

EFFECT OF LOW LEVEL LASER THERAPY (LLLT) AS AN ADJUNCT TO PLATELET RICH FIBRIN (PRF) ON SCIATIC NERVE REGENERATION IN RAT MODEL

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ABSTRACT

Peripheral nerve injury (PNI) is a challenging injury in which healing relies on many factors. End-to-end neurorrhaphy (EEN) is the gold standard technique used in management of these injuries. Platelet rich fibrin (PRF) and low level laser therapy (LLLT) have the ability to enhance nerve regeneration after neurorrhaphy. The aim of this study is to evaluate the effect of LLLT as an adjunct to PRF use after end-to-end neurorrhaphy on sciatic nerve regeneration in rat model.

Material and methods: This double-blind randomized study was performed on 20 Albino Wistar rats in the animal house of Misr University for Science and Technology. Animals were divided into two groups; group I in which nerve repair was performed using EEN with PRF membrane, in group II the same treatment was performed in addition to intraoperative application of LLLT diode laser. Within each group, animals were divided into two subgroups where rats were sacrificed after one and three weeks follow-up period. Assessment was performed using electromyography measurements and area percent evaluation of S100 protein immunostain at one and three weeks follow-up periods.

Results: Comparison showed that electromyography measurements improved in group II more than group I with significant difference in the first week follow-up period. At three weeks follow-up period group II showed enhanced values than group I but with no significance. Histologic examination showed more enhanced regeneration with group II at both follow-up periods with significant difference.

Conclusion: Application of LLLT enhances peripheral nerve regeneration of sciatic nerve in rat model

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INTRODUCTION

Peripheral nerve injury (PNI) is a common traumatic lesion, according to Seddon^[1], PNI was categorized into three types, neuropraxis, axonotmesis and neurotmesis. The process of nerve healing is a multifactorial one that depends basically on skills and technique of the surgeon, the type of injury and the physiological condition of the patient^[2].

End-to-end neurorrhaphy is the most used and is considered the gold standard technique used in management of PNI that was associated with short operative time and the best results when proper technique is performed by skilled surgeon^[3,4].

Wistar rats sciatic nerve model is a common model for nerve regeneration studies because of close similarity to humans regarding nerve morphology and peripheral nerve regeneration, moreover they are simple to handle at low cost^[5].

About twenty years ago, platelet rich fibrin (PRF) gained the attention of researchers with its ability to enhance nerve regeneration after neurorrhaphy. This is due to its ability to act as a biophysical/biochemical milieu that can deliver growth factors^[6], as platelet-derived growth factor (PDGF), platelet derived endothelial cell growth factor, transforming growth factor- b1 (TGF-b1), epidermal growth factor, and vascular endothelial growth factor. These growth factors promote cellular proliferation and differentiation which is associated with improved nerve regeneration^[7-9]. PRF is a natural autologous fibrin matrix that can be prepared without the addition of heterogenous materials. Moreover, PRF can be easily obtained as a membrane with a prime advantage in nerve repair and regeneration thanks to the conduit-like that works as a growth guidance that prevents growth of collagen fibers to the inside of the repaired nerve with improved regeneration quality^[7, 10-12].

One of the newly recruited modalities that has an enhanced effect of tissue regeneration is the low-level laser therapy (LLLT)^[13]. Since more than three decades, LLLT was subjected to intense investigations to evaluate its effect on healing process especially its anti-oedema with its anti-inflammatory properties, also the ability of increasing nerve mitotic activity that leads to enhancement of nerve regeneration and healing^[13-15]. The ability of LLLT to perform its action is based upon the ability of the soft tissue photoreceptors to absorb the laser beam which in turn activates a biological cascade through increase production of adenosine triphosphate and increased mitochondrial respiration leading to enhancement of growth, healing and nerve regeneration with prevention of nerve degeneration^[14, 16].

Successful neurorrhaphy is not a matter of simple surgical judgement regarding the apparent clinical alignment of the proximal and distal ends of the nerve, or what can be considered a tension free suture, it must be based on sound measures. One of the well-established reliable methods is the use of electromyography measurements to detect nerve conduction as, latency in conduction in milliseconds, amplitude of voltage in millivolts and reaction of degeneration^[17-19]. Immunohistochemical examination of the repaired segment is another sound prove of nerve regeneration that has been used in many studies^[20-22]. S100 protein is encoded by a family of S100 genes and contains two calcium-binding sites and has highly conserved amino acid sequences in vertebrates^[23]. S100 protein has been used to indicate a variety of intracellular and extracellular functions as proliferation of Schwann cells^[24-26].

The aim of this study is to evaluate the effect of low level laser therapy (LLLT) as an adjunct to Platelet Rich Fibrin (PRF) use after end-to-end neurorrhaphy on sciatic nerve regeneration in rat model.

