

# Effectiveness of Low Level Laser Therapy and Low Intensity Pulsed Ultrasound on Neuropathic Pain among the Experimentally Induced Peripheral Nerve Injury in Rat Model

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## Abstract

**Background:** Peripheral nerve injury creates an impact on the inflammatory process, leading to an elevation in Hypoxia-inducible factor-1 alpha levels, which contributes to the development and progression of neuropathic pain. An innovative approach to alleviating neuropathic pain involves targeting inflammation.

**Purpose:** This study aims to compare the effect of Low-Level Laser Therapy (LLLT) and Low-Intensity Pulsed Ultrasound (LIPUS) on neuropathic pain among the experimentally induced peripheral nerve injury in rat model.

**Methods:** Rats with induced sciatic nerve crush injury were randomly divided into three groups: control, LIPUS treatment, and LLLT treatment. Mechanical paw withdrawal threshold analysis was performed before the injury and from POD 2nd, 7th, 14th, and 21st to assess effectiveness of the treatment.

**Result:** Low-Level Laser Therapy (LLLT) group showing statistically greater improvement on POD 14 and 21 compared to both the LIPUS group and the control group in terms of paw withdrawal threshold measurements among rats with crush-induced neuropathic pain ( $P < 0.001$ ).

**Conclusion:** This study found that the use of LLLT has a beneficial effect in the management of neuropathic pain and control of analgesia compared to LIPUS by inhibiting the upregulation of HIF-1 $\alpha$  synthesis during ischemia, hypoxia, and inflammation. However further studies are recommended to analyze long term results in larger sample to control neuropathic pain

**Key Words:** Injury, Inflammation, Laser, Ultrasound, Rats, Sciatic nerve

## Introduction

Trauma to the peripheral nerve induces

inflammation leading to neuropathic pain which is commonly perceived in the skin that is innervated by

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the inflamed peripheral nerve<sup>1</sup>. Following the nerve injury there will be a phenomenon known as action potential that is ectopic and nerve hyper excitability. Injured nerve provides changes in both structural and functional actions<sup>2</sup>. One of the significant changes is generation of abnormal action potential in the location where they are not intended to produce. These abnormal action potential leads to pain signals that are spontaneously carried to brain even there is no external stimulus. Another significant change is hyper excitability of the injured nerve. After injury nerve becomes more vulnerable and responsive even to mild stimulus leads to constant painful sensation<sup>3</sup>.

Degeneration of axon due to degradation of myelin, proliferation of glial cell, compromise of blood-nerve barrier, permeation and proliferation of macrophages are all symptoms of the complex process known as Wallerian degeneration, which is brought on by metabolic processes or mechanical damage to peripheral nerves. Macrophages emits cytokines and other substances that influence myelin phagocytosis, Low level laser therapy has gained popularity because of its positive effects on arthritis, neuropathic pain and nerve regenerating effects<sup>4,18</sup>. Hypoxia inducible factor-1 $\alpha$  are produced whenever oxygen is lacking resulting in ischemic environment or during inflammation. It is necessary to maintain the process of inflammation by enhancing the proinflammatory cytokines secretion such as IL-1b and TNF. It is helpful in carrying out the pathway of inflammation. Inhibiting the hypoxic state and downregulating the inflammatory effects are useful strategies in order to prevent neuropathic pain<sup>5</sup>.

HIF-1 $\alpha$  is an essential molecule that controls the development of proteins involved in oxygen regulation and cellular survival, enabling cells to become adapted to low-oxygen environments. It is a key target for therapeutic approaches since its abnormal function has been linked to numerous illnesses<sup>6</sup>. Low Level Laser Therapy (LLLT) showed positive effects on chromatolysis, a process that occurs after trauma and motor neuron degeneration. In rat sciatic nerves that have been crushed, the severity of chromatolysis was reduced. The molecular basis of LLLT's action involves the increased synthesis of several molecules with neuroprotective properties, such as calcitonin gene-related neuropeptide and transforming growth

