THE EFFECT OF COMBINED RED AND INFRARED LASERS ON HISTOPATHOLOGY COLLAGEN FORMATION IN DIABETIC FOOT ULCER: A RANDOMIZED CONTROLLED TRIAL

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Article History: Received: 05.06.2023 **Revised:** 10.07.2023 **Accepted:** 12.07.2023

Abstract

The aim was to investigate the effects of combined red and infrared lasers on histopathology collagen formation in diabetic foot ulcer (DFU). Forty-five DFU patients both sexes, age range of 18–60 years were divided randomly into three equal groups. Laser group (A) received laser therapy in sequential beam mode, while (B) received laser therapy in separate beam mode. The control group only received conventional wound management, which was also provided to both the laser and the exercise groups. All laser groups were offered 12 sessions over 8 week inform of 2 sessions of laser therapy/ week in 1st month and one session/ week in the 2nd month. Wound surface area (WSA), the percentage of complete healing and collagen formation percent were evaluated pre- and post-treatment. Statistical significance was set at $P < 0.05$. There was a significant decrease within all the groups' comparison, and there was a significant decrease in WSA of group A compared with that of group B and group C post treatment ($p < 0.001$). There was a significant decrease in WSA of group B compared with group C post treatment ($p < 0.01$). The percentage of complete healing in group A, B and C was 60%, 13.3% and 0% respectively. There was a significant increase in the percentage of complete healing in group A compared with group B and C ($p < 0.001$), which indicate the increase of collagen formation due to the healing process of DFU and illustrated by histopathology pictures. Sequential mode in group (A) and with separate mode beam group (B) were found to be the most effective methods for DFU healing by decrease wound surface area, with sequential mode beam group (A) offering superior performance in the percentage of complete healing and collagen formation percent in the management of DFU.

Keywords

Diabetic foot ulcer, infrared laser, laser combined effect, collagen formation

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INTRODUCTION

The International Diabetes Federation (IDF) classified Egypt among the top 10 countries in the world with the highest prevalence of diabetes, where about 9 million adults between 20 and 79 years of age were living with DM in 2019. The number of patients with DM in Egypt has increased rapidly from about 4.5 million in 2007 to 7.5 million in 2013, and is expected to increase to 13.1 million by 2035. **(1)**

Diabetic foot ulcers (DFUs) markedly contribute to morbidity of diabetic patients; they prolong hospital stays and account for nearly 20% of all diabetes-related hospitalizations. **(2)**

Diabetes is a vascular disease. Peripheral arterial disease which is a macrovascular complication is a known cause of diabetic foot. The endothelial dysfunction that occurs in diabetes leads to reduced production of nitrous oxide (NO) which is a dependable vasodilator and an inhibitor of smooth muscle proliferation. **(3)** hyperglycemia promotes increase levels of fibrinogen and plasminogen activator inhibitor which impairs fibrinolysis. **(4)**

DFU begins as acute wounds, but the healing process is interrupted and stalled in all phases of wound healing due to hyperglycemia, chronic inflammation, impaired angiogenesis, microvascular and macrovascular dysfunction including peripheral neuropathy and peripheral arterial disease that result in a hypoxic wound environment. **(5, 6)**

Laser therapy reduced inflammation and significantly increased collagen deposition, showing that alterations in the dynamics of cicatricial fibrogenesis, acceleration in the replacement of collagen I molecules by III and

promoted early collagen remodeling. **(7)** laser therapy progress in the healing of diabetic foot, increase in tissue perfusion of the lesion, which favors greater blood supply and nutrition of the injured area, besides the stimulation of neovascularization and cell proliferation. **(8)**

Difference between Low Level Laser Therapy and High intensity Laser Therapy an output power of less than 0.5 Watts is classed as Low Level Laser Therapy LLLT (class III in the USA) whereas lasers with an output powers greater than 500mW or 0.5 Watts are termed High Power Laser Therapy HILT (Class IV lasers in the USA). HPLT creates heat on the surface of the skin due to their higher power density (irradiance). LLLT is often referred to as "Cold Lasers" since they do not create a heating sensation during treatment. **(9)**

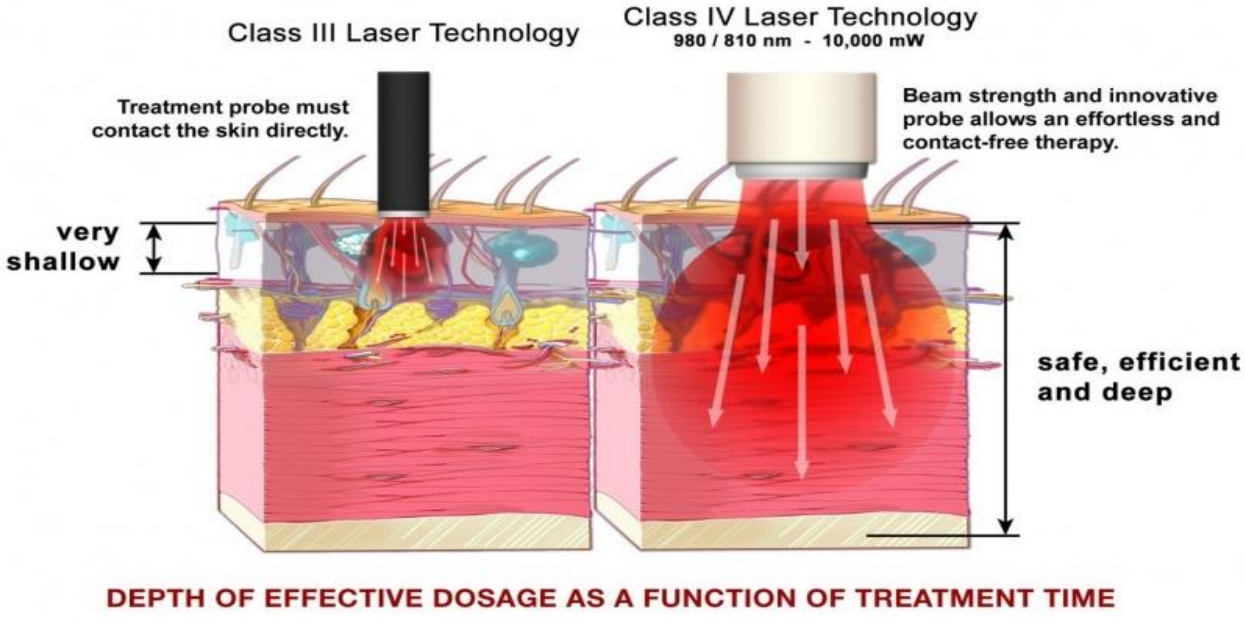


Figure (1): Difference between Low Level Laser Therapy and High Power Laser Therapy