MATERIALS AND METHODS

The study is a double-blind randomized study performed on 20 Albino Wistar rats in the animal house of Misr University for Science and Technology. The selected Albino Wistar rats were 10-12 weeks old and weigh about 250 grams (250-300 g) of both genders. Animals were kept in numbered boxes and were divided into two groups, group I and group II (10 animals per each group). Within each group, animals were divided in two subgroups each contains five animals. Subgroups were named as w1 and w3, where *w* represents "week". In the first subgroup "w1" within group I and II, rats were sacrificed after one week which is the first follow-up period. In the other subgroup w3 within group I and II, rats were sacrificed after three weeks, the second follow-up period. Animal identity was only known to the veterinary staff members, so surgeon, physiotherapy team or the pathologist do not know to which group the animal belongs. Housing, feeding and medication along the whole period of the study were under the supervision and responsibility of the veterinary staff members.

The study conducts two treatment lines; the first line which was represented as "Group I" in which nerve repair was performed using end-to-end neurorrhaphy then PRF was applied as wrapping membrane at the area of neurorrhaphy. Group II represented the second line of treatment in which nerve repair was managed in the same way as group I in addition to using intraoperative LLLT diode laser at the repair area. Two parameters were used to assess sciatic nerve regeneration postoperatively. The first parameter was electromyography measurements that were performed preoperatively, at one week and three weeks follow-up periods respectively. The second parameter was examination of S100 protein stained sections that was performed at one week and three weeks follow-up periods.

Preoperative preparation: Anaesthetization was performed via intraperitoneal injection using

xylazine hydrochloride (20mg/kg) and ketamine (50mg/kg) to prepare the animal for the preoperative electromyography measuring before surgery. Shaving of the lower back and left hind limb of the rat preceded the insertion of a concentric needle in the hamstring muscle. By using of Cadwell Sierra II Wedge EMG system (Kennewick, Washington, USA), stimulation of the sciatic nerve was performed using a stimulator and the effect on hamstring muscle was recorded. Application of electrical stimulation to the sciatic nerve was performed at nerve trunk at its proximal portion at its origin from the spinal segments L4-L6. Two parameters were recorded preoperatively for both groups, the latency in conduction in milliseconds and the amplitude of voltage in millivoltages.

Surgical procedure

Aseptic technique was followed using betadine application at the site of skin incision followed by exposure of vastus lateralis and biceps femoris muscle on the left leg. After retraction, sciatic nerve was identified and then transected with lancet (fig. 1a & b).

Group I

The transected sciatic nerve was sutured under microscope using microsurgical instruments. End-to-end neurorrhaphy was performed using 8-0 pro-line suture, two simple interrupted sutures at sides of the nerve at 0 and 180 degrees. Simple approximation of proximal and distal ends of transected nerve was obtained with no tension (fig. 2a).

After optimum neurorrhaphy was achieved, preparation of PRF was initiated. Delaying of PRF preparation aimed to avoid dryness of the PRF. Veterinarian staff member used orbital plexus of the rat to collect 1 ml in a plain clot activator tube. Centrifuging of collected blood was performed using electric centrifuge (Model: 80-1B Electric Centrifuge 4000rpm/min, Jiangsu, China) at 4000 rpm for 5 minutes.

