Effectiveness of low-level Laser Therapy and Low Intensity Pulsed Ultrasound on Motor Recovery among Experimentally Induced Peripheral Nerve Injury in Wistar Rats

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Abstract

Background: Damage or disease can result in peripheral nerve injury, which is a frequent clinical issue. It frequently renders a person disabled by causing severe sensory loss and motor deficiencies along with pain, tingling, burning and other nagging sensations. A form of ultrasound called Low-intensity pulsed ultrasound, which uses far less energy than normal ultrasound (3 W/cm²). A biological reaction is triggered by low-level laser light having a wavelength of 808nm and a power range of 1-1000 mW.

Purpose: The Study is to find out the effect of low-level laser therapy and low intensity pulsed ultrasound on motor recovery among experimentally induced peripheral nerve injury in Wistar rats.

Methods: Eighteen rats were chosen in total, skin hair was removed to expose the sciatic nerve area, and the crush injury was done by hemostatic forceps for 30 seconds, the group 1 is ultrasound, group 2 is laser and group 3 is control group. The Step Length Ratio (SLR) was used as an outcome measure to assess motor recovery.

Result: All three groups are statistically significant (p<0.001) was analysed using one-way ANOVA during intervention period (7th, 24th, 21st day) but LLLT & UST has shown better improvement in their functional index than the control group.

Conclusion: According to the results, LLLT has more impact in motor recovery than LIPU also each treatment has substantial impact on accelerating nerve regeneration and shortening the recovery time.

Keywords: low level laser, rat study, compound muscle action potential, motor recovery and step length ratio.

Introduction

Peripheral nerve injuries caused by trauma are thought to occur in roughly 300,000 cases annually.¹ The most common causes of peripheral nerve damage are stretching, division, compression, crush and avulsion. Instead of utilizing of easiest, modern options for treating, reconstructing, morphologic changes and regeneration were rarely fully achieved due to the influence of hurdles like type and degree of damage injury and its damage, diameter, length.

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of denervation of the damaged nerve fibres.

Some cohort study stated, a prevalence rate of 1.8 to 3.3% and as the age increases so as the prevalence also increased to 82.7-86.1% in the age 16 to 59 years for women also its 78.6%-80% in men and primarily associated with RTA. Nerve injuries primarily affect working people in social system of human. In addition, it affects an individuals’ quality of life because motor deficiencies brought on by the injury and associated processes may last more with impaired motor functions and frequently leading to lifetime disability and morbidity.

A significant nerve in the lower limbs that controls both motor and sensory activities is the sciatic nerve. Injury to peripheral nerves can damage motor and sensory abilities, making it more difficult to ambulation. Atrophy of the muscles and a decrease in the interior components of the muscular fibres are being main effects such injury.

The morphofunctional recovery of injured peripheral axons is caused by the regenerative neuroplasticity, which consists of three processes. That is, Wallerian degeneration: When the axonal membrane ruptures it causes vigorous influx of calcium ion that results because Wallerian degeneration, next phase is, Chromatolysis: During the process of regeneration of axon related to axonal stability and synaptic transmission, proteins which causes the gene encoding is increased. Axonal growth cone: This growth is caused by regeneration of the axonal stump which is present in the proximal region. These are the stages this process has—1) consolidation 2) protrusion 3) engorgement also is similarly 4 triggered by the entry of Ca2+ ions into the neuron. A necessary factor for the growth of axon and a required element for functional recovery and motor development which is the controller of dynamic movement of cytoskeleton structures.