HILT can penetrate skin, subcutaneous fats, muscle, tendons and bones according to the location treatment, there are 3 key effects of the therapeutic HILT; photo acoustic, bio modulation and thermic according to the wavelength used as 1064 nm which may reach 10 cm deep into the tissues. Oxygen processed by cell in mitochondria by cascade of respiratory enzymes and delivered ATP synthase that synthesize cell energy fuel ATP. Faster exchange of oxygen and metabolites occur due to laser radiation inside mitochondria which increase ATP production so fasten RNA and DNA synthesize which increase healing process and edema reduction in the treatment area. Thermic effect due to increase temperature that cause vasodilation, as consequence blood perfusion. **(10)**

Photbiomodulation therapy (PBMT) using red and near-infrared (NIR) wavelengths is being considered as a promising technique for speeding up the rate of diabetic wound healing, eradication of pain and reduction of inflammation through the alteration of diverse cellular and molecular processes, activate effective biological responses especially activating mitochondrial proteins inducing NF-κB release, ATP respiration, ROS and fibroblast differentiation. **(11)**

high intensity laser therapy HILT can stimulate joints more deeply and treat wider area than low level laser therapy LLLT **(12)** Although low-power laser (Class III) therapy has been employed for more than two decades' high-power laser (Class IV) therapy has been implemented in the clinical setting in the last years due to its greater depth of penetration and the possibility of delivering higher doses with lower exposure times. **(13)**

High-intensity laser therapy (HILT). This modality is convenient, noninvasive, and painless, stimulates efficiently deepen tissue, and provides anti-inflammation, analgesics, demonstrates similar influences of the common laser while with more focused and powerful photoenergy effects, with a more sufficient concentration of endogenous chromophores throughout the treatment program depending on the wavelength. **(14)**

Aim

studies regarding the effectiveness for HILT have been limited. Thus, it is necessary to investigate the effectiveness of HILT in DFU patients. The aim of the present study was to clinical trial regarding the effectiveness of HILT in treatment of DFU.

Patients and methods

All of the patients signed a written declaration of informed consent relating to participation and the

publication of results. The protocol for this study was approved by the local research ethics committee (NILES-EC-CU 23/3/3) and registered with clinical registration trial (NCT05739214). The study was carried from May to December 2022. This study was carried out on diabetic foot ulcers patients in the national institute of laser sciences, Giza, Egypt. The study was conducted with 45 patients who were randomized into sequential mode of laser, separate mode of laser and control groups; the three groups were of equal size (n = 15 in each group). Measurements were obtained before and after the intervention period, which was 8 weeks. All of the patients were able to receive the same conventional therapy in the form of dressing with irrigated by saline, and excision of slough or dead tissue if needed. The inclusion criteria were as follows: aged between 18 and 60 years; both gender; Diabetic patients type II; Ulcer lasting longer than two months; diabetic foot ulcer (DFU) grade 1 (Superficial diabetic ulcer, ulcer limited to the dermis, not extending to the subcutis) or grade 2 (Ulcer of the skin extending through the subcutis with exposed tendon or bone and without osteomyelitis or abscess formation) according to the Wagner classification **(15)**. The Exclusion criteria were as follows: Patients with fixed ankle deformity as Charcot foot or stiffness; Patients with any type of osteomyelitis associated with DFU; the presence of active infection requiring hospitalization, gangrene, systemic diseases such as collagen-vascular diseases, renal failure, evidence of ischemia; BMI < 30 kg/m2 as Obesity can cause poor perfusion due to vascular insufficiencies; an altered population of immune mediators may lengthen the inflammatory process & decrease oxygenation of subcutaneous adipose tissue which is liable to be infected **(16).**

Power of the study

Sample size calculation was performed using G*POWER statistical software (version 3.1.9.2; Franz Faul, Universitat Kiel, Germany) and revealed that the sample size required for this study was N=45. Calculation is made with α=0.05, β=0.2, effect size = 0.48.

Assessment of eligibility

Fifty-six both gender patients were recruited to participate in the study. During assessment for eligibility, six patients were excluded because two of them develop vascular disease and the other four refused to participate in the study and the other declined to participate (Fig. 2). Allocation was concealed using sealed opaque envelopes.

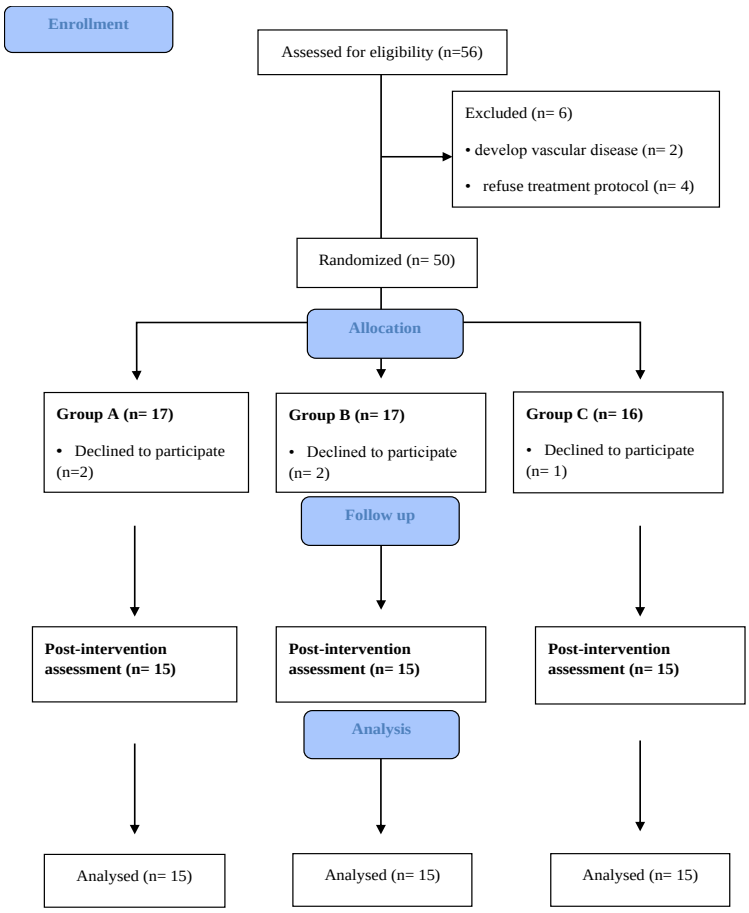


Figure 2. CONSORT flow chart for the numbers of patients included in the three groups.

