The Effects of Low Level Laser Treatment on Recovery of Nerve Conduction after Sciatic Nerve Compression Injury (Experimental study)

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Abstract: Aim of the work: The objective of study is to determine the effect of the low level laser therapy on nerves regeneration after a compression injury of rat sciatic nerve. Study design: The injury of the sciatic nerve was induced by using portable deadweight device with a load of 5000g applied over a length of 5 mm 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. The animals were divided into three groups, the animals in group III received LLLT daily for 28 consecutive days we used A Semi-conductor Diode AlGaInP Laser, formed a control console and a handheld probe. The diameter of the probe is 18 mm and it was used to deliver the LLLT. Functional nerve assessment was done using the foot step forms and sciatic nerve was dissected for histological examination to evaluate the regeneration of the sciatic nerve. Results: Found that significant differences of the times factor (F= 4765.53, P= 0.001), groups factor (F= 2222.54, P=0.0001), Also we found a significant differences of the interaction between times and groups factors (F=742.63, P=0.001) when sphericity assumed, Pillai's trace showing a significant differences for the time factor, group factor and the interaction between time and group factors. The histological assessment improve that the most axons of the Group III sections showed regeneration and a uniform arrangement. Conclusion: The Low level laser therapy is a useful way for treating the injuries of the nerves and when we use the LLLT at the early stage of the injury of the nerve it will helps in the minimizing of the loss of nerve functions.

Introduction
1 – Nerve tissues: The development of nerve tissues is a process in which the cellular interaction follow a specific sequence of Schwann cells¹,² develops in early stage, then proliferate and migrate along the nerve axons radially into the nerve fibers bundles.
2- Nerve degeneration: The targets of nerve degeneration are the axon, Schwann cells, and the myelin sheaths. Inflammatory neuropathies lead to focal degeneration of the myelin sheath with relative preservation of the axon. The Regeneration mechanisms restore the conduction of the nerve fibers byremyelination. On the other hand, the axonal damage can be caused by axotomy, crush, ischemia, or inflammation and can leads to loss of continuity of axonal integrity and nerve fibres degeneration at the distal part to the crush site, this is called Wallerian degeneration (WD)³.
3- Nerve regeneration: In case of nerve damage with no tension on the nerve and end-to-end nerve repair is not possible, it is necessary to use a nerve graft. The graft must be from a nerve and must be autogenous which cause minimal disability. After nerve grafting paraesthesia and amputation neuroma of the site of donation are takes place. The basement membrane has an important role in nerve regeneration, and trials have been made to use the basement membrane scaffolds of skeletal muscles as abridging grafts³-⁹. Since Ide et al. reported that amputated nerves could regenerate and showed that axons grew through the remaining lumen of the basement membrane of Schwann cells, The nerve guide must be integrated into the surrounding tissues and must guide the nerve fibers towards the distal nerve stump to secure success. Preventing fibrous tissue from invading the nerve gap, to be stable as long as the regenerating nerve fibers are not mature enough, is also expected, and finally to disappear rather than...
being removed, to avoid the risk of the repaired nerve.

4) Low level laser:

Clinical applications for laser light are used in many branches of medicine and surgery. High power densities are commonly used to produce a thermally destructive effect and selective photocoagulation. The development of lasers during the last 43 years has produced many wavelengths of laser light to be used in researches to investigate for a variety of surgical and medical procedures. Because of their extremely high powers and clinical effects, these lasers can be labeled high-level lasers. There is also a class of lasers, called low-level lasers (LLL), which were developed to use a purely phototherapeutic process. The therapy performed with such lasers is called low-level laser therapy (LLLT), and the lasers are described as therapeutic lasers. These lasers have several other names, such as soft laser, low-reactive laser, low energy laser, and low-intensity level laser. Therapies that use these lasers are often referred to as biostimulation or biomodulation. The biomodulation is more appropriate, because the therapy not only can stimulate but also can suppress biologic processes.

2. Materials and Methods:

Animals and Grouping:

Thirty adult male Wistar rats, weighing between 200 g and 250 g, were involved in this study. The rats were kept in discrete boxes and ate a standard rat food and allowed a free access to water.

