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Evaluation of Low-Level Laser Therapy with and without Platelet Rich fibrin on Sciatic Nerve Regeneration in Rats

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Abstract
Background: Peripheral nerves are structures that, when damaged, can result in significant motor and sensory disabilities. Several studies have used therapeutic resources with the aim of promoting early nerve regeneration.
Aim of the study: evaluate the effect of low-level laser therapy and platelet rich fibrin on nerve regeneration after compression.
*Material and Methods: 45 Wistar rats were randomly divided into three groups following right sciatic nerve compression, as follows: (1) control gp **(gp I)**: The nerve was compressed without further treatment options, (2) Laser group **(gp II):** The nerve was compressed followed by laser application to the site of injury immediately and for 8 postoperative weeks and (3) Laser and PRF Group **(gp III):** The nerve was compressed followed by adding PRF to the site of energy and laser application to it immediately and for 8 postoperative weeks. 808 nm AlGaInP low level laser with power of inergy 110 mw /cm ^{2} was used. Functional evaluation was done prior to surgery and functional and histological evaluation were done after 1, 2, 4, 6 and 8 postoperative weeks.*
Results: group III showed the best results for nerve repair, faster neurological improvement and muscle re-innervation and decreased muscle atrophy. This is followed by group II and lastly was group I.
Conclusion: Low level laser and platlet rich fibrin provide promising methods for accelerating \nerve regeneration.
***Keywords**: Laser therapy, low-level laser, Nerve regeneration, Platelet rich fibrin.*

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I. Introduction

Trauma frequently causes damage to peripheral nerves. The process of healing of peripheral nerves and degree of recovery after injury is slow and even incomplete. Peripheral nerve injury can cause loss of sensation, paralysis or disability to the affected organ affecting human normal lifestyle leading to irritability, discomfort and many other social problems [1]. Several techniques in peripheral nerve repair have been improved in the past decades trying to achieve early recovery and accelerate healing time [2-6].

Low-level laser (LLL) is one of the most important inventions in the last years and has shown positive results in treating inflammation, nerve injuries and many other medical and dental problems [7-10]. LLL promotes blood flow, formation of new blood vessels and stimulates the production of adenosine triphosphate (ATP) in the irradiated area, thus accelerating healing. Other studies demonstrated that it has no effect on nerve regeneration [11]. Up till now the ideal parameters for laser application such as dose, method of application, timing of application, wave length, pulsed or continuous, the power and energy used are still unknown and are not standardized [12].

Platelet-rich fibrin (PRF), another innovation in the last years, is an autologous fibrin matrix, contains many growth factors that are involved in wound healing and bone regeneration. There are various clinical and experimental studies demonstrating that PRF has a positive effect on bone and wound healing [13-15].

Some studies on nerve regeneration concluded that PRF improved regeneration and functional recovery after nerve injury [16-18], while others demonstrated that it has no effect [19]. So, further investigations and studies are required to determine the appropriate PRF criteria that aid in nerve regeneration.

The aim of this study is to evaluate the effect of low-level laser therapy with and without PRF on regeneration of rat sciatic nerve after compression.

II. Materials and Methods

Forty-five adult Wister rats were used in this study with body weights ranging from 250 to 300 gm. They were kept under controlled optimum light and temperature conditions, with standard chow, water and libitum for 8 weeks (study period). Animals were divided into three groups, one control group and two study groups, each group contained 15 animals and the groups were divided according to the procedures as following:

- Control Group (gp I): The nerve was compressed without further treatment options.
- Laser group (gp II): The nerve was compressed followed by laser application to the site of injury immediately and for 8 postoperative weeks.
- Laser and PRF Group (gp III): The nerve was compressed followed by adding PRF to the site of energy and laser application immediately and for 8 postoperative weeks.

This study was done at Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Tanta University and Medical Technology center, Medical Research Institute, Alexandria University.

Laser device: AlGaInP LLL was used in this study. It emits a continuous wave length of 808 nm and delivers power energy of 110 mw /cm 2 to 1 cm 2 of area.

PRF preparation: Six milliliters of blood were obtained in a sterile tube without anticoagulant from each rat. Immediately after the blood was drawn, the tubes were centrifuged for 10 minutes at 3000 rpm in a centrifuge device. The blood was separated into three layers: cellular plasma at the top, red blood cells at the bottom and PRF clot in the middle [20, 21].