factor beta-1. Additionally, LLLT demonstrated decreased nitric oxide activity, which is a neurotoxic compound. LLLT has the potential to promote nerve repair and neuroprotection by modulating various molecular pathways involved in nerve regeneration and protection against neurotoxicity. It is also helpful in decreasing neuropathic inflammation by inhibiting the secretion of HIF-1 $\alpha$ <sup>7, 18</sup>. Low intensity pulsed ultrasound proved its biological effects by promoting the production of certain cytokines consisting of angiogenesis, as well as anti-inflammatory cytokines and other components involved in tissue healing<sup>20</sup>. This suggests that it has beneficial effects on pain relief and tissue healing may be mediated, at least in part, through its modulation of these cytokines and healing factors in the treated area<sup>8, 19</sup>.

### Aim

The study aimed to assess the effects of low-level laser therapy and low-intensity pulse ultrasound on neuropathic pain in crush injured rats.

### Material and Methods

**Study Design:** Experimental design.

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

**Sample:** The study involved 18 male Wistar rats from Mass Biotech (Chengalpattu).

#### Inclusion Criteria

- Wistar rats
- Adult age group
- Healthy rats without the sign of illness or distress
- Average of 250grams

#### Exclusion Criteria

- Physically deformed rats
- Aggressive rats,
- Previous Experimental Exposed rats
- Signs of wound infection

#### Materials Used

- Laser: Provided by TECHNOMED ELECTRONICS.

- Ultrasound: Provided by RAPOSTIM MEDI TECH.
- Monofilament.

**Quarantine and Housing:** The rats were quarantined for 7 days in a controlled room with a temperature and a light/dark cycle of 12 hours. Three rodents were housed in single cages. The rats were provided with water and pelleted feed during the study.

### Surgical Procedure

During the study, rat surgery was conducted under anesthesia with ketamine hydrochloride (70mg/kg) and xylazine hydrochloride (10mg/kg). The rats were placed in a ventral recumbency position, and a 3cm incision was made on the skin to expose the right sciatic nerve, which was then crushed for 30 seconds using hemostatic tweezers with a force of 54 N, resulting in a 3mm long crush injury<sup>9</sup>. The surgical site was closed with silicon 3/0 thread, and the rats were placed in individual cages. To manage post-operative pain, a single subcutaneous dose of meloxicam (1mg/kg) was administered (Figure 1).

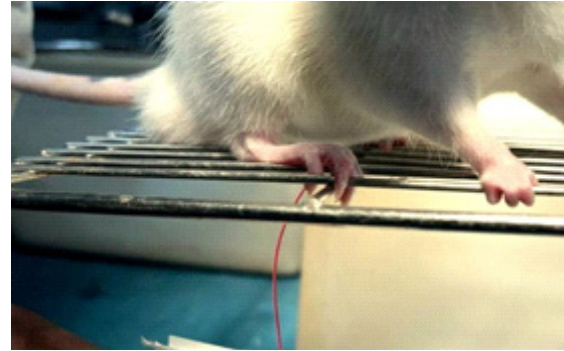


**Fig-1: Exposure of sciatic nerve**

### Outcome measures

**Mechanical paw withdrawal threshold:** The mechanical withdrawal threshold of rats is assessed using Von Frey filament on POD 7, 14, 21. Rats response to mechanical stimuli is observed in the present study. Rats were placed on the metal mesh plate (40x40cm dimension) and nine filaments (1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 26.0 g) were used to apply perpendicularly to the hind paws on the plantar surface for 3 to 5 seconds until the filament bend to S shape. Stimulation is initiated on 1g filament, and if no withdrawal response is observed, next filament

is used, until the rat shows a withdrawal response. The response might be escaping, shaking, licking, squeaking. When there is no withdrawal response shown by the rat, the estimated threshold for a rat is 26g, considering the study's sensitivity<sup>10, 21</sup>.