Sciatic Nerve Anatomy and Dissection:

The sciatic nerve axons in the rats arise from spinal segments L3-L6. It has 4 main branches; the first one is the sural nerve, and it contains 4% motor and 96% sensory fibers, the second one is the lateral sural nerve, which is a coetaneous branch, the third one is the common peroneal nerve, and the last one is the tibial nerve.

The right sciatic nerve was isolated in animals included in group 3 to induce the crush injury. Anesthesia was induced using a mixture of Hypnorm, Dormicum and sterile water, at a ratio of 1:1:2, and a dose of 0.3 ml/100 g body weight. Under sterile conditions, the surgery was performed through a posterolateral approach. Skin was shaved and prepared with an antiseptic solution then incised (Fig. 1).

Nerve Injury:

The right sciatic nerve was freed then compressed at the same level of the sciatic notch, 3mm distal to the superior gluteal branch of the sciatic nerve (Fig. 2).

Crushing was induced to the sciatic nerve using a portable device which called deadweight device and using a load of 5kg applied on a length of 5mm for 10 minutes. This load which applied and the length and time sequences is to cause extensive degeneration in the axons of the rat sciatic nerve.

Low Level Laser Therapy:

A photon plus Semi conductor Diode AlGaInP Laser, consisting of a control console and a handheld delivery probe with a probe diameter of 18 mm was used to deliver the LLLT. The device delivers a 110mW output in a 810nm continuous wavelength.

Functional Assessment:

The SFI is certified as a thematic and quantitative mode to estimate healing of sciatic nerve after damage. It transforms function into numeral values permitting statistical analysis of the collected observation. The distance between the first and fifth toes (toe spreading, TS), the distance between the second and fourth toes (intermediary toes, IT) and print length (PL) were measured as described by de Medinaceli et al. The SFI was calculated according to the following equation as described by Bain et al:

\[ SFI = -83.3 \left( \frac{EPL - NPL}{NPL} \right) + 109.5 \left( \frac{ETS - NTS}{NTS} \right) + 12.3 \left( \frac{EIT - NIT}{NIT} \right) - 8.8 \]

Histological Evaluation:

Animals were euthanized after 28 days following the nerve trauma. The sciatic nerve was then isolated and dissected for histological examination by light microscope and electron microscope and Computer Assisted digital image analysis (Digital morphometric study).

Statistical analysis:

Figure (1): A photograph showing the incised skin in the posterolateral approach.

Data were tabulated, and then analyzed using statistical package for social science (SPSS) version 17.0. For the analytical statistics, the significance of difference was tested for the axon count and average axon total area using numerical parametric univariate analysis of variance (ANOVA) to compare between more than two groups followed by post-hoc LSD for
multiple comparisons. Partial correlation was used to correlate the relation between axon count and average axon total area.

The multivariate two-way repeated measure ANOVA statistical test was used for foot print measurements followed by Bonferroni post-hoc for multiple comparisons. The factors for ANOVA were 1) Time and 2) groups, Statistical tests were based on a type 1 error value of 5% (α=0.05) and a power of 0.85 sample size.

3. Results:
1. Clinical findings:
The wounds healed without complications, and all the animals continued to increase in weight satisfactorily during the 28 post-operative days without any indication of discomfort apart from right hind limb lameness.

2. Functional Assessment
Functional assessment was undertaken at 1, 3, 10, 20 and 27 days postoperatively. The ANOVA test for foot print measurements revealed significant differences regarding the times factor (F= 4765.53, P=0.001), groups factor (F= 2222.54, P=0.0001) and the interaction between times and groups factors (F=742.63, P=0.001) when sphericity assumed (Table 1).