Outcome measures

Wound surface area (WSA):

Ulcer surface area was calculated by A metric ruler was used to measure the length of the ulcer. The measurement was from open ulcer edge to open ulcer edge at the distant point. Area (cm^2) = length (cm) x width (cm). Ulcer size was measured at the beginning of the study, after 4 weeks, and after 8 weeks at the end of the study. Percent of cases with complete ulcer closure in each group

The percentage of complete healing

The percentage of complete healing calculated to compare the number of ceases with complete ulcer closure within each group in two months of treatment protocol

Collagen formation:

Collagen formation that indicated wound healing done by histopathology using tri masson Chrome stain at the beginning and the end of study for whom their ulcer still open (partial healing) after 8 weeks of the treatment in all the groups

Intervention

patients in group A and B received 2 sessions of laser therapy/ week in 1st month and one session/ week in the 2nd month aiming for complete wound closure, patients received & infrared laser therapy plus conventional wound care treatment: Use red (650 nm accelerates surface healing and tissue regeneration) and infrared lasers therapy (980 nm for wound decontamination, improve circulation, and lymphatic drainage, 915 nm enhances O2 delivery, 810 nm increases ATP production) **(17).** Parameters are described in table 1.

Laser device used

Summus, platinum P4, class laser therapy Diode laser 4 wavelengths (980, 915, 810, and 650), peak power up to 24 watts & frequency up to 20 kHz, Country of made in the USA. Summus device is the 1st laser device that delivers a combination of 4 wavelengths in the same beam at six phases in the same session with auto parameter calculation for synchronization of 4 wavelengths according to wound size to fulfill all laser tissue interaction for proper wound healing.

Group (A) sequential mode of laser: received laser therapy with 4 wavelengths of red and infrared lasers in a synchronized mode in one beam calculated automatically by the device software plus received conventional wound care treatment informs of (debridement, irrigation with saline, daily dressing) & off-loading if needed .

Group (B) separate mode of laser : received laser therapy with 4 wavelengths of red and infrared lasers in separate modes plus received conventional wound care treatment informs of (debridement, irrigation with saline, daily dressing) & off-loading if needed .

Group (C) control: received conventional wound care treatment informs of (debridement, irrigation with saline, daily dressing) & off-loading if needed.

Table 1: parameters of laser therapy

Parameters	Red laser	Infrared laser		
Wave length	650 nm	980 nm	915 nm	810 nm
Power	200 mW	1.5 W	1.5 W	1.5 W
Fluence	10 J/ cm2	25 J/ cm2	25 J/ cm2	25 J/ cm2
Hand piece radius	5 cm	5 cm	5 cm	5 cm
Spot size (area)	7.5 cm2	7.5 cm2	7.5 cm2	7.5 cm2
Time of irradiation	120 s	120 s	120 s	120 s
Application	Non-contact technique as it is an open wound to prevent infection with a distance between lenses & wound not exceeding 1 inch.			
Light dose LD	LD= $\frac{\text{power (watt)}}{\text{area (cm2)}}$ x time (seconds)			

STATISTICAL ANALYSIS

ANOVA test was conducted for comparison of subject characteristics between groups. Chi-squared test was conducted for comparison of sex distribution between groups. As the data was not normally distributed, Kruskal-Wallis test was conducted for comparison of the median values of wound surface area between groups and was followed by Mann–Whitney test to identify the significance difference between each two groups. Wilcoxon Signed Ranks Test was conducted for comparison of pre and post treatment in each group. Chi-squared test was conducted for comparison of percentage of healing between groups. The level of significance for all statistical tests was set at $p < 0.05$. All statistical analysis was conducted through the statistical package for social studies (SPSS) version 25 for windows (IBM SPSS, Chicago, IL, USA).

RESULTS

- Subject characteristics:

Table (2) showed the subject characteristics of group A, B and C. There was no significant difference between groups in age, BMI and sex distribution ($p > 0.05$). The percentage of subject characteristics distribution among the groups is illustrated in figures (3, 4, 5).

Table 2. Basic characteristics of participants.

	Group A	Group B	Group C	F-value	p-value
	mean ± SD	mean ± SD	mean ± SD		
Age (years)	49.66 ± 7.54	48.06 ± 6.61	48.46 ± 7.18	0.21	0.81
BMI (kg/m²)	24.07 ± 3.17	24.26 ± 4.07	23.93 ± 3.17	0.03	0.96
Sex, n(%)					
Females	7 (47%)	7 (47%)	9 (60%)	$\chi^2 = 0.71$	0.7
Males	8 (53%)	8 (53%)	6 (40%)		

SD, standard deviation; χ^2, Chi-squared value; p-value, level of significance

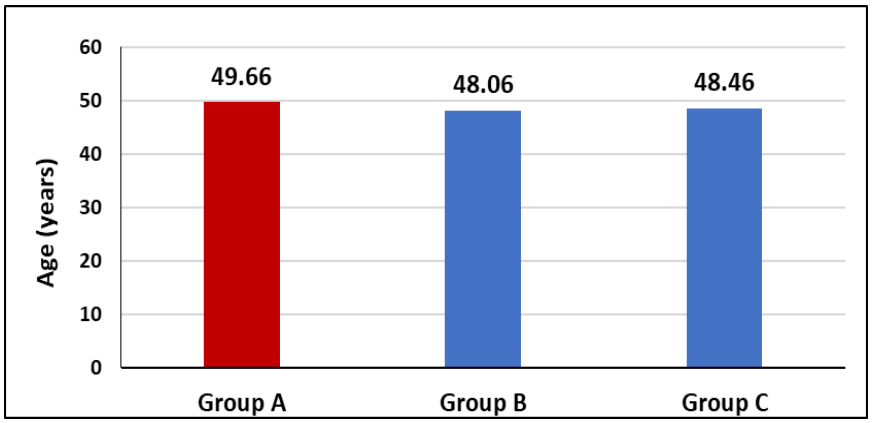


Figure 3. Mean age of group A, B and C.

