:

Rats received anaesthetic medication with 10 mg/kg xylazine and 70 mg/kg ketamine at 54N pressure.

Surgical procedure:

Rats were placed in ventral recumbency, and a 3-cm incision was made to reveal the right sciatic nerve. A crush injury was made 10 mm above the nerve’s bifurcation, and sterile saline solution was used to maintain the nerve’s moistness15.

Post operative management:

Rats received penicillin-procaine prophylactic dose and nalbuphine hydrochloride analgesic.

Intervention:

Six injured posterior right sciatic nerves were randomly assigned to three treatment groups: control, LLLT, and LIPUS.

Control Group:

The animals in this group (n = 6) did not receive any special treatment; instead, they received standard care.

Low-Level Laser Therapy Group:

Six animals underwent compression injury to the sciatic nerve and low-level laser irradiation using a GaAIAs system from Technomed Electronics. The group was irradiated transcutaneously for 1 minute at the suture site. Laser therapy may accelerate nerve tissue regeneration in damaged peripheral nerve tissue, starting post-operatively and continuing on alternative days16.

Low Intensity Pulsed Ultrasound Group:

LIPUS is an affordable, non-destructive therapeutic strategy for peripheral nerve regeneration in rodents, promoting regeneration and reconstruction through ultrasonic gel application17.

Data analysis

In statistical analysis, ANOVA analysis used mean and standard deviation to compare groups in a control and experimental setting.
Results

For the purpose of assessing the pre- and postoperative sensory recovery at POD 7, 14, and 21 days, Hot-Plate and Cold-Plate Tests were performed.

Effect Of LLLT And LIPUS On Heat Sensitivity Among CCI Rats:

In graph 1, The hot plate test measures thermal response in rats before and after surgery. Heat sensitivity decreases on day 7. Both experimental groups showed significant improvement after treatment, but LLLT showed a greater statistical significance (p-value <0.001) when compared to LIPUS data. Latency increased with thermal stimulation, with the LASER group showing 10s to 21s latency, ultrasound group showing 9s to 19s, and control group showing 9s to 13s latency. The ANOVA and tukey test were used for multiple comparisons. The control group showed a statistically significant difference of 0.001 in multiple comparisons, with a mean difference of 18. The POD 21 vs POD 14 group showed a non-significant difference, while the POD 14 vs POD 7 group showed a non-significant difference.

In LLLT, p values showed a statistically significant result of 0.001, with mean differences in multiple comparisons. POD2 vs POD 2 was 18, POD 7 vs POD 12 was 12, POD 21 vs POD 7 was 6, and POD21 vs POD7 was 0.3. Non-significant results were found in POD21 vs POD 14 and POD 21 vs POD 7. In LIPUS, p values were less than 0.001, with a non-significant mean difference of 6 in POD 21 vs POD 14.

Effect Of LLLT And LIPUS On Cold Sensitivity Among CCI Rats:

The cold plate test measures latencies response to cold stimuli. Before surgery, cold sensitivity was similar, with a mean response of 27 seconds. After crush injury, cold sensitivity decreased. Rats treated with LLLT and LIPUS showed significant improvement on day 14-21, but LLLT data have shown a greater statistical significance (p value <0.001). Latency increased to 9s to 26s in LASER, 10s to 19s in ultrasound, and 8s to 21s in control groups.

The TUKEY test was used for multiple comparisons on ranks after ANOVA measurement. Statistically significant results were found in LLLT, with mean differences in baseline vs POD 2 and POD 7 values. POD21 vs POD7 values were 0.03, and POD 14 vs POD 7 values were 6. Non-significant results were found in POD21 vs POD 14.

LIPUS shows statistically significant p values <0.001 in multiple comparisons, with baseline vs POD2 having 18 differences, POD 7 having 12, and POD 21 vs POD 7 having 12. However, nonsignificant mean differences were observed in POD 21 vs POD 14, POD 14 vs POD 7, and POD 21 vs POD 14.
The control group showed a statistically significant 0.001 p value, with mean differences in multiple comparisons between baseline and POD 2 and POD 7 and POD 21. Non-significant results were found in POD 21 vs POD 14 and POD 14 vs POD 7.

**Discussion**

The main goal of this research is to examine how well these two treatments promote nerve regeneration.

This study will assess the efficacy of LLLT and LIPUS in promoting nerve regeneration using various tests, including nerve conduction investigations, functional evaluations, sensory assessments, and histological examinations of regenerated nerves. Both LLLT and LIPUS have shown promise in reducing pain and restoring sensory function, although their parameters may vary across studies and rat species.

According to a 2016 study by Ching-Hsia Hung et al., therapeutic ultrasound and treadmill exercise both help reduce the pain that peripheral nerve damage causes in rats. The researchers came to the conclusion that the combination of TU and TT reduces neuropathic pain and appears to be associated with the increased levels of pro-inflammatory IL-6 and Iba1 as well as the anti-inflammatory IL-1012.

In a 2012 study titled Effects of modified Ultrasound Parameters on the Restoration of Sciatic Nerve Injury, Zhamak et al. came to the conclusion that changing ultrasound parameters with 20 groups had an impact on the recovery of peripheral nerve injury. The effectiveness of ultrasonic parameters on the restoration of injured patients are therefore being carefully examined and compared in this study for the first time.

Suganthirababu P et al., suggested that ulnar nerve stimulation with low-level laser radiation increased peak to peak amplitude and distal latency.

A study by Chen et al. (2014) found that low-level laser therapy (LLLT) reduced inflammation and also neural regeneration in chronically compressed rat dorsal root ganglia.

In 2017, Mohammadreza Mashhoudi Barez, conducted a study on the stimulating impact of low-level laser therapy on regeneration of the sciatic nerve in rats. They discovered that laser phototherapy at a precise wavelength of 780 nm can hasten the regeneration of damaged peripheral nerve tissues.

Turner et al.’s 2019 study found facial grimacing, nest building, and grooming as useful indicators for pain assessment in mice, offering potential for analgesic therapies21. This discussion aims to explore and analyze the effectiveness of LLLT and LIPUS in the context of sensory recovery in an experimentally induced peripheral nerve injury rat model.

**Conclusion**

This research studied the effectiveness of LLLT and LIPUS on improving nerve regeneration and sensory recovery. In terms of neuromotor rehabilitation following compression injury of sciatic nerve, the results showed that laser therapy produced better recovery results than ultrasound treatment. As a result, more research should be done to develop appropriate standards for sensory recovery. Although many clinical studies have shown the effectiveness of LLLT in treating neuropathic pain and sensory recovery, there is still insufficient research. Future research is desired to fully understand and evaluate the long-term effectiveness of ultrasonic and laser therapy.

**Ethical Clearance:**

The study followed ethical standards for animal treatment and was approved by Institutional Animal Ethics Committee IAEC NO: BRULAC/SDCH/SIMATS /IAEC/01-2-23/11 for the experimental procedures.

**Funding:** This study is a self-funded study.

**Acknowledgements**

We thank R. Arun Kailash, Vice President of Marketing at Technomed Electronics, for providing the laser system and Preeta Sahoo from Elite Equipments for providing the ultrasound system, for their invaluable assistance in the study’s successful execution.

**Conflict of Interest:** No conflict of interest.
References