<table>
<thead>
<tr>
<th>Tests of Within-Subjects Effects</th>
<th>Tests of Within-Subjects Contrasts</th>
<th>Tests of Between-Subjects Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mauchly's W</td>
<td>Approx. Chi-Square</td>
<td>Sphericity Assumed (F ratio and P value)</td>
</tr>
<tr>
<td>Time</td>
<td>.130</td>
<td>15.138</td>
</tr>
<tr>
<td>Group</td>
<td>.583</td>
<td>4.320</td>
</tr>
<tr>
<td>Time * Group</td>
<td>.000</td>
<td>62.434</td>
</tr>
</tbody>
</table>

Table 2: Bonferroni post-hoc for foot print measurements.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>Time * Group</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3666.09 (0.001)</td>
<td>4612.68 (0.001)</td>
<td>2345.65/0.001</td>
<td>-8.33±0.72</td>
<td>-8.26±0.74</td>
<td>-8.33±0.43</td>
<td>-8.48±0.41</td>
<td>-8.44±0.39</td>
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<td></td>
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</table>

Means with same superscript letter in the column indicate a statistical significance at 0.5 P level. *Means with the same superscript symbol in the row indicate a statistical significance at 0.5 P level.

In addition, significant differences were also found when linearity assumed regarding times factor (F=8431.69, P= 0.001), groups factor (F=2658.74, P=0.001) and the interaction between times and groups factors (F=5369, P=0.001) (Table 1).

Pillai’s trace revealed a significant differences for the time factor (F=3666.09, P=0.001), group factor (F=4612, P=0.001) and the interaction between time and group factors (F=2345.65, P=0.001) (Table 2).

Meanwhile, Bonferroni post-hoc pairwise statistical test for foot print measurements revealed significant differences between T1&T2, T1&T3, T1&T4, T1&T5, T2&T3, T2&T4, T2&T5, T3&T4, T3&T5 and between T4&T5 for groups II and III and non-significant differences for group I between all examination periods (Table2). Moreover, significant differences were found between groups I &II, I& III and between II&III within each examination period (Table 2).
3. Histological examination:
   a. Light microscopic findings:
      The transverse section of the sciatic nerve for group I showed the normal healthy composition of nerve tissues, consisting of a peripheral layer of connective tissue coat (epineurium) surrounding the nerve. The fibrous perineurium wrapping individual nerve fascicles and deep to the epineurium, perineural epithelium was seen composed of several layers of flattened cells. A thin layer of connective tissue called the endoneurium was seen wrapping individual myelinated nerve fibers. The fibers were composed of an axon surrounded by myelin sheath and Schwann cell (Figs.3).

      Figure (3): Photomicrograph of sciatic nerve transverse section for group I showing perineurium (P) surrounds individual nerve fascicles, endoneurium (E) surrounding individual myelinated fibers. The fiber is composed of an axon (N) surrounded by myelin sheath (M) and Schwann cell (S) (H&EX200).

      Group II transverse section showed loss of the normal architecture of sciatic nerve tissue. The nerve fascicle illustrated marked absence of large caliber axons associated with myelin degradation. In addition, loss of normal connective architecture was clearly seen. The fibroblasts were irregularly distributed with irregular arrangement of collagen fibers and the blood vessels were congested (Figs.4).

      Figure 4: Photomicrograph of group II transverse section showing loss of normal nerve tissue architecture. The nerve fascicle illustrates marked absence of large caliber axons (N) associated with myelin degradation (M) and a decrease in the number of Schwann cells (S). Loss of connective tissue integrity is clearly obvious for both perineurium (P) and endoneurium (E) (H&EX200).

   b. Digital image results
      One-way ANOVA for axon count revealed a significant difference between all groups (F= 258.15, P = 0.001). LSD pairwise comparison revealed significant differences between groups I & II, I & III and between II and III (Table 3). One-way ANOVA for average axon total area revealed a significant difference between all groups (F= 430.04, P= 0.001). LSD pair wise comparison revealed significant differences between groups I & II, and between II & III. Non-significant difference was found between group I & III (Table 4). Strong positive significant correlation for group III was found between axon count and average axon (Table 5).

<table>
<thead>
<tr>
<th>One-way ANOVA</th>
<th>LSD post-hoc test</th>
</tr>
</thead>
<tbody>
<tr>
<td>F ratio</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>258.15</td>
<td>0.001</td>
</tr>
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</table>

Means with different superscripts in the same row are statistically significant at 0.5 P level.
### Table 4: One-way ANOVA and LSD post-hoc test for average axon total area.