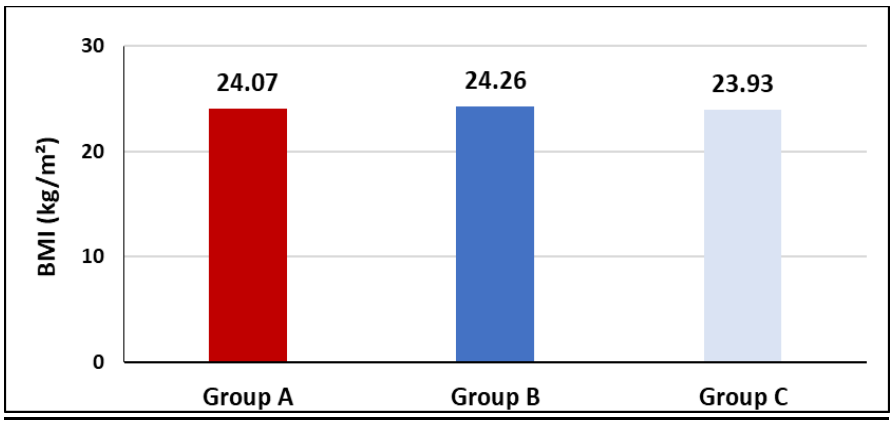


Figure 4. Mean BMI of group A, B and C.

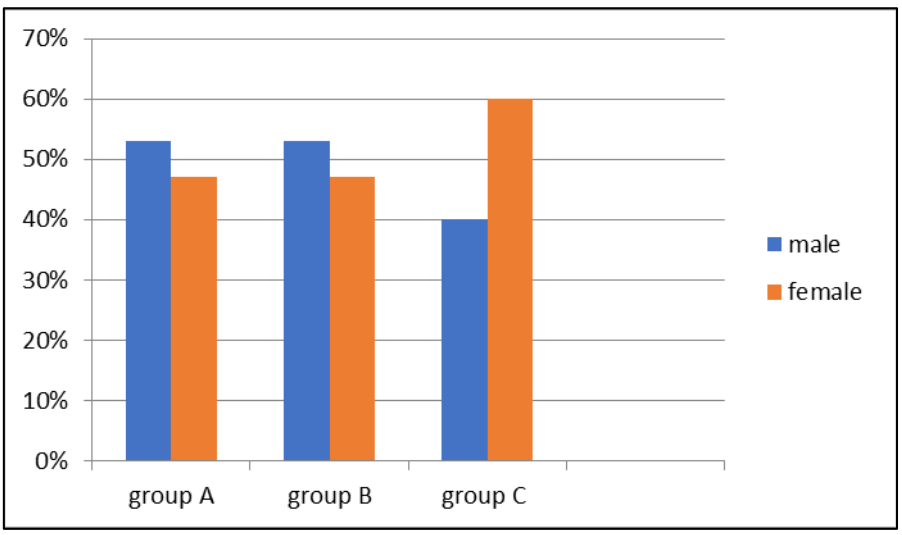


Figure 5. Sex distribution of group A, B and C.

Effect of treatment on wound surface area:

Within group comparison

There was a significant decrease in wound surface area of group A ($p < 0.001$), group B ($p < 0.001$) and group C ($p < 0.01$) post treatment compared with that pretreatment. (Table 3)

Between group comparison

There was a significant decrease in wound surface area of group A compared with that of group B and group C post treatment ($p < 0.001$). There was a significant decrease in wound surface area of group B compared with group C post treatment ($p < 0.01$). (Table 3). The figure (6) shows Median values of wound surface area pre and post treatment of group A, B and C

The percentage of complete healing in group A, B and C was 60%, 13.3% and 0% respectively. There was a significant increase in the percentage of complete healing in group A compared with group B and C ($p < 0.001$). (Table 4) and figure (7).

Table 3. Median values of wound surface area pre and post treatment of group A, B and C:

	Group A	Group B	Group C	p-value		
	Median (IQR)	Median (IQR)	Median (IQR)	A vs B	A vs C	B vs C
Wound surface area						
Pre treatment	21 (22.5, 12.6)	17.5 (21.5, 10.8)	13.25 (21, 7)	0.42	0.14	0.32
Post treatment	0 (2.4, 0)	4 (7, 2.5)	11.4 (15.2, 6.3)	0.001	0.001	0.002
Z- value	-3.41	-3.41	-2.35			
p-value	**p = 0.001**	**p = 0.001**	**p = 0.01**			

IQR, Interquartile range; U- value: Mann-Whitney test value, - value: Wilcoxon signed ranks test value; p-value, Level of significance

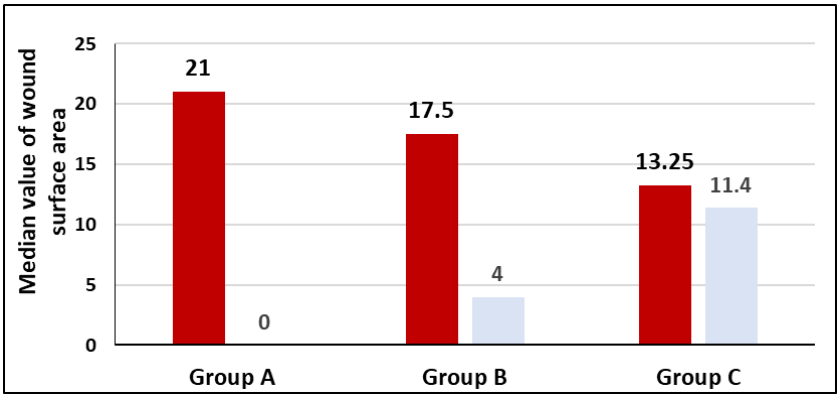


Figure 6. Median values of wound surface area pre and post treatment of group A, B and C

Table 4. Comparison of percentage of healing between group A, B and C.