Compression device: A custom made portable deadweight device, as described by Pachioni et al. [22] was used in this study for the crushing of the sciatic nerve of rats (fig. 1) The crushing load used in this experiment was 5000 gm applied for 10 minutes, according to the survey by Mazzer et al. [23] in 2006, it promotes an axonotmesis.

Fig. 1: Showing the portable deadweight device as described by Pachioni et al.

Walking track: constructed according to parameters described by De Medinaceli et al. [24] (fig. 2). This track was used to facilitate sampling of the hind footprints, which is necessary to calculate the Sciatic Functional Index (SFI). The footprints were obtained in the pre-operative period and after 1, 2, 4, 6 and 8 weeks post-operatively. The footprints were collected using stamp ink. The ink-soaked paws left footprints on strips of paper cut to the same dimensions as the track (43×8.5 cm), three footprints per paw on average. The formula proposed by Bain et al.[25] in 1989 was used to calculate SFI. This formula transforms function into numeral values permitting statistical analysis of the collected data. This index was statistically, not empirically, derived. It was created based on the coefficients derived from multiple linear regression analysis.

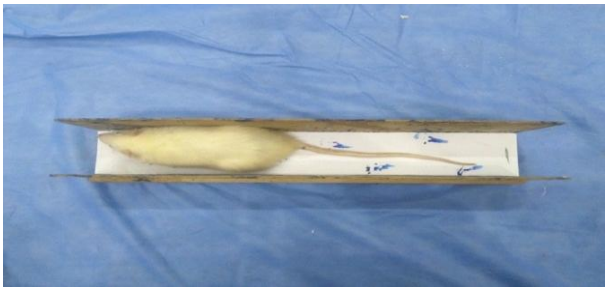

Fig. 2: Showing training of the rat on walking inside the track and footprints.

Pre-operative evaluation

All animals were trained for ten consecutive days on the walking track, pre-operative prints were taken and SFI records were calculated.

Surgical procedure

All rats were operated on under general anesthesia with intramuscular injection of 50 mg/kg of body weight ketamine. Hair was shaved from the right hind limb and mid back and iodine antiseptic solution was painted on the surgical site. Rats were placed in a prone position (fig. 3). The sciatic nerve was exposed at the dorsocaudal region, an incision was made starting 0.5 cm laterally from the animal's midline and extending laterally for 3 cm toward the tibiofemoral articulation. The femoral biceps and gluteus muscles were separated using blunt dissection to allow access to provide exposure of the right sciatic nerve (fig. 4), then the nerve was placed on the compression device and compressed at a proximal segment 5 mm from its bifurcation (fig. 5, 6, 7 (A&B))

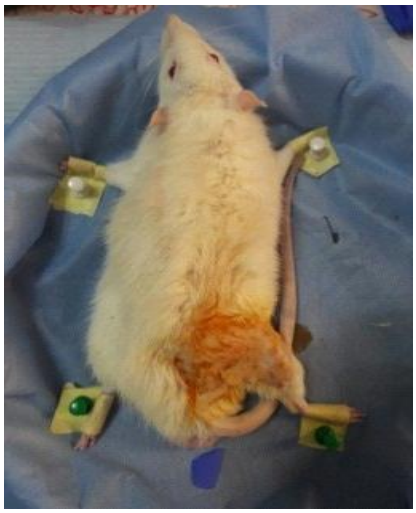

Fig. 3: Showing rat placed in a prone position, shaved hair from the right hind limb and mid back and Painting surgical area with iodine antiseptic solution

Fig. 4: Showing exposure of right sciatic nerve.

Fig. 5: Showing sciatic nerve before compression

Fig. 6: Showing crushing of rat sciatic nerve by portable dead weight device with load 5000gm applied for 10 minutes.

A B

Fig. 7A, B: showing crushed sciatic nerve.