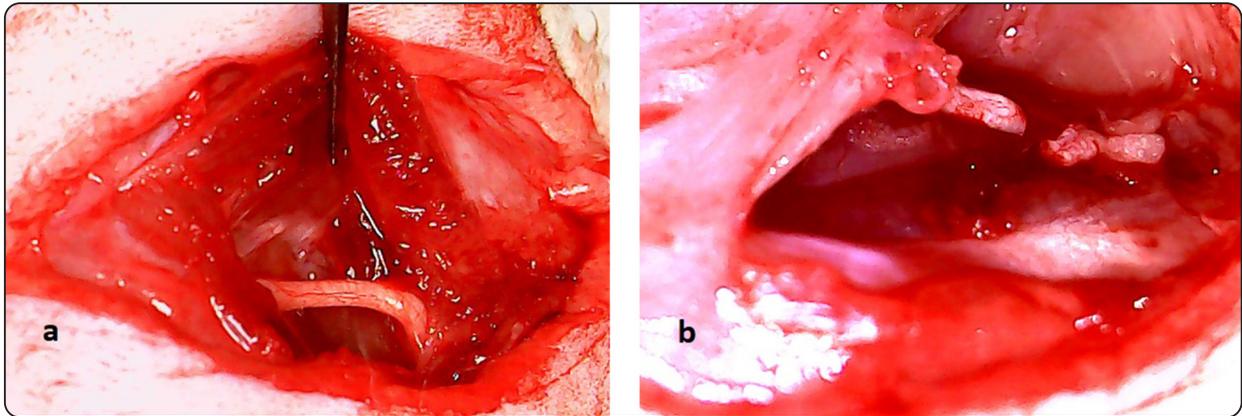


Fig. (1): a: Identified sciatic nerve, b: Transected sciatic nerve

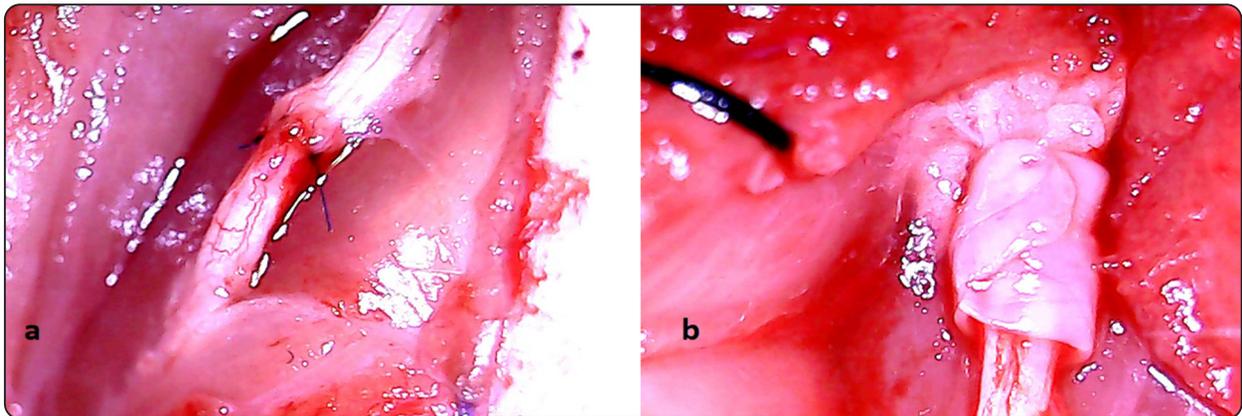


Fig. (2): a: end-to-end neurorrhaphy, b: PRF membrane wrapped around the sutured area.

As a result of centrifuging, blood is separated into three layers: the uppermost layer that contains acellular plasma, the lowermost layer that contains red blood cells (RBCs), and PRF clot in the middle layer. Using forceps and scissors, PRF clot was separated from the RBCs to be pressed between two glass slaps to form a membrane. Under the microscope, the membrane was wrapped around the sutured area and tightened to provide intimate contact between the nerve and the membrane (fig. 2b).

Skin closure was performed with interrupted sutures with 3/0 silk. Postoperative medications were prescribed by the veterinarian staff members.

Group II

The exact procedures were followed as in group

I, then LLLT with diode laser was applied to the site of PRF membrane (fig. 3)

An iLase soft tissue diode laser handpiece was used (iLase™, P/N 5400230 Rev. H, BIOLASE, Inc. USA). We used 940 nm diode laser with output power of 0.5W (watt), continuous mode for 10 seconds in non-contact mode of application. After LLLT application, the skin was closed in an interrupted fashion using 3/0 silk and postoperative medications was prescribed by the veterinarian staff members as performed in group I.

Follow-up was performed at the first week for the subgroups “w1” in both groups I and II and at the third weeks for the subgroups “w3” in group I and II postoperatively. Cadwell Sierra II Wedge EMG sys-

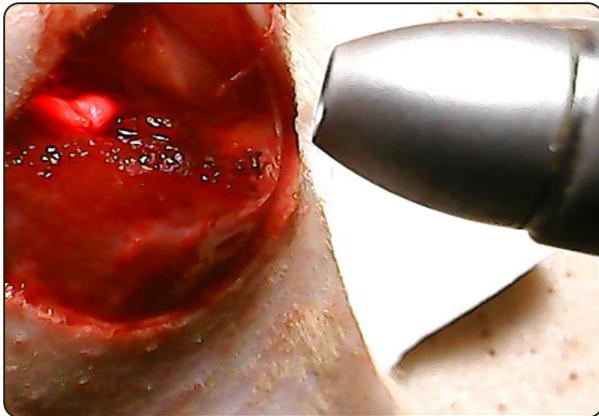


Fig. (3): LLLT with diode laser at the site of PRF membrane.

tem was used to record the electromyograph measurements and response from the left operated leg of the rat. Intraperitoneal injection Ketamine/Xylazine in the same doses and technique used for the preoperative measurements and surgical procedures was used to record measurements at the follow-up periods. Three parameters were recorded at the follow-up periods for both groups, the latency in conduction in milliseconds, the amplitude of voltage in millivolts and the reaction of degeneration of the sciatic nerve which is the resultant of postoperative amplitude divided by the preoperative amplitude ($\text{amplitude}_{\text{post}} / \text{amplitude}_{\text{pre}}$).

At the first and third weeks postoperatively, euthanasia was performed for the corresponding subgroups w1 and w3 in each group respectively using an anaesthetic overdose. Rat specimens were fixed in 10% buffered formalin for 24 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections were cut into 3 microns thick and placed on positively charged (opti-plus) slides to be stained with S100 protein immunostain. Immunostaining was performed according to Ventana benchmark autostainer (USA). The computer image analyser system, Leica Qwin 500 software (Leica Microsystems LTD, CH9435 Meerbrugg Type: DFC295 (12730469), Input: 12v/170 MA, Serial number: 0557060916, Switzerland) was

used to measure the area percent for S100 protein immunoeexpression. Three fields from each slide were chosen in a standard measuring frame using a magnification $\times 40$ by light microscopy transferred to the monitor's screen.

Statistical analysis

The data was analyzed and processed with IBM SPSS Statistics Software. The normality test indicate that the data were normally distributed. The changes occurring in latency and amplitude were calculated by subtracting the post treatment readings from the baseline readings; this difference was used for comparison of the changes between the two intervention groups. The readings analyzed as parametric variables since they were continuous variables. The test for difference between two independent groups, independent sample T test was used. For the changes in the repeated measures at two points, pair sample t Test was used. For testing the difference between groups (more than two groups), ANOVA test was used, furthermore, Tukey Post Hoc Test was used to verify significance between each individual pair. P values ≤ 0.05 were considered significant, while P values ≤ 0.001 were considered highly significant.