	Group A	Group B	Group C	χ^2	p-value
	N (%)	N (%)	N (%)		
Complete healing	9 (60%)	2 (13.3%)	0 (0%)		
Partial healing	6 (40%)	13 (86.7%)	10 (66.7%)	24.73	0.001
Non healed	0 (0%)	0 (0%)	5 (33%)		

χ^2, Chi-squared value; p-value, level of significance

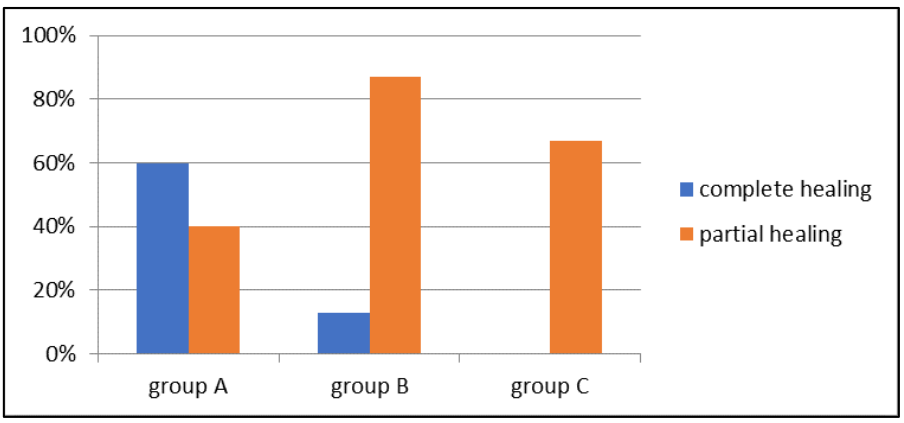
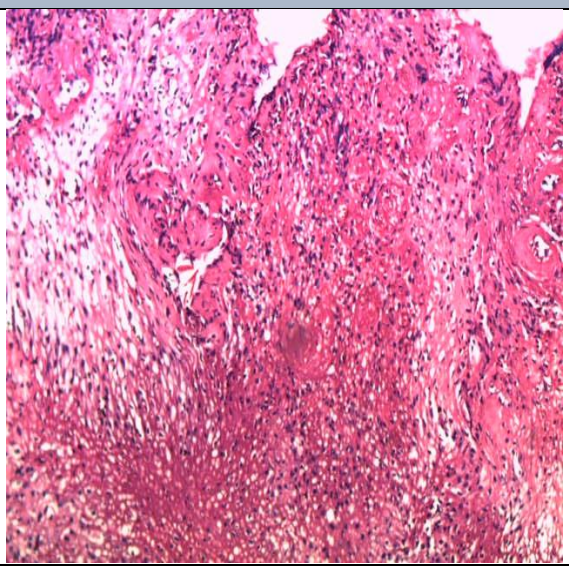
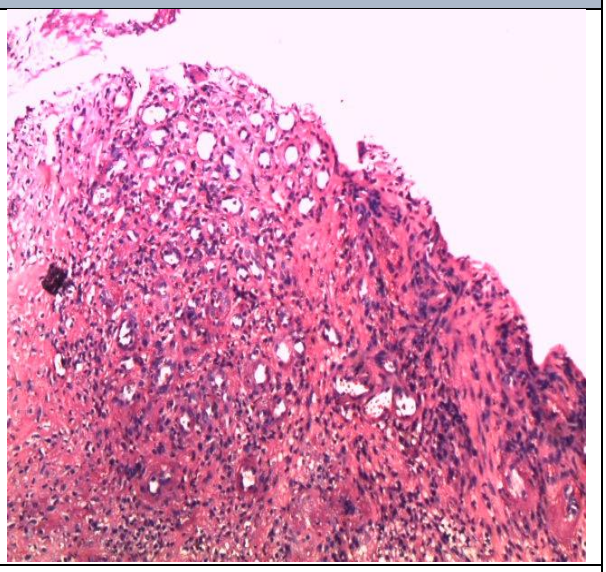
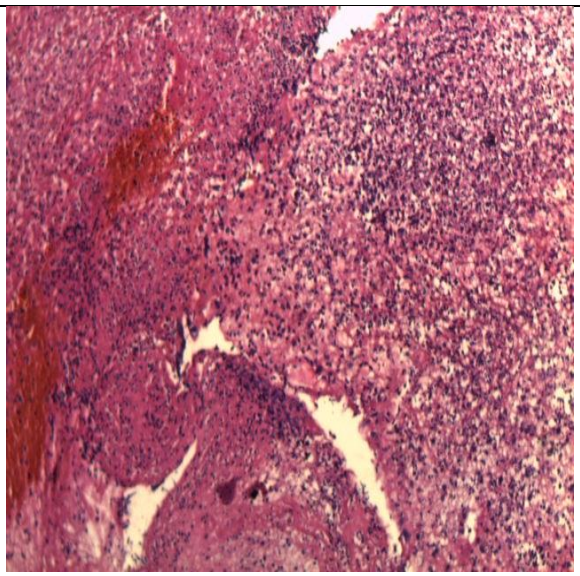
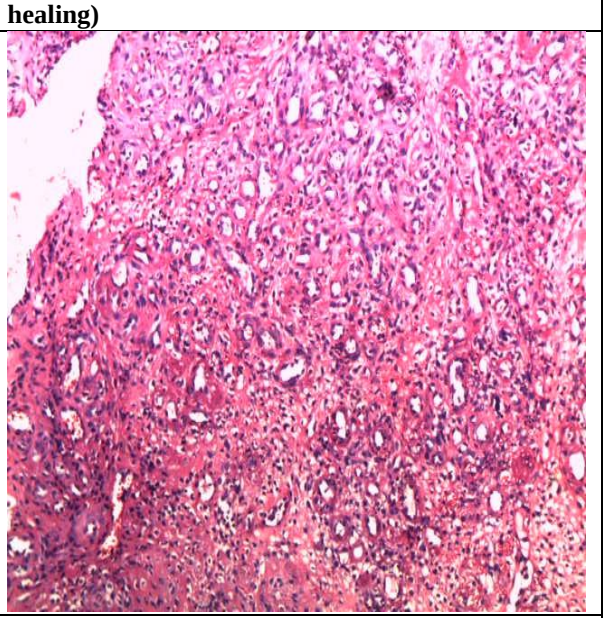
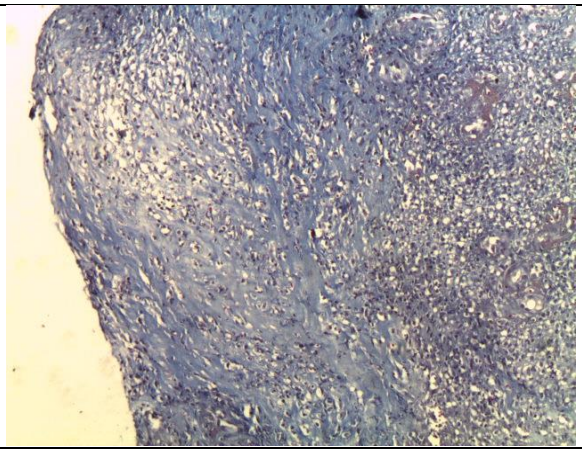
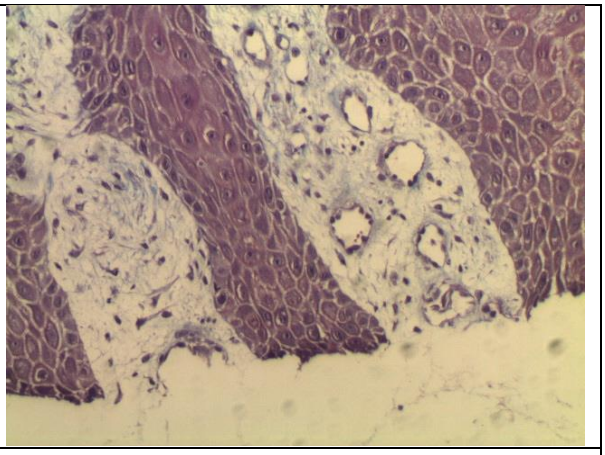
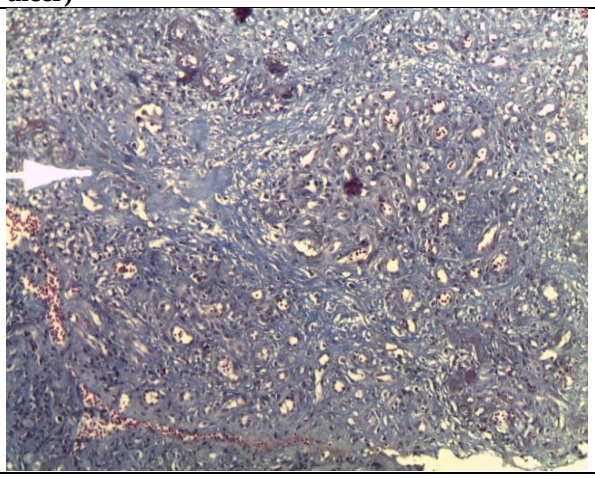
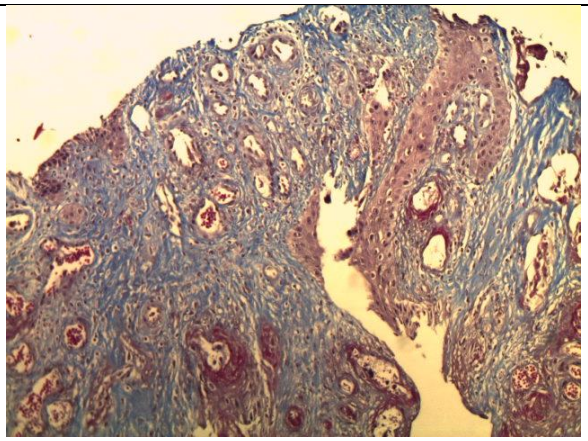