Then, animals were randomly divided into 3 groups, according to the procedures that will be carried out:

- **Control group (gp I):** No further treatment options were done after nerve compression.
- **Laser group (gpII):** Laser was applied immediately after surgery and daily for 8 postoperative weeks. The radiation was applied for 60 seconds delivering power energy of 110 mw/cm2 to the area of injury. The radiation source was kept in contact and perpendicular to the skin surface. A single point in the middle of the surgical incision was irradiated (transcutaneous method). (Fig. 8)

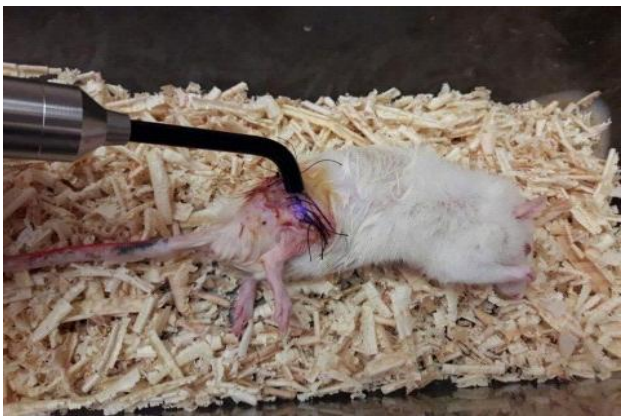

Fig. 8: showing laser application immediately after surgery.

Laser and PRF group (gpIII):

PRF clot (fig. 9) was transformed into PRF membrane (fig. 10) and was wrapped around the area of nerve injury (fig. 11) followed by laser application immediately after surgery and for 8 postoperative weeks with the same parameters as gp II.

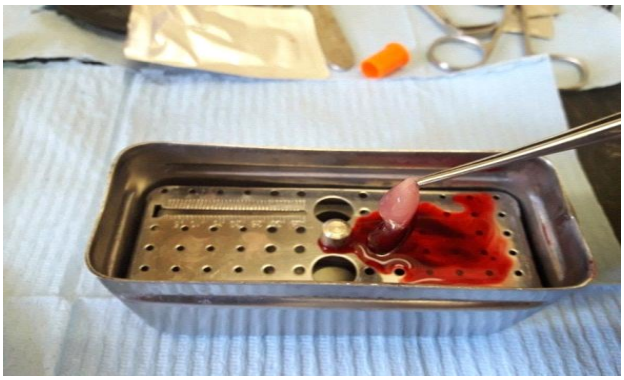

Fig. 9: showing PRF.

Fig. 10: Showing PRF membrane.

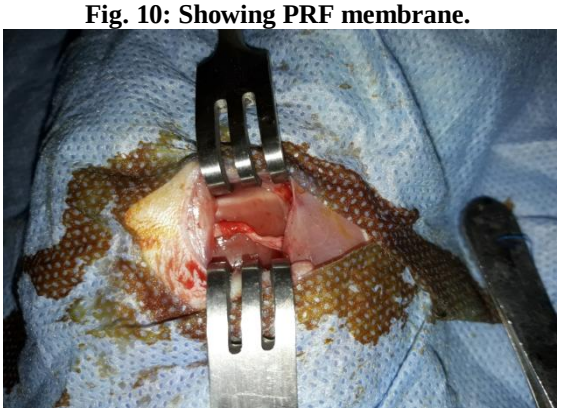

Fig. 11: PRF wrapped around crushed area of sciatic nerve.

In all rats, the wound was closed in layers. A 3/0 vicryl suture was used to close the wound and the skin was painted with iodine antiseptic solution. The rats were kept in their cage with the free access to water and food until complete recovery. The rats were kept on a single dose of antibiotic (Penicillin G 2000 u / 100 gm body weight) for three days [26].

Post-operative evaluation

Recovery of the sciatic nerve was evaluated both functionally and histologically:

- **Functional evaluation:**

Rats in all groups underwent a postoperative SFI record after the following post-operative intervals 1, 2, 4, 6 and 8 weeks using SIF formula described before.

- **Histological evaluation:**

Two rats from each group were euthanasized randomly by over dose of ketamine after the following post-operative intervals 1, 2, 4, 6 and 8 weeks after SFI evaluation to evaluate histological changes in comparison with functional development along the period of treatment. 7 mm of the sciatic nerve including area of injury and the adjacent muscle were removed and submitted to routine histological procedures using light microscope.

III. Results

I- Clinical assessment:

Only 30 rats survived the entire study period. They all tolerated the surgical procedure intended for each group. Wounds healed without any complications. They all continued to increase in weight satisfactorily during the 8

post-operative weeks until date of scarification. The total number of rats underwent complications was 15 rats, 9 rats died in the 1st week of surgery without any obvious cause and the other 6 rats underwent autotomy of their fingers and were excluded from the study (fig. 12).