RESULTS

Electromyography measurements

Preoperative measurements of the two groups were recorded and analyzed. We found that there was no significant difference between the mean values of latency in conduction, in group I it was (0.93 ± 0.15) while with group II, mean of values were (1.03 ± 0.21) with $P > 0.05$. Similarly, there was no significant difference between the mean values of amplitude of voltage in group I (11.07 ± 1.6) and group II (10.03 ± 1.55) with $P > 0.05$, indicating that the rats were evenly distributed on the two the group with no difference between them at baseline, as seen in table (1).

TABLE (1): Mean and Standard deviation (SD) of the preoperative latency in conduction and amplitude of voltage in group I and group II

	Preoperative latency in conduction (msec)		P value
	Mean (msec)	SD	
Group I	0.93	0.15	0.206
Group II	1.03	0.21	
	Preoperative amplitude of voltage (mV)		P value
	Mean (mV)	SD	
Group I	11.07	1.6	0.159
Group II	10.03	1.55	

P<0.05 is considered significant*

*P<0.001** is considered highly significant*

P>0.05 is non significant

On comparing results within each group at the first week postoperative follow-up period (subgroups w1), results within group I showed that, there was an increase in the average mean of latency in conduction from (0.85 ± 0.09) to (3.82 ± 1.4) high significant. For amplitude of voltage, there was marked reduction in the average mean from (11.7 ± 1.01) to reach (1.62 ± 0.15) with $P < 0.01$. Within group II showed a highly significant increase in the average mean of the latency in conduction from

(0.94 ± 0.07) to be (1.9 ± 0.21) with high significant decrease in the average mean of amplitude of voltage from (10.75 ± 1.17) to (2.66 ± 0.2) with $P < 0.01$ as summarized in table (2).

At the third week postoperative follow-up period (subgroups w3), results within group I demonstrated that, average mean of latency in conduction showed increase from (1.0 ± 0.16) up to (1.8 ± 0.45) and reduction in the average mean of amplitude voltage from (10.37 ± 1.88) to (2.65 ± 0.56) both with high significant value with $P < 0.01$. Similarly, within group II, increased average mean of the latency from (1.12 ± 0.26) up to (1.29 ± 0.19) was observed highly significant. At the same time high significant decrease in the average mean of amplitude from (9.32 ± 1.66) to (3.61 ± 0.58) was observed with $P < 0.01$, table (3) showed the results.

For comparison of results between the two groups regarding latency in conduction and amplitude of voltage, difference was calculated by subtracting the postoperative value from the preoperative value ($\text{value}_{\text{post}} - \text{value}_{\text{pre}}$) then mean of these values was calculated and analyzed at the two follow-up periods one and three weeks respectively.

At one week follow-up period (Subgroups w1), the average *mean of difference* in latency in conduction within group I was (2.97 ± 1.34) which is higher than what was observed in group II was (0.95 ± 0.126) meaning that improvement occurred

TABLE (2): Mean and SD of the latency in conduction and amplitude of voltage at preoperative and one week follow-up period within group I and group II

One week follow-up period (Subgroups w1)		Preoperative Mean \pm SD	One week postoperative Mean \pm SD	P value
Latency in conduction (msec)	Group I	0.85 ± 0.09	3.82 ± 1.4	0.008*
	Group II	0.94 ± 0.07	1.9 ± 0.21	0.000**
Amplitude of voltage (mV)	Group I	11.7 ± 1.01	1.62 ± 0.15	0.000**
	Group II	10.75 ± 1.17	2.66 ± 0.2	0.000**

P<0.05 is considered significant*

*P<0.001** is considered highly significant*

P>0.05 is non significant

TABLE (3): Mean and SD of the latency in conduction and amplitude of voltage at preoperative and three weeks follow-up period within group I and group II

Three weeks follow-up period (Subgroups w3)		Preoperative Mean±SD	Three weeks postoperative Mean±SD	P value
Latency in conduction (msec)	Group I	1.0 ± 0.16	1.8 ± 0.45	0.004*
	Group II	1.12 ± 0.26	1.29 ± 0.19	0.009*
Amplitude of voltage (mV)	Group I	10.37 ± 1.88	2.65 ± 0.56	0.000**
	Group II	9.32 ± 1.66	3.61 ± 0.58	0.000**

P<0.05 is considered significant P<0.001** is considered highly significant*

P>0.05 is non significant

with group II was higher than with group I (*as result is closer to the preoperative value*), with high significance $P < 0.01$ and mean difference between groups equal 2.02 msec in favor of group II. For amplitude of voltage, we found that there was more improvement in group II more than obtained in group I hand as *mean of difference* of amplitude was (-8.09 ± 1.0) in group II while it was (-10.14 ± 0.96) in group I with is statistically significant $P < 0.05$ with mean difference between the two groups equals 2.05 mV in favor of group II. The third electromyography parameter to compare was the reaction of degeneration (RD) which was significantly higher in group II with mean of (24.84 ± 1.54) in comparison to group I which recorded only mean of (13.44 ± 0.96) with a mean difference between groups equals to 11.4 and $P < 0.01$.