Low Level Laser Therapy (LLLT) made an attracted interest on a potential therapy varieties of nerve injury restoration since it is non-invasive and uses low-energy laser light to stimulate cellular processes. This LLLT is a therapeutic method that has mostly utilized to encourage nerve development and hasten the functional improvement and recovery of PNS(peripheral nervous system ). The potential effects of phototherapy on neurotrophic factor release, myelination, axonal growth, and nerve cell proliferation, all of which help promote nerve regeneration. therefore, some specific parameter able to reach deep the target and cause biological reactions that have an impact on the chemical changes and physical changes in exposed tissues. By speeding up mitosis and improving the effectiveness of tissue regeneration, LLLT encourages the differentiation of myofibroblastic in the initial stages of healing mechanism. It which in turn increases the ATP synthesis in a cells’ mitochondria, which favours cellular processes that are pro-regeneratively activates electron transport chain and accelerates the entire process. Step length was defined as the distance between a hind paw strike and the strike of the opposite hind paw. No distinction was made between the left and right measurements for stride and step lengths.

After sciatic nerve crush injury in rats, low intensity pulsed ultrasound contributes to quick functional and histologic improvement as well as increased brain-derived neurotrophic factor expression. High frequency sound waves are used in therapeutic ultrasound, a non-invasive medical procedure, to promote tissue healing and restoration. It has been utilised to treat ailments like musculoskeletal injuries and wound healing in a number of medical specialties. Discovering efficient strategies to quicken neuron regeneration and healing since peripheral nerve injuries can cause serious functional deficits. Since therapeutic ultrasound has demonstrated promise in accelerating tissue repair in other medical applications, it has been researched as a potential strategy to improve nerve regeneration. This study offered important proof in favour of therapeutic ultrasound use as a viable therapy to quicken motor recovery following peripheral nerve damage. looking into the possibility of using low-intensity ultrasound to encourage the activity of Schwann cells and improve nerve repair in the setting of peripheral nerve regeneration.

The increased demand of neurological lesions has compelled the medical community to look for practical remedies that might speed up the healing of the wounded nerve and stop the progression of muscle atrophy brought on by nerve injury.
AIM

The aim of the study is to compare the effectiveness of low level laser therapy and therapeutic ultrasound on motor recovery among experimentally induced peripheral nerve injury in Wistar rats.

Materials and Methods

The study was carried out between July and November of 2022. 18 male adult Wistar rats that were 3 months old, weighed 150–300g were selected by simple random technique and received surgically induced peripheral nerve (sciatic nerve) damage were used in the experiment. Every animal received the same surgical procedure. They were fed pellets and given water while being housed in cages with one other animal apiece. The rats were weighed and then randomly split into three groups of six rats in each group. Group 3 received a control group, while Groups 1 and 2 received ultrasound and laser therapy, respectively.

Selection criteria

a) Inclusion criteria:
   • Wistar rats at age between 6(0.5) months to 48 months (4.0)
   • Only Male rats were included
   • Physically active rats will be included

b) Exclusion criteria:
   • Rats with physical deformity and skin problems are excluded
   • Rat with psychological distress more aggressive rats

Outcome Measure:

Step length ratio (SLR): Step length is the distance between the heels of one foot to the heel of the other while walking. It is an easy way to assess motor function in rats. We used a wide wooden ruler, where the rat was made to walk to see the distance of each step length. A permanent marker is used to mark the metatarsal heads of the hind foot and front foot of the rat. Then the distance between hind foot’s metatarsal head to the front foot is recorded, the step length of each side’s hind foot was taken at floor level using the wooden ruler as a runway. The distance is noted for both the experienced and normal side. Step length ratio was then calculated by dividing the step length of the experimented side by the normal side. This was done before the surgery (PRE-OP), 7th day of post OP (PRE-TEST), 14th day (POST-TEST) and on the 21st day of post OP (POST-TEST) for all the three groups.

PROCEDURE:

18 animals were given intramuscular and intraperitoneal injections of 5% ketamine hydrochloride (70 mg/kg body weight) and 2% xylazine hydrochloride (10 mg/kg body weight) in a 1:4 ratio to induce anaesthesia. Meloxicam was administered subcutaneously in a dose of 1 mg/kg to treat the pain. By carefully shaved and dissecting between the gluteus maximus and quadriceps muscles, a 3 cm long postero-lateral longitudinal thigh incision was made, exposing the right sciatic nerve. 54 N of crush injury was then caused for 30 seconds using hemostatic forceps. Then suturing done followed by pain management.