**Fig 2: Represents application of filament to the plantar surface of the hind paw**

**Grouping:** In the study, rats with induced crush injuries were divided into three groups: control group, LIPUS group, and LLLT group. Neuropathic pain was evaluated using mechanical paw withdrawal threshold test. The treatment protocols were followed until POD 21 for all three groups.

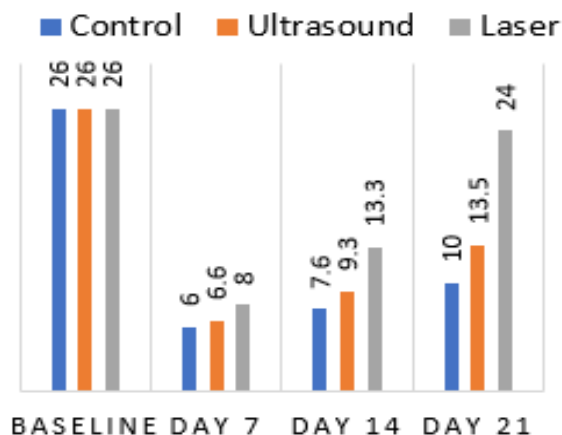
1. Control Group: Rats in this group received no specific treatment after the crush injury was induced.

2. Low-Intensity Pulsed Ultrasound (LIPUS) Group: Rats were positioned right laterally, and treatment began on Postoperative Day 2 (POD 2) using the water bag technique. Ultrasound with a frequency of 1MHz and power density of 1.4 W/cm<sup>2</sup> was applied in a pulsed mode using a finger probe. The treatment was given for 4 minutes per day on alternate days until POD 21

3. Low-Level Laser Therapy (LLLT) Group: Rats were treated with Gallium Arsenide laser therapy using a red infrared beam with a wavelength of 808nm. The laser was applied in continuous mode with a power of 200mW and energy density of 3J/cm<sup>2</sup>. The beam area had a length and width of 1cm, and the tissue depth of treatment was 1cm. The treatment duration was 1 minute, applied in a grid method. Treatment started on POD 2 and continued until POD 21, with the laser beam applied perpendicular to the treatment area for 1 minute.

## Data analysis

The study presented data as mean  $\pm$  standard deviation and Repeated Measures ANOVA followed by Bonferroni t-test has been used and analyzed in SPSS, Significance threshold of P value  $< 0.05$  is considered significant.



**Graph 1: X-axis shows quantification of pain on baseline, POD 7, 14, 21 and Y-axis shows varied thickness of Von Frey filament in grams.**

## Results

### Impact of LLLT & LIPUS on Mechanical Allodynia:

In mechanical paw withdrawal threshold, baseline measurements between the groups has no significant difference. On 7<sup>th</sup> POD, threshold has been found to be decreased in crush injured rats, reduction in the withdrawal threshold has been found in CCI rats, which indicates that the mechanical allodynia is present, it is one of the neuropathic pain type where a stimulus which is nonpainful provides exaggerated pain response. After the intervention, threshold has been increased in both the LLLT group and LIPUS group on 14<sup>th</sup> & 21<sup>st</sup> POD. Repeated-measures ANOVA has been used for statistical analysis.

Following ANOVA multiple group comparison at one particular point was done using Bonferroni t-test. In LLLT all the multiple comparison procedure showed statistically significant result of p value  $< 0.001$  with a mean difference between baseline vs POD 7 is 18, baseline vs POD 14 is 12.66, baseline vs POD 21 is 1.83, POD 21 vs POD7 is 16.16, POD21 vs POD14 is 10.833, POD14 vs POD7 is 5.33.

LIPUS also showed statistically significant result with p value of  $< 0.001$  in multiple comparison procedure with a mean difference between baseline vs POD7 is 19.33, baseline vs POD 14 is 16.33, baseline vs POD 21 is 12, POD 21 vs POD 7 is 7.33, POD 21 vs POD 14 is 4.33, POD 14 vs POD 7 is 3.