Figure (7): Percentage of healing of group A, B and C.

The histological pictures (H&E stain picture and Masson trichrome stain picture) of pre and post laser treatment in laser groups (A, B) show the laser effect on collagen formation which indicated by the partial to complete healing with 2 months' time line of treatment as shown in Table (5).

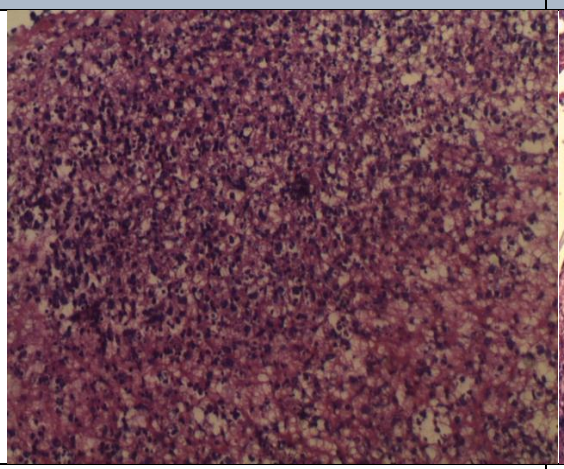
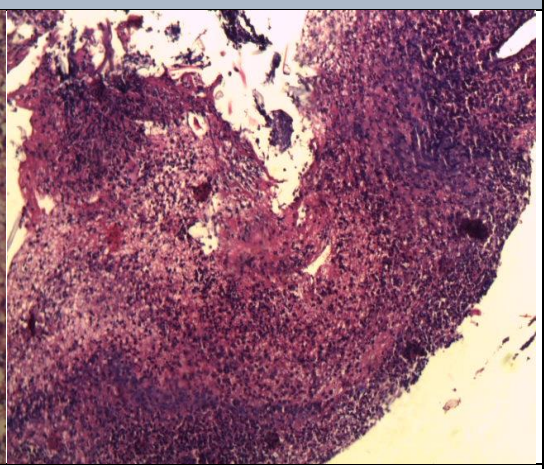
Table (5): histological pictures of pre and post laser treatment in laser groups (partial to complete healing)