Ultrasound group (Group 1)

The crush site was the only target for the pulsed-wave ultrasound, which covered a range of frequencies, durations, and spatial peaks with time-averaged intensities. With a finger probe and a pulsed intensity of 0.4 W/cm2 in a 1:1 ratio, the ultrasound used had a frequency of 1 MHz and was applied transcutaneously. The treatment duration is 4 minutes. The transducer was placed around 2 to 3 cm distant from the sutured area over the glove water bed in order to enable effective transmission of ultrasound into the target area. The treatment focused on the site of the crush injury and started on the second postoperative day (postoperative day 2). It lasted for 21 days in total. On days 7, 14, and 21 following surgery, the step length ratio was carried out and compared to the pre-injury assessment to gauge motor recovery. Beginning on the third postoperative day, a 4-minute treatment session was administered once a day for 21 days.
**Laser group (Group 2)**

For LLLT, a portable Aluminium Gallium Arsenide Laser Diode was employed. The laser met the following requirements: an energy density of 3 J/cm², an area length, width, and tissue depth of 1 cm, a wavelength of 808 nm (infrared light), a power of 200 mW, and continuous mode. Each session’s exposure period was set to 60 seconds. Rats in the experimental group 2 had laser irradiation with a focus on the nerve damage location that had been surgically identified. The contact point approach was used to apply a laser pen at a 90-degree angle to the skin during the first 21 days. According to Monte-Raso, this method proved quantitative, trustworthy, and reproducible in rat sciatic nerve operating settings.

**TABLE 1: Pre and Post-test mean values of step length ratio for LLLT, LLPU and Control group.**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Pre and post-test values</th>
<th>7th Day</th>
<th>14th Day</th>
<th>21st Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLLT GROUP</td>
<td>Pre-OP</td>
<td>1.01</td>
<td>0.4</td>
<td>0.73</td>
</tr>
<tr>
<td>LLPU GROUP</td>
<td>0.96</td>
<td>0.38</td>
<td>0.59</td>
<td>0.74</td>
</tr>
<tr>
<td>CONTROL</td>
<td>1</td>
<td>0.4</td>
<td>0.51</td>
<td>0.61</td>
</tr>
</tbody>
</table>

**Fig 2: LASER therapy**

**Control group (Group 3)**

Control group also underwent the same surgical procedure and no treatment was provided to the animals in the control group.

**DATA ANALYSIS:**

The assessed values of step length ratio was statistically analysed for their normality has been done by evaluating the values with Shapiro - wilk test, equal variance with the help of Brown forsyth test, significance among 3 groups were identified using one way repeated measure using ANOVA and pair comparison between the pre op value and 7th,14th and 21st day measure was analysed using Bonferroni and Holm multiple comparison test. The Tukey HSD test was used to compare the difference in final measure (21st day) between the three groups. SPSS statistical tools and Microsoft Office Excel were used to analyse and record the data. The ANOVA is used to analyse the SFI and SLR values of within-groups, significance threshold is fixed as 1% (p <0.01).

**GRAPH 1: Mean difference of SLR among LLLT group, USD group and Control group**

**Result**

The study was randomly assigned among 18 rats assigned into 3 groups which have been equally separated as 6 rats in each as LLLT group, Control group and USD group. step length ratio was measured and recorded before the surgery and post-op day of 7, day 14 and day 21 (Table 1). This indicates that all three groups have a similar baseline index and there is no potential bias among allocated subjects. One-way repeated ANOVA (analysis of variance) is used to interpret the difference of F-statistics and standard error within a group for Step length ratio (SLR) values. Comparing the mean difference of all three groups, 21st day value Group A with a mean difference of -12.43 is the least of all three groups where Group B has -13.76 and Group C has - 21.11 which is very higher than the other two groups. Multiple group comparison by tukey test between three groups has not shown significant difference between group A and group B while group C is significant with other two groups indicating significant difference. Step
length ratio (SLR) values among all the three groups were found statistically significant comparing its pre-OP and 21st day value. The mean difference of group A is 0.17, group B is 0.23 and group C is 0.39 (Graph 1). This shows that the LLLT group is very close to normal value showing significant difference than the other two groups.