At three weeks (Subgroups w3) follow-up period, it was observed that the average mean of difference in latency in conduction within group I was (0.79 ± 0.29) which is higher than what was observed in group II; (0.17 ± 0.08) and the mean difference between the two groups equals 0.62 msec denoting that improvement occurred with group II was greater than that occurred with group I, yet statistically significant difference was not obtained. Again, we found that the average *mean of difference* in amplitude of voltage an obvious improvement with group II to be (-5.71 ± 1.42)

which is of higher value and closer to preoperative values than recorded with group I; (-7.72 ± 1.33) with high mean difference between the two groups equals 2.01 mV, p value was close to 0.050, yet no significance was obtained as P was 0.051.

Finally, on comparing the reaction of degeneration (RD) there was significantly higher value in group II with mean of 38.92 ± 2.72 in comparison to group I which recorded 25.46 ± 1.07 with a mean difference between groups equals to 13.46 and $P < 0.01$.

Finally, based on the previous results values and analytical analysis we observed that there was improvement along the follow-up periods starting from the first week to reach the third week postoperatively with both groups regarding the three electromyography measurements.

Histopathologic examination of S100 protein immunostained sections

Histopathologic examination of S100 protein immunostained sections in group I showed a small bundle of nerve tissue at one week postoperative follow-up period (Subgroup w1) (fig.4 a & b). While at three weeks postoperative follow-up period (Subgroup w3), more regeneration was noticed showing many small bundles of nerve tissue between the two ends of the surgically transected nerve (fig.4 c).

TABLE (4): Mean and SD of difference regarding latency in conduction, amplitude of voltage and reaction of degeneration (RD) between the two groups at one- and three-weeks follow-up periods.

One week (Subgroups w1)	Group I	Group II	Mean of Difference between groups	P value
	Mean of difference \pm SD	Mean of difference \pm SD		
Latency	2.97 \pm 1.34	0.95 \pm .176	2.02	0.002*
Amplitude	-10.14 \pm 0.96	-8.09 \pm 1.0	2.05	0.046*
RD ^s	13.44 \pm 0.96	24.84 \pm 1.54	11.4	0.000**
Three weeks (Subgroups w3)	Group I	Group II	Mean of Difference between groups	P value
	Mean \pm SD	Mean \pm SD		
Latency	0.79 \pm .29	0.17 \pm 0.079	0.62	0.508
Amplitude	-7.72 \pm 1.33	-5.71 \pm 1.14	2.01	0.051
RD ^s	25.46 \pm 1.07	38.92 \pm 2.72	13.46	0.000**