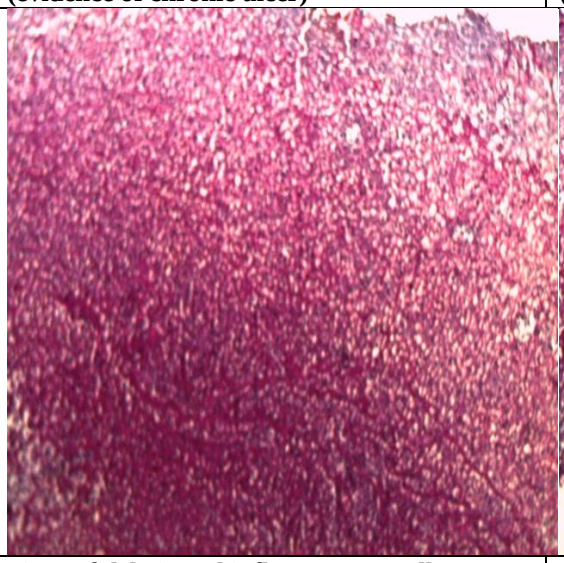
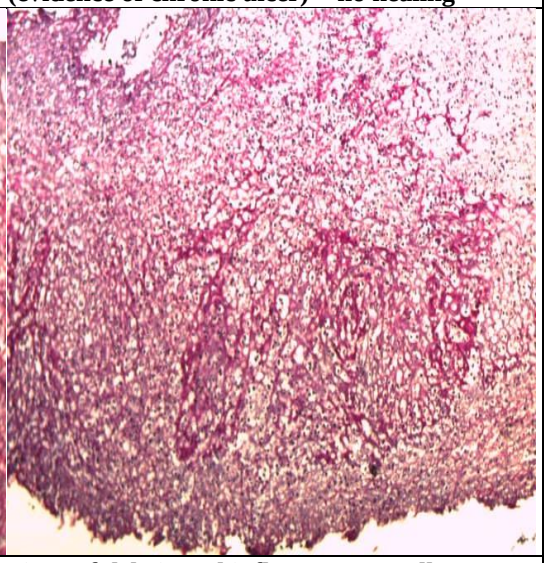
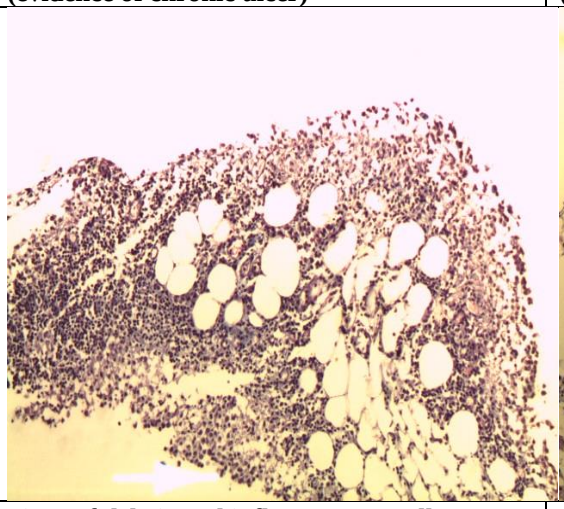
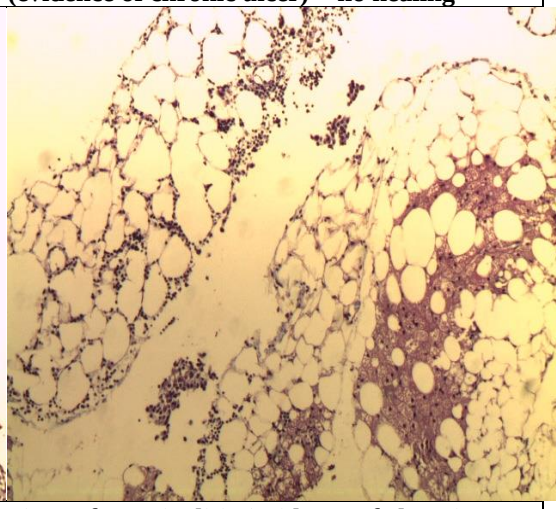
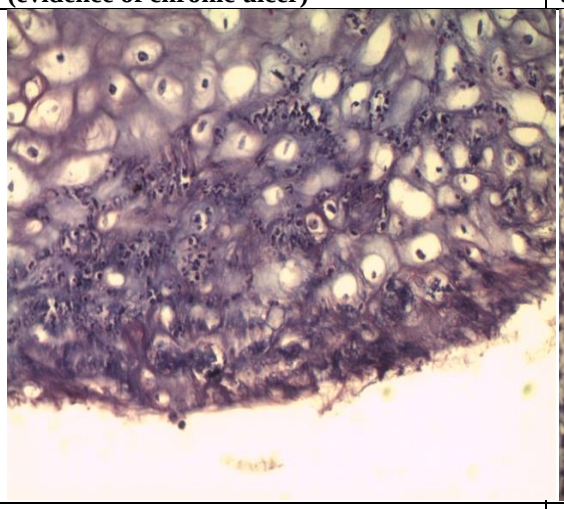
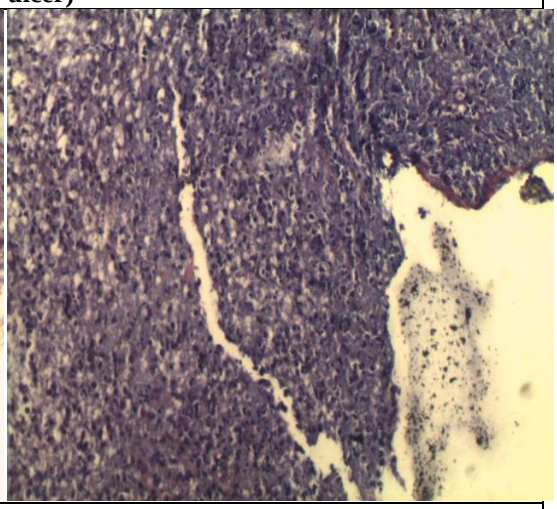
Laser groups	Pre laser treatment	Post laser treatment
H&E stain picture		
Description	**Signs of debris and neutrophil (evidence of ulcer)**	**Signs of granulation tissue formation (Evidence of healing)**
H&E stain picture		
Description	**Signs of debris and neutrophil (evidence of chronic ulcer)**	**Signs of granulation tissue formation (Evidence of healing)**

Masson trichrome stain picture		
Description	**Signs of debris and neutrophil (evidence of chronic ulcer)**	**Signs of fibrosis formation (evidence of healing)**
Masson trichrome stain picture		
Description	**Signs of debris and neutrophil (evidence of chronic ulcer)**	**Signs of fibrosis formation (evidence of healing)**

The histological pictures (H&E stain picture and Masson trichrome stain picture) of pre and post laser treatment in control group (C) show the response and the activity of collagen formation for using only conventional wound treatment which presented by the non-healing pictures with 2 months' time line of treatment as shown in Table (6).