**Discussion**

Nerve fibres regenerate on average at a rate of about 1 mm per day if the cellular and extracellular elements that make up a good substrate for axonal regeneration are present, as after a crush injury. Sciatic nerves that were exposed to low intensity laser irradiation had considerably higher levels of GAP-43. This implies that laser therapy may encourage GAP-43 expression, which is known to be essential for nerve regeneration. An axonotmetic grade of injury results from acute and chronic injuries of increased severity and necessitates not just remyelination but also axonal regeneration in order to recover. Examples of these injuries include diabetes, severe forms of carpal tunnel syndrome, and some types of trauma. Motor recovery can take up to two years for proximal lesions that necessitate axons to travel great distances before they reach target muscles or sensory receptors. According to surgical decompression for entrapment neuropathies and surgical exploration and repair following traumatic nerve injuries are common therapies for incapacitating nerve injuries. Drugs for peripheral neuropathies, including various neurotrophic factors, have not yet shown effective in clinical trials.

The prolonged and inaccurate effective reinnervation of motor and sensory target structures are significant barriers to a full recovery. The creation of novel therapies that hasten and enhance the healing process would have positive clinical effects. Many of these studies lacked details on essential characteristics such as dose, apparatus power, application duration, and method of application. This makes it difficult to comprehend their procedures, which makes it difficult to replicate their findings. It also makes it challenging to compare different studies. The results of these investigations appear to be inconclusive and debatable, which motivated us to conduct this study to determine whether LLLT may actually stimulate the recovery of sciatic nerve in functional aspect by using a rat model with crush injury of sciatic nerve.

The rats were split into two groups after being injured: one group underwent low level laser treatment to the damaged sciatic nerve, while the other group served as a control and did not receive laser treatment. At particular times following the injury, the researchers evaluated the levels of GAP-43 in both groups' damaged sciatic nerves. The GAP-43 has increased in LLLT group indicating nerve regeneration.

Two groups in rats were created: a control group and an experimental group. The site of the nerve injury, the experimental group received therapeutic ultrasound treatment, but the control group did not. It is important to remember that this was an animal study, and more investigation would be necessary to establish the applicability and efficiency of therapeutic ultrasonography in human neuron regeneration. The effect of ultrasonic radiation exposure within a relatively limited intensity band that is inversely proportional to dose has been suggested by experimental evidence shows that when therapeutic ultrasound is applied in low intensity and in small dosage, it enhances the regeneration of nerve meanwhile high intensity irradiation would inhibit the regeneration. Low-intensity ultrasound has a variety of effects on Schwann cells, according to studies. It can boost their growth, encourage growth factor release, and improve the expression of specific neurotrophic factors, all of which are crucial for nerve regeneration and repair. Prathap S et al., concluded that low level laser irradiation can increase the conduction velocity of motor nerves in diabetic neuropathy.

Hence, we decided to take the most used parameter in LLLT and LLPU to find its significance in motor recovery of sciatic nerve in rats.

**Conclusion**

According to the results, LLLT has more impact on motor recovery than LIPU. Also each treatment has a substantial impact on accelerating nerve regeneration and shortening the recovery time. Step length ratio has shown significant difference in motor recovery in LLLT than the LIPU group and control group had very less result than the other two groups.
Ethical Clearance: This original experimental study on rats was approved by the Ethical Committee, BRULAC/SDCH/SIMATS/IAEC/01-2023/11, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, India

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Conflict of Interest: Nil

Reference