P<0.05* is considered significant *P*<0.001** is considered highly significant

P>0.05 is non significant

RD^s: reaction of degeneration

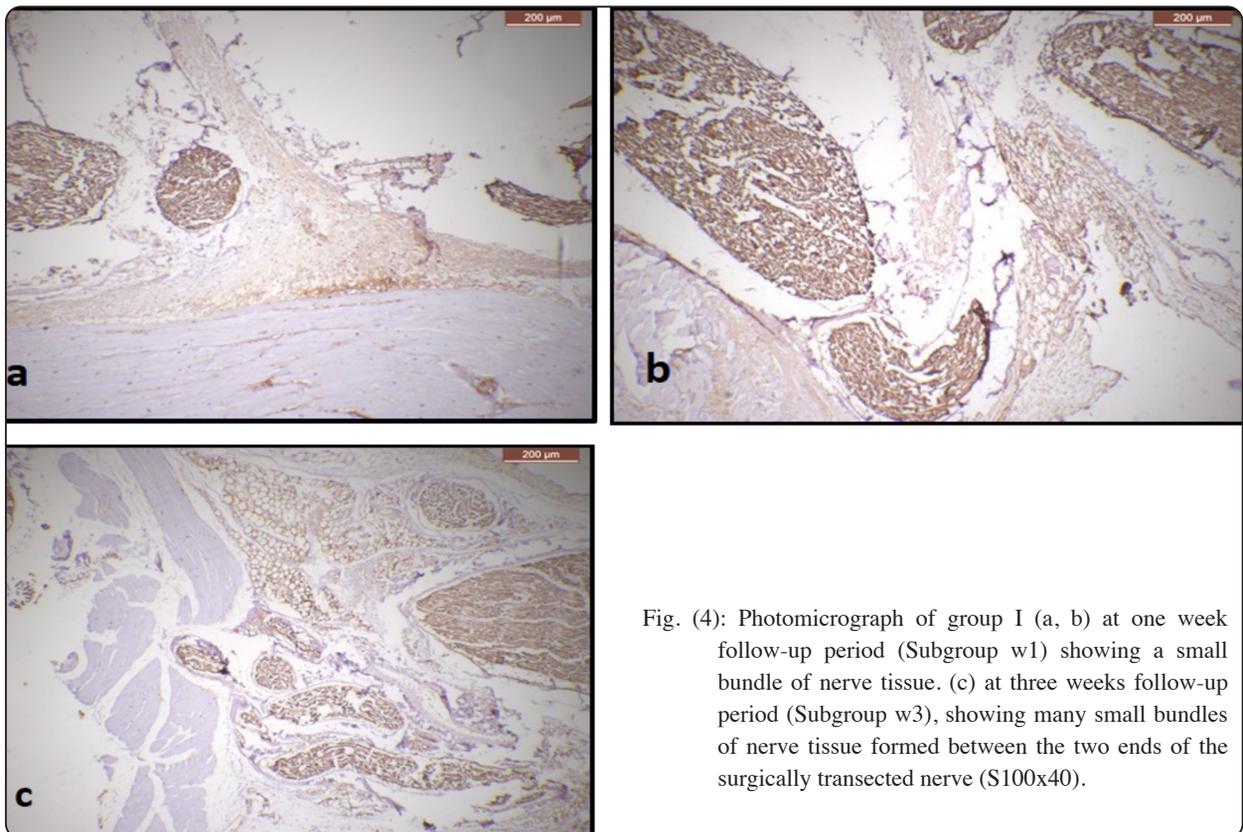


Fig. (4): Photomicrograph of group I (a, b) at one week follow-up period (Subgroup w1) showing a small bundle of nerve tissue. (c) at three weeks follow-up period (Subgroup w3), showing many small bundles of nerve tissue formed between the two ends of the surgically transected nerve (S100x40).

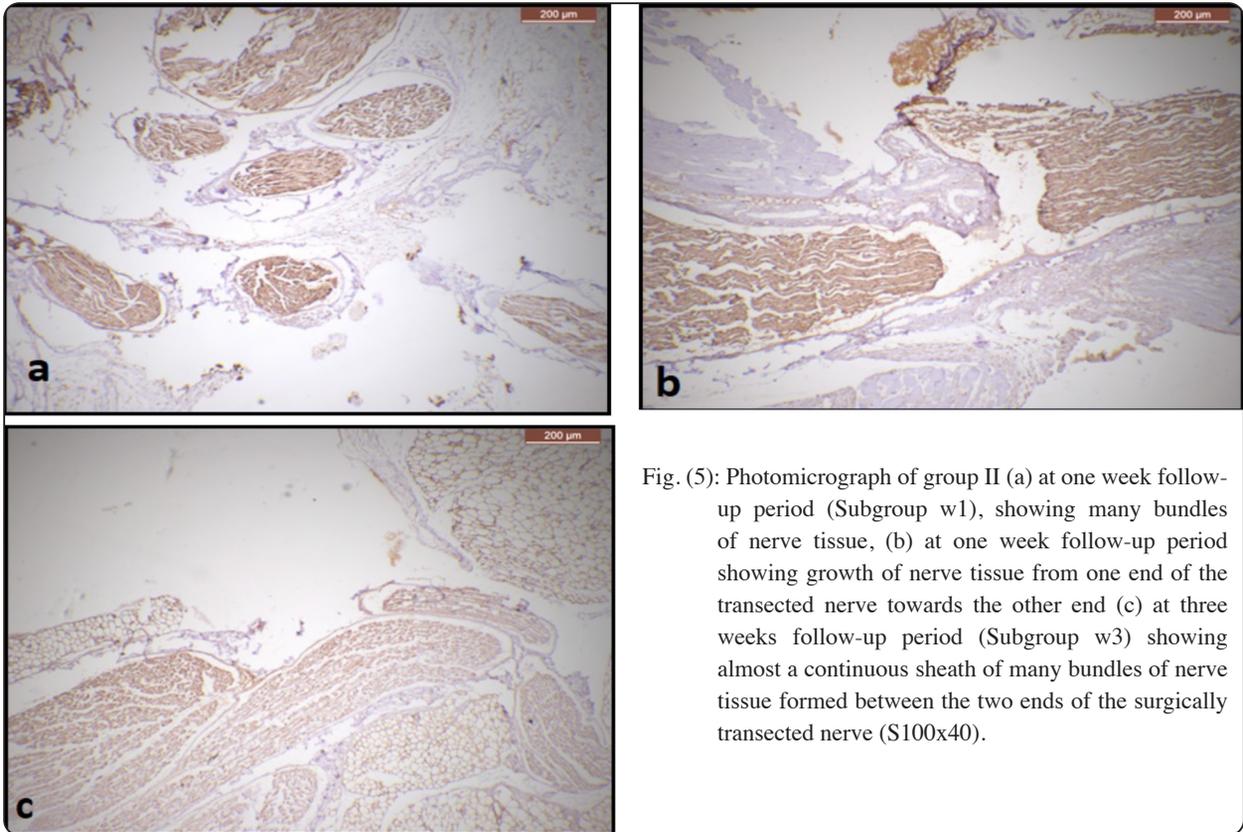


Fig. (5): Photomicrograph of group II (a) at one week follow-up period (Subgroup w1), showing many bundles of nerve tissue, (b) at one week follow-up period showing growth of nerve tissue from one end of the transected nerve towards the other end (c) at three weeks follow-up period (Subgroup w3) showing almost a continuous sheath of many bundles of nerve tissue formed between the two ends of the surgically transected nerve (S100x40).