Table (6): histological pictures of pre and post laser treatment in conventional wound treatment group after 2 months (no healing)

Conventional group	Pre conventional treatment	Post conventional treatment
H & E stain picture		
Description	**Signs of debris and inflammatory cells**	**Signs of debris and inflammatory cells**

	(evidence of chronic ulcer)	**(evidence of chronic ulcer) = no healing**
H & E stain picture		
Description	**Signs of debris and inflammatory cells (evidence of chronic ulcer)**	**Signs of debris and inflammatory cells (evidence of chronic ulcer) = no healing**
Masson trichrome stain picture		
Description	**Signs of debris and inflammatory cells (evidence of chronic ulcer)**	**Signs of panniculitis (evidence of chronic ulcer)**
Masson trichrome stain picture		
Description	**Signs of debris, inflammatory cells and dead skin (evidence of chronic ulcer)**	**Signs of debris and inflammatory cells (evidence of no healing)**

DISCUSSION

Application of HILT for pain and functional ability of management of DFU showed significant improvement compared to control groups. We were unable to find any previous reviews evaluating the effectiveness of HILT for the management of DFU, but a systematic review for LLLT in management of DFU was available.

HILT groups showed more significant decrease in wound surface area in both groups than the control group, due to the deep penetration of photons inside tissue, combined effect of red and infrared laser used in treatment protocol. Which give HILT superiority above LLLT application in deep penetration and decrease exposure time.

In group A we found significant increase in number of cases with complete wound closure due to enhance collagen formation the mechanism of beam radiation as in group A we used sequential beam that radiate the 4 wavelengths in the same which enhance the absorption of wavelengths between itself inside tissue in shorter time and decrease tissue resistance to deliver the same joules/ cm compared to separate beam mode in group B which radiate the 4 wavelengths separately in longer time of exposure with much scattering.

The proliferation of the laser irradiation through the body realizes easily deep penetration, achieves the spread out in the tissue, enhances the oxidative response of mitochondria, generates ATP, RNA, and DNA, enhances photobiological impacts on the affected tissues, stimulates collagen production of the muscle tendons, and accordingly improves the status of chronic nsLBP patients and their daily life activities. **(18)**

HILT has been known to reduce heat accumulation in tissues and to have photothermal and photochemical effects in deep tissues for limited periods. These properties favor treatment of deep tissues and structures by increasing cell metabolism, vascular permeability, and blood flow **(19)**

Laser therapy for diabetic foot ulcer has been investigated in many previous clinical studies. Kaviani et al. found that laser therapy could hasten the healing of diabetic foot ulcer and reduce the time needed to achieve complete wound healing as compared with a placebo control group **(20)**

In recent years, cell studies have indicated that Laser therapy may moderate the adverse effects of hyperglycemia on vascular endothelial cells and lead to a reduction in TNF-α concentration and enhancement of fibroblast proliferation **(21)**

Collagen I is a major protein in the extracellular matrix that constitutes most of the connective tissue during wound healing. However, diabetes can cause fibroblast proliferation disorders and impaired collagen synthesis. An in vitro study found that Laser therapy could increase cell viability, cell migration, proliferation, and collagen synthesis. **(22)**

Laser therapy induces macrophages to release factors that stimulate fibroblast proliferation. In addition; Laser therapy can promote the production of interleukin-1 alpha (IL-1α) and interleukin-8 (IL-8), which can stimulate the migration of keratinocytes**. (23)**

Laser therapy also increases the expression of platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β). **(24)** PDGF stimulates mitogenicity and chemotaxis of fibroblasts and smooth muscle cells and chemotaxis of neutrophils and macrophages, playing a role in wound healing. Wound healing is a complex process, and TGF-β has been shown to regulate these different steps by acting on multiple cell types and to promote the wound healing process in the body. **(25)**

Laser therapy also increases the ulcer granulation rate of patients with DFUs. In general, Laser therapy promotes ulcer healing by increasing the synthesis of collagen and extracellular matrix, recruiting-related cytokines and growth factors, and promoting the migration, proliferation and differentiation of different cell types. **(26)**

It was also found that wound repair were higher when laser therapy was adjusted with higher power intensity and shorter wavelengths which give the advantage of using class IV laser therapy than class IIIb. But the increase in application frequency from three to five weekly sessions did not result in changes in wound repair, but higher frequencies can inhibit the repair of these lesions. **(27)**

CONCLUSION

Both high intensity laser therapy (HILT) with sequential or separate beam radiation providing better diabetic foot ulcer healing for patients who have undergone chronic DFU, compared with conventional therapy of wound management in decrease wound surface area, with superior performance in the case of the percentage of complete healing and collagen formation percent in the management of DFU. These findings call for a further study to investigate the combined effects of different types of laser with different type of laser beam radiation on healing of DFU.

Credit authorship contribution statement

Conceptualization; HFA, MSB
Data curation; HFA, MSB
Formal analysis; MIM, HAS
Funding acquisition; HFA, MSB
Investigation: AMM, MIM
Methodology; HFA, MSB, AMM
Project administration; MSB, MIM
Resources; Software; AMM, MSB
Supervision: MSB, MIM
Validation: HAS, HAS
Visualization; HSA
Roles/Writing - original draft; HFA, MSB
Writing - review & editing; HFA, MSB

Compliance with ethical standards

Conflicts of interest: The authors declare that they have no conflicts of interest.

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