In control group, mean difference of multiple comparison between baseline vs POD7 is 20, baseline vs POD 14 is 18.66, baseline vs POD21 is 16, POD 21 vs POD 7 is 4, POD 21 vs POD 14 is 2.66 showed significant result of p value  $< 0.001$ . However, mean difference between POD 14 vs POD 7 is 1.33 showed a non-significant p value of 0.182

Intervention groups showed significantly effective results, however low level laser therapy group showed statistically more improvement than the LIPUS group & control group in paw withdrawal threshold measurements among crush injured rats for neuropathic pain ( $P < 0.001$ ) (graph 1).

## Discussion

The current research offers compelling evidence supporting the positive impact of laser irradiation and ultrasound therapy on neuropathic pain. These treatments demonstrate the ability to reduce inflammation, stimulate tissue regeneration, and influence biochemical reactions such as HIF-1 $\alpha$  and proinflammatory cytokines, all of which contribute to their beneficial effects. In the later stages of the experiment, behavioural changes can indicate allodynia, which is experiencing pain from non-painful stimuli. Researchers efficiently identified these changes by measuring withdrawal thresholds using von Frey filaments. On day 7, it was not able to differentiate between the two groups. On the other hand, gait analysis demonstrated high specificity and successfully detected differences between the two groups at earlier time points in the study<sup>21</sup>.

Many studies revealed that pro-inflammatory cytokines were elevated following CCI, but their overexpression was significantly reduced by LLLT. Numerous treatments have been developed to prevent or manage neuropathies, as they can significantly impact patients' social, economic, and medical well-being. LLLT has recently got popularity in various medical and rehabilitation fields and is

being explored as a potential option for neuropathic pain (NP) management. Based on the results of previous study, LLLT appears to reduce DRG glial cell activation, inhibit CCI-induced behavioral hypersensitivity, and suppress pro-inflammatory cytokines. The study's authors hypothesize that glial cell involvement may be one potential mechanism behind these beneficial effects of LLLT in neuropathic pain<sup>11</sup>. Another study stated that LLLT may impact HIF-1 $\alpha$  activity and could be a cutting-edge and practical therapeutic approach to reducing hypoxia in tissue, ischemic process and inflammatory processes thereby inhibiting neuropathic pain as well as promoting nerve regeneration<sup>12</sup>. Previous research found that in order to improve neural function, LLLT is highly helpful by elevating the level of VEGF protein and NGF, thereby repairing the myelin sheath among injured nerve tissues<sup>16</sup>. According to MonteRaso et al., LIPUS ultrasound is believed to primarily affect the tissues supporting axons. It accelerates neural tube regeneration and facilitates the early removal of barriers that may impede the formation of new axoplasm. By promoting a conducive environment for nerve regeneration, LIPUS can contribute to the overall recovery and restoration of nerve function<sup>13,17</sup>. Another study stated that the antinociceptive effect of LIPUS is believed to have a biophysical basis, as neither a placebo effect nor an impact arising solely from massage was observed. This suggests that the pain relief seen in the rats after LIPUS treatment is not due to psychological factors or general physical manipulation but is likely a result of the specific biophysical effects of LIPUS on the cells and mediators involved in pain modulation<sup>14,15</sup>. Although all clinical trials demonstrated the effectiveness of LLLT in managing neuropathic pain, there were discrepancies regarding the application parameters. In conclusion, the study revealed that LLLT has positive benefits in alleviating neuropathic pain, but further research with rigorous scientific methodologies is necessary to develop treatment protocols that can fully capitalize on the effects of LLLT & LIPUS in neuropathic pain management.

### Conclusion

The study's conclusion highlights that low level laser therapy (LLLT) provides more beneficial effects compared to low intensity pulsed ultrasound in

altering the inflammatory process at the injury site, promoting tissue healing, and reducing neuropathic pain in crush injured nerves. However, the researchers recommend further studies to analyze the long-term results using larger sample sizes to ensure the treatment's efficacy and safety.

**Ethical clearance:** The study commencement took place after receiving approval from the Institute Animal Ethics Committee (IAEC) with the reference number

BRULAC/SDCH/SIMATS/IAEC/01-2023/11.

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