Regarding group II, histopathologic examination showed many larger bundles of nerve tissue after one week of follow-up (Subgroup w1) as seen in (fig. 5a). One rat showed a small area of nerve tissue growing from one end of the cut nerve at that period as shown in (fig. 5b). At three weeks postoperative follow-up period (Subgroup w3), we could detect the best regeneration that appeared as almost a continuous sheath of many bundles of nerve tissue formed between the two ends of the surgically transected nerve (fig. 5c).

Statistical analysis of area percent values for S100 protein immunorexpression

Comparing area percent values for S100 protein immunorexpression revealed that within group II, the percent values was higher than that within group I. The highest values were seen in group II at three weeks (Subgroup w3) then at one week (Subgroup w1) follow-up periods respectively to be followed

by values obtained from group I at three weeks (Subgroup w3) follow-up period, with the lowest value was detected within group I at one week (Subgroup w1) follow-up period. The difference between the previous four groups was highly significant ($P < 0.001^{**}$) as shown in table (5).

Pairwise comparison between each two groups revealed the effect of time on enhancing nerve regeneration where a significant difference between rats in group II at one- and three- weeks follow-up periods respectively with ($P < 0.05^{*}$). Similarly, there was a significant difference between rats within group I at one- and three- weeks follow-up periods respectively with ($P < 0.05^{*}$). The type of treatment also affected regeneration as there was a highly significant difference between values in group I and II at the two follow-up periods with ($P < 0.001^{**}$). Moreover, there was no significant difference between rats in group II at one week follow-up period and those in group I at three weeks follow-up period ($P > 0.05$) as seen in table (6).

TABLE (5): Area percent values for S100 protein immunoeexpression in the studied groups (ANOVA test)

	Group I one week (Subgroup w1)	Group I three weeks (Subgroup w3)	Group II one week (Subgroup w1)	Group II three weeks (Subgroup w3)	P value
Mean ±SD	9.28±1.53	14.17±1.55	17.01± 1.95	23.01±3.01	P<0.001**

P<0.05 is considered significant* *P<0.001** is considered highly significant*

P>0.05 is non significant

TABLE (6): Pairwise comparison of area percent values for S100 protein immunoeexpression in the studied groups (Post Hoc Tukey test)

	Group I one week	Group I three weeks	Group II one week	Group II three weeks
Group I one week (Subgroup w1)	-	P<0.05*	P<0.001**	P<0.001**
Group I three weeks (Subgroup w3)	P<0.05*	-	P>0.05	P<0.001**
Group II one week (Subgroup w1)	P<0.001**	P>0.05	-	P<0.05*
Group II three weeks (Subgroup w3)	P<0.001**	P<0.001**	P<0.05*	-

P<0.05 is considered significant*

*P<0.001** is considered highly significant*

P>0.05 is non significant

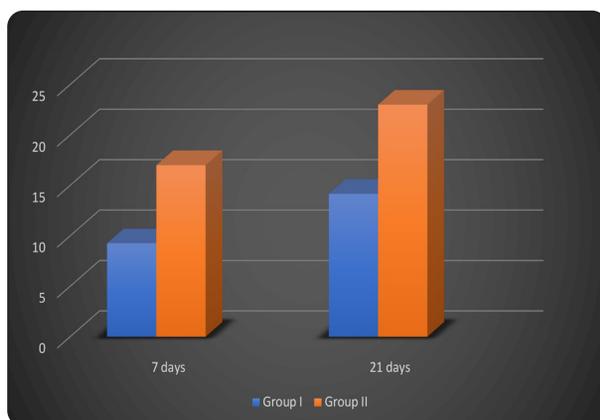


Fig. (6): Bar chart showing area percent values for S100 protein immunoeexpression in the studied groups.

DISCUSSION

Peripheral nerve injuries are common injuries that are associated with sensory or motor function loss. Despite the advances in surgical repair techniques and addition of modern adjuncts, yet regeneration is suboptimal. This may be due to slow axonal regeneration rate and associated degenerative processes even after optimum surgical repair^[27].

Wistar rats used in this study is a common animal model in nerve regeneration studies due to proximity of the structural morphology to humans with a very fast healing and regeneration process that starts within few^[15,28].

PRF attracted the attention of researchers and is defined as a next-generation platelet concentrates that has a powerful regenerative efficiency. This is due to the potentiality of the produced growth factors which enhances the process of nerve regeneration. Yet, few research concerning nerve regeneration were performed on it^[29, 30].

The idea of using PRF as a conduit like membrane is a promising method to enhance nerve regeneration as mentioned by Kokkalas^[31]. In this study we aimed to achieve the advantages provided by the presence of this conduit as a guidance to nerve fibers during growth, benefit from the presence of growth factors and prevent fibrosis and adhesion which is coinciding with different studies^[8, 10, 31].

Application of LLLT has shown positive effects and enhancement of nerve regeneration^[13]. In order to study this effect, we relied on both electrophysiological measurements and immunohistochemical analysis of S100 protein immunostain of nerve tissue which was also recommended by authors^[16, 32].

In our study we used 940 nm m diode laser with output power of 0.5W (watt), continuous mode for 10 seconds only in non-contact mode of application. According to these parameters, the energy density *-which is an important parameter*^[33], equals 5 Joules/cm²^[33, 34]. This was coinciding with many studies concerned with biostimulation and nerve regeneration that showed optimum results at the power off 0.5W and should not exceed it^[35-37].