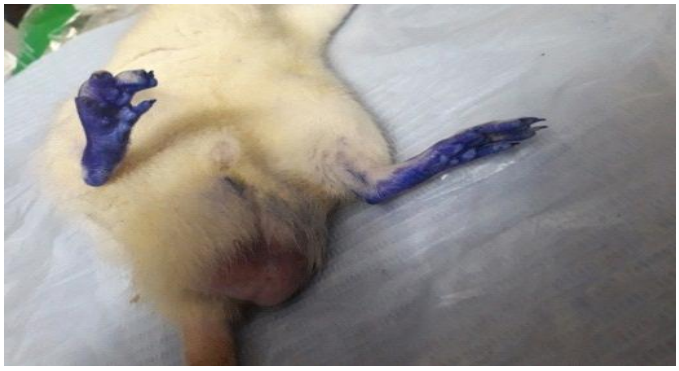

Fig. 12: Postoperative photograph showing finger autotomy of right operated foot.

II- Functional assessment and statistical analysis:

Only 30 rats survived the entire study period. So, statistical analysis was obtained from results of the survived 30 rats.

- Group I: included 10 rats (after death of 3 rats and fingure autotomy of 2 rats).
- Group II: included 10 rats (after death of 1 rat and fingure autotomy of 4 rats) .
- Group III: included 10 rat (after death of 5 rats) .

The results of this study were based on the SFI values for the tested rats, calculated by SFI formula. Values of SFI of the three tested groups were collected preoperatively and postoperatively at the different follow up periods (after 1, 2, 4, 6, and 8 weeks) from rats footprints

After one week: Based on Sig. value, it was observed that the difference of SFI between groups (I versus II) [Sig = 0.125] and (II versus III) [Sig = 0.026] was statistically not significant at 1% level of significance, whereas the difference of SFI between groups (I versus III) [Sig = 0.008] were statistically significant at 1% level of significance for the benefit of group III.

After two weeks: Based on Sig. value, it was noted that the difference of SFI between groups (I versus II) [Sig = 0.017] and (II versus III) [Sig = 0.011] was statistically not significant at 1% level of significance, whereas the difference of SFI between groups (I versus III) [Sig = 0.002] were statistically significant at 1% level of significance for the benefit of the third group.

After four, six and eight weeks: Based on Sig. value, there was not a significant difference in the SFI values between the three tested groups [Sig = 0.104, 0.158 & 0.104 respictively]. ANOVA test and post hoc test revealed that the difference between the three groups were statistically significant at 1% level of significance for the benefit of gp III.

Based on the Sig value, it was observed that the difference of SFI between groups (I versus II) and (II versus III) were statistically not significant at 1% level of significance, whereas the difference of SFI between groups (I versus III) were statistically significant at 1% level of significance for the benefit of the third group (fig. 13)

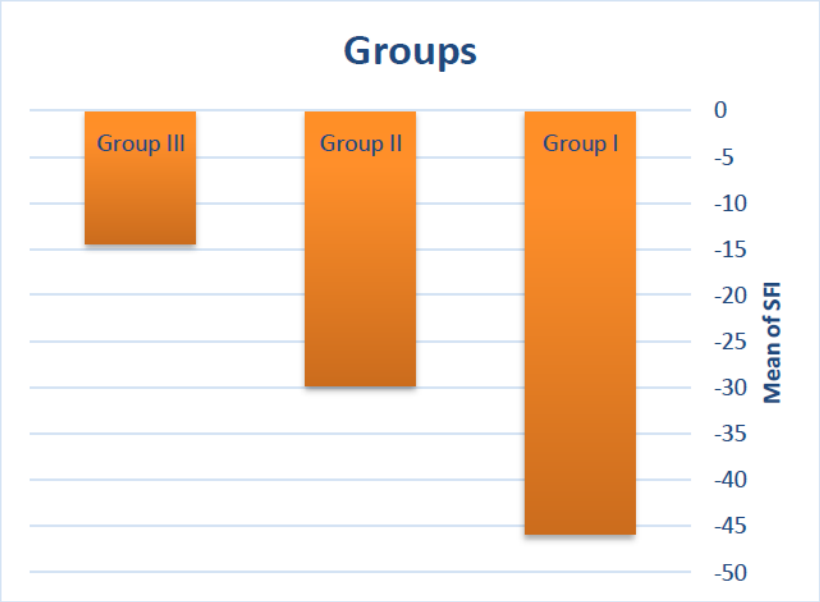

Fig. 13: Showing the mean plots, indicating that group III is better than group II followed by group I