<table>
<thead>
<tr>
<th>F ratio</th>
<th>P value</th>
<th>Mean±SD</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>430.04</td>
<td>0.001</td>
<td></td>
<td>11.48±0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.84±0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.95±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.76±3.59</td>
</tr>
</tbody>
</table>

Means with different superscripts in the same raw are statistically significant at 0.5 P level.

### Table 5: Partial correlations for group III between axon count and average axon total area.

<table>
<thead>
<tr>
<th>Normal Variables</th>
<th>Count</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Count</td>
<td>Correlation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Average</td>
<td>Correlation</td>
<td>.964</td>
</tr>
<tr>
<td></td>
<td>Significance (2-tailed)</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Df</td>
<td>27</td>
</tr>
</tbody>
</table>

### Figure 5: Photomicrograph of group III showing regeneration of most axons (N) and the axon shave uniform arrangement, reduction of spaces between the axons that surrounded by myelin sheaths (M). Few areas of degenerated axons are clearly seen (asterisks). Regeneration of the Schwann cells (S) and increasing its numbers. Perineurium (P) surrounds individual nerve fascicles and endoneurium (E) surrounding individual myelinated fibers (H&EX200).

### 4. Discussion:

The damage of peripheral nerves in not uncommon. Causes include traumatic injuries, inflammatory disorders, and chemotherapy. Such damage can result in secondary muscle atrophy. Consequently, this disorder carry a devastating effect on the patient’s psychological status and quality of life. (24,25)

Different animal models are used for the research of nerve damage. However, rats remain the most commonly used, owing to the availability, low cost and easy manipulation. A key selection factor in this particular research is the nerve trunk distribution, which is similar to that in humans. (26)

Different methods have been advocated to induce compressive nerve damage in animal models; including tourniquets, forceps and hemostats. (27-29) The crushing injury was selected in this research to induce the axonotmesis type of nerve injury, to preserve the continuity of the endoneural tube to allow nerve regeneration by the reactive Schwann cells. (30-32) Following nerve injury, Wallerian degeneration takes place at the distal end of injury, while the proximal end undergoes retrograde degeneration. (33)

Timing of laser application is crucial for its effect. In accordance with our study, Dahlin (34) and Belchoir et al. (35) reported a positive functional improvement following sciatic nerve injury when the laser is used soon after injury. This early intervention also minimizes the immediate functional loss.

How LLLT could enhance nerve recovery is unclear. However, multiple biological processes are reported to be enhanced following the application of LLLT. LLLT is reported to accelerate Wallerian degeneration and growth of the newly formed nerve fibers. (36) Laser is also reported to enhance cultured rat Schwann cells regeneration in vitro. (37) On the molecular level, LLLT is also reported to increase the blood flow and to promote the formation of cellular ATP, which accelerate cellular mitotic activity and result in superior angiogenesis. (38) LLLT is also reported to stimulate neurotropic growth factors such as growth-associated protein 43 (GAP-43) and fibroblast growth factor. (39,40)

In contrast with our results, Bagis et al. (41) reported no beneficial effect of a 904nm LLLT on injured rat sciatic nerve. The same results were also reported by Carla et al. (42), who utilized a 808-nm LLLT.

The use of LLLT following surgical correction has been reported with promising results. In contrast, Chen et al. (43) reported a negative effect of 904nm GaAs laser applied to rat sciatic nerve with a 10-mm
gap. This could be attributed, however, to the delayed application of laser used in Chen et al. study, beginning a week after nerve surgical repair.

The use of LLLT for clinical improvement of neural deficiency is reported. Promising, however, limited results are described. However, the most beneficial type of laser, as well as the wave length, and time of application are yet to be determined.

Conclusions

Low level laser therapy is a promising modality for treating crushing nerve injuries. The Early use of LLLT following nerve injury minimizes the immediate functional loss.

References:

22. Endo C, Barbieri CH, Mazzer N, Fazan VS. A laserterapia de baixa intensidade acelera a