Few studies have compared the effect of PRF and LLLT on nerve regeneration to each other. In this study, this comparison was done and follow-up was performed up to three weeks which is an adequate period for nerve tissue to be nearly normal in rats due to the quick spontaneous regeneration process, as stated by Endo^[38] and Raso^[39].

Based on our electrophysiological measurements, we found that group II in which LLLT was applied, there was a significant improvement in

measurements at the first follow-up period. Despite we could not achieve a significant difference (with amplitude of voltage (P=0.051) at the three weeks follow-up period, yet obtained results showed noticeable improvement with group II over that seen in group I. Recorded enhancement in regeneration is correlated with what was found by Rochkind^[40], Marcolino^[5] and others^[13, 41, 42] who observed the improvement with LLLT application but with different electrophysiologic measurements.

Again results of the first and three weeks follow-up periods in this study coincide with that study conducted by Shen^[43], who showed a significant improvement in the electrophysiological measurements in the group with LLLT within his selected follow-up periods.

Regarding the statistical analysis of the results on comparing between the two groups, the observed improved measurements at the three weeks follow-up period for group II over group I did not reach statistical significance. This was observed by other authors^[15, 44] who could not find a significant difference regarding using of LLLT.

In this study, area percent values for S100 protein immunoexpression revealed that nerve regeneration in rats in group II with LLLT application was highly significantly greater than that within group I at both follow-up periods denoting that LLLT is more effective on enhancement of nerve regeneration than PRF membrane at both time intervals. This result was confirmed by histopathologic examination of tissue sections, as group II showed many larger bundles of nerve tissue after one and three weeks.

The best regeneration results were seen at the three weeks follow-up period in group II showing almost a continuous sheath of many bundles of nerve tissue formed between the two ends of the surgically transected nerve. This is in accordance with Sene^[15] and Rochkind^[40] who proved in their studies that LLLT was able to promote nerve regeneration and intense axonal growth.

In a matching study conducted by Serafim^[36], he found that application of diode laser led to accelerated organized nerve regeneration. Moreover, other related studies^[38, 45-48] found that application of LLLT is associated with improvement in nerve regeneration with reduction in fibrosis and increased number of nerve fibers at the site of injury. In the study by Shen^[43], histological and immunohistochemical analysis showed that application of LLLT leads to formation of more compact and complete form of regeneration with higher expression of S100 protein in comparison to the group not managed with LLLT which is coinciding with our results.

On the other hand, in a study conducted by Chen^[49], results were surprising to the author according to his words, where nerve regeneration and the number of healed nerve fibers were reduced compared to the non-laser irradiated group. Not only the histological results but also the electrophysiological measurements were better in the control group rather than the LLLT treated group. The same was reported with Bagis^[50] and Wu^[51] who showed no positive effects after use of LLLT on nerve injuries. This is in addition to Bayram^[10] who did not find any histomorphometric or functional improvement in peripheral nerve regeneration through using of local PRF membrane.

According to this study, we found that regeneration in group II at one week follow-up period was greater than those in group I at three weeks follow-up period, but the difference was not statistically significant. This denotes that the effect of PRF is enhanced by time as its effect after 21 days was very close to the effect of LLLT after 7 days. Histopathologic examination of tissue sections revealed many bundles of nerve tissue in both groups, that appeared larger in diameter in rats in group II. Another observation we noticed that there is an improvement in electromyography measurements results with group II on comparison to those with group I at three weeks follow-up period but with no significant difference between the two groups. We

may attribute this to the rapid effect of the LLLT that appears at one week follow-up period with the need of repeated application at intervals to achieve the continuous effective enhancement in regeneration. While in group I, improvement continued as noticed by Mourad^[52] but at a lower level with weaker effect on regeneration as the effect of PRF is time related.

Finally, on comparing the results based on follow-up intervals, we found that improvement in electromyography measurements and area percent values for S100 protein immunoeexpression are more obvious and higher at three weeks follow-up period than that at one week follow-up. This may be attributed to the effect of time on enhancing nerve regeneration in both groups. This is in accordance with Gordon^[53] who observed enhanced normal regeneration by time as well as after electric stimulation after 14 days compared to 4 days. Barbois^[41] found a promoted sciatic functional index after LLLT application over the evaluated periods (7, 14 and 21 days) in rat models.

LLLT improves the healing and regeneration of the nerve tissue especially after improvement of the quality of laser technology based on previously mentioned studies as well as results of this study. Nevertheless, there is no consensus about the optimal protocol for LLLT in case of PNI with no standardization regarding LLLT parameters i.e., wavelength, period of application, energy density, contact or non-contact, continuous or pulsating and other crucial items^[5, 33, 49, 54]. This lack of standardization makes comparison of methodologies, results and explanations difficult^[41] leading to the need of further studies to obtain an efficient safe standard protocol for LLLT^[46].

CONCLUSION

We can conclude that application of LLLT enhances peripheral nerve regeneration of sciatic nerve in rat model as seen in our study, where group II showed improved results than group I. LLLT can be used as an adjunct to PRF after end-to-end

neurorrhaphy as it improves electrophysiological and histological results. Further studies are needed to develop a standard protocol for LLLT application in management of nerve injury.

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