From the previous data, based on statistical analysis for functional assessment, it can be observed that group III (laser and PRF group) has the best results for functional improvement, followed by group II (laser group), then group I (control group) which is the least group.

III- Histological assessment

Histological findings at the different follow up periods for the three groups showed that group III has the best results for nerve repair, faster neurological improvement and muscle re-innervation and decreased muscle atrophy. This is followed by group II (laser group) and lastly was group I (control group).

By examination of the repaired sciatic nerves under light microscope at the end of the experiment after 8 weeks, there was axonal regeneration in the three groups. The axonal regeneration in group II was better than that in group I which show less fibrosis and less inflammatory reaction. Also, muscle atrophy in group II was less than that in group I. Group III (Laser and PRF group) showed the best results among the three tested groups.

IV. Discussion

Sciatic nerve regeneration after injury is a slow process with a limited degree of functional recovery. Accelerating the rate of nerve regeneration and improving the degree of nerve repair is a clinical challenge. Several treatment protocols have been proposed to induce healing after compression, but there was no gold standard treatment due to lack of ideal parameters required to get the most benefit from the technique. Management of nerve after compression injury remains a controversial topic [2-6, 27, 28] .

In this study, the experimental model of choice was the rat as it provides an inexpensive source of mammalian nervous tissue of identical genetic stock that is easy to work with and well-studied by many authors [29]. The sciatic nerve was choose in this study as it has large size that facilitates experimental surgery, easy surgical access and few collateral branches. This was according to studies achieved by Sadakah et al.[2] and Varejao et al.[30].

LLLT was used in this study as according to Morais et al.[7] , Belchior et al.[8] , Rochkind et al.[9] and Serafim et al.[10] it has a neuroinductive and neurostimulatory effect that is used to enhance tissue healing and induce early functional recovery. This was opposite to Bagis et al.[11, 31] who found that LLLT has no effect on regeneration of nerve tissue. This negative effect may be due to lack of ideal parameters that is required to enhance nerve regeneration.

PRF was used as according to Martina et al. [16] it improves healing and functional recovery in sciatic nerve injury. Also, Chuang et al.[17] found PRF and adipose tissue–derived stem cells decreases axonal and myelin damage after sciatic nerve injury and appears to have a positive effect on the recovery of walking function after injury, thus paving the way for nerve damage repair, speeding up the regeneration of nerves and improving recovery quality. This was opposite to Bayram et al.[19] who found that local PRF application did not show any improvement in recovery of crush nerve injuries and Senses et al.[32] who reported that PRF decreases functional recovery in sciatic nerve injury.

Walking track analysis was used in our study for assessing functional recovery according to De Medinaceli et al.[24]. Foot prints obtained from walking track analyzed in the form of SFI provides a noninvasive method of assessing the functional status of the sciatic nerve during the regeneration process. This was in agreement with studies by Sarikcioglu et al.[33].

The Sciatic Functional Index (SFI) was used in our study as an index for evaluation of the functional recovery of the sciatic nerve after injury. This was in agreement with study performed by Monte-Raso et al. [34] who reported the SFI as a reliable, reproducible and quantitative method for evaluating functional recovery providing both numerical values and allowing statistical analysis of the results.

Axonotmesis in our work was achieved by a crushing load of 5000 gm which was applied for 10 minuts to insure that axonotmesis has occurred as reported by Mazzer et al [23]. This was in contrast with study performed by Lundborg et al.[35] who used tourniquets, study performed by Gutmann et al.[36] who used forceps and study performed by Hasegawa et al.[37] who used hemostats, in all of these trials the crushing load or crushing force was not measurable and it did not guarantee that axonotmesis has occurred. So, the results of these researches were not predictable.

All rats regained functional recovery at the end of this experiment and the mean values of SFI after 8 postoperative weeks approximated the mean SFI preoperative values. It was clearly obvious that functional recovery of group II (laser group) was faster than group I (control group), and this coincided with the mean values of SFI for both groups at the different follow up periods. This was in agreement with studies performed by Belchior et al.8 who reported that laser phototherapy improved functional recovery of rat sciatic nerve after compression in contrast with the control group and Serafim et al.10 who reported that laser phototherapy increased functional recovery scores at 7, 14 and 21 days postoperatively.

It was also clear that functional recovery of group III (laser and PRF group) was clinically faster than group II and group I, and this coincided with the mean values of SFI records obtained during the postoperative follow up periods for the three groups. Up till now, there is no meta-analysis that demonstrates the combined effect of LLLT and PRF together on regeneration of rat sciatic nerve after compression. Also, there is no meta-analysis demonstrates the comparison between effect of laser and PRF on regeneration of rat sciatic nerve after compression.

Martina et al. [16] found that PRF improves healing and functional recovery after sciatic nerve injury compared to the control group. Also, Chuang et al.[17] found that PRF have a positive effect on the recovery of walking function after injury, speeding up the regeneration of nerves and improving recovery quality. This was in agreement with our group III results (laser and PRF group) that it had better results than group I (control group).

Histological results showed that healing of group II (Laser group) was better than group I (control group), there was significant decrease in Wallerian degeneration in the laser group after LLLT in comparison with the control group, there was increasing in axonal growth, regeneration and reduction of inflammatory cells in comparison to the control group. Moreover, the quantity of healthy Schwann cells in the injured area increased, showing that they really play an important role in reconstruction and restoration of the injured peripheral nerve to its original shape. These findings were in agreement with results obtained by Mashhoudi et al.[38] who reported faster healing, increased Schwann cells and decreased wallerian degeneration and inflammatory cells in laser group in comparison with the control group.

Our histological results also showed that healing of group III (laser and PRF group) was better than group II (laser group) and group I (control group). Although, there was no literature studied the combined effect of laser and PRF together on regeneration of nerve tissue or compare it with effect of laser alone, Gladson et al.[39] found that the isolated or associated use of the PRF with physical exercises did not produce statistical quantitative functional or morphometric changes in the median nerve compression model. However, in the morphological evaluation it was possible to distinguish defined characteristics of the repair process for the treated groups. This was in agreement with our results which showed decreased degeneration time, decreased edema and inflammatory cells and increased regeneration of nerve fibers and proliferating Schwann cells in earlier weeks in the (PRF and laser) group compared to laser and control groups.

In our study, muscle atrophy or wasting has occurred after injury to the supplied nerve. This was in agreement with Adrian et al.[40] who reported that denervation atrophy occurs when the muscle nerve is interrupted and the muscle tissue no longer receives stimulation signals from the nervous system and represents a debilitating condition. The most pronounced muscle atrophy and decrease in fiber diameters was observed during the 1st week of denervation, these findings were in agreement with studies performed by Appell et al.[41] and Bodine-Fowler et al.[42] who reported that the gross amount of atrophy occurs during the first week after muscle disuse or denervation.

These data proves our findings that group I (control group) showed the most signs of muscle atrophy which continued up to the 4th and 6th post-operative weeks until nerve regeneration started to occur. Group II (laser group) showed less signs of muscle atrophy than group I as nerve regeneration and muscle re-innervation

start earlier in the 2nd and 4th postoperative weeks. Group III (PRF and laser group) showed the least signs of muscle atrophy and earlier return of function due to earlier nerve regeneration and earlier muscle re-innervation than group II and group I.

V. Conclusion

- Low level laser provide a promising treatment modality for nerve injuries, laser also is available nowadays in most of our clinics and provides a noninvasive technique for regeneration and biostimulation.
- PRF which is autologous substrate provide a promising, cheap and biological treatment for treating nerve injuries, improve and accelerate healing and regeneration.
- Using PRF together with Laser provides better results instead of using laser alone.

VI. Recommendation

Further investigations are required:

- To determine the most appropriate laser parameters and PRF criteria to get the best results.
- To study the combined effect of using LLL and PRF together in treating nerve injuries to get the best results.
- To compare between the effect of LLL and PRF each alone on regeneration of nerve injuries.
- With increased number of study samples to get appropriate statistical significance between study groups.

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