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ORIGINAL ARTICLE

Low-level laser therapy as an adjunct to conventional therapy in the treatment of diabetic foot ulcers

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Abstract Foot ulcers are serious complications of diabetes mellitus (DM) and are known to be resistant to conventional treatment. This study was conducted to evaluate the efficacy of low-level laser therapy (LLLT) for the treatment of diabetic foot ulcers in a tertiary care centre (Department of Surgery, Mahatma Gandhi Memorial Medical College and Maharaja Yashwantrao Hospital, A.B. Road, Indore). A total of 30 patients with type 2 DM having Meggitt-Wagner grade I foot ulcers of more than 6 weeks duration with negative culture were studied. Patients were randomized into two groups of 15 each. Patients in study group received LLLT (660 ± 20 nm, 3 J/cm^2) along with conventional therapy and those in control group were treated with conventional therapy alone. The primary outcome measure was the absolute and relative wound size reduction at 2 weeks compared to the baseline parameter. Percentage ulcer area reduction was 37 ± 9% in the LLLT group and 15 ± 5.4% in the control group ($p < 0.001$). For ~75% of wounds of the treatment group, wound area reduction of 30–50% was observed. In contrast, for the control group, ~80% of wounds showed a wound area reduction of <20% on day 15. Further, the wounds with initial wound area 1000–2000 mm^2 seems to have better final outcome than the groups with larger areas. The treated groups showed higher amount of granulation than the control group. The results suggest that LLLT is beneficial as an adjunct to conventional therapy in the treatment of diabetic foot ulcers.

Keywords Adjuvant therapies · Diabetic foot ulcers (DFU) · Low-level laser therapy (LLLT) · Wound healing · Ankle brachial index (ABI)

Introduction

The incidence of diabetes mellitus (DM) has increased dramatically over the past two decades and is becoming a global public health threat [1, 2]. According to epidemiological studies, the number of patients with DM have increased from about 30 million cases in 1985 to 285 million in 2010 and it is estimated that by 2030, more than 360 million people will have DM [3, 4]. Patients with DM are prone to multiple complications such as diabetic foot ulcer (DFU) [5–7]. It is estimated that 15% of patients with diabetes will suffer from DFU during their lifetime [8–11].

DFU is considered as a major source of morbidity and can lead to infection, gangrene, amputation and even death if necessary care is not provided [12–14]. Overall, the rate of lower limb amputation in patients with DM is 15 times higher than patients without diabetes and approximately 50–70% of all lower limb amputations are due to DFU [8]. Furthermore, DFU is responsible for substantial emotional and physical distress as well as productivity and financial losses that lower the quality of life [15]. Risk factors for DFUs include males, DM of more than 10 years' duration, peripheral neuropathy,

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abnormal foot structure, peripheral arterial disease, smoking, previous history of ulcers or amputations and poor glycaemic control. About 15% of patients with DM are likely to develop foot ulcers during their lifetime and about 6–40% of them may require an amputation [16].

Although the fundamental pathophysiological factors leading to DFUs remain incompletely understood, the triad of neuropathy, ischaemia and infections is commonly considered the most important [17]. These ulcers show decrease in both angiogenic response and deficient growth factors resulting in delayed healing [18]. Non-healing DFUs are resistant to conventional treatment [19]. Several adjuvant therapies which have been tried to stimulate healing process are ultrasound, laser therapy and other forms of photobiomodulation, electrical stimulation, hyperbaric oxygen and vacuum-assisted closure [20, 21]. Although laser therapy has been investigated since the 1990s for possible improvements in the healing of wounds [22–31], lack of reproducible results [32, 33] have hampered its widespread use. For better comprehension of the efficacy of laser therapy for wound healing, better designed experiments using different laser wavelengths and fluence have been carried out more recently using animal models. These studies show that laser therapy, also known as low-level laser therapy (LLLT), modulates the expression of inflammatory mediators and leads to a reduction in edema, leukocyte influx, and oxidative stress [34–37]. Further, LLLT has been shown to stimulate neovascualrization [38, 39] and collagen remodeling [39, 40] which result in faster wound healing. Some of these studies also suggest that a wavelength around 660 nm gives better results compared to longer wavelengths [41]. A detailed study with varying doses of 632.8 nm He-Ne laser irradiation suggested that a dose of 3 J/cm^2 lead to better results [42, 43]. We have therefore carried out a randomized placebo-controlled study on the effect of red light (660 ± 20 nm) on the healing of DFUs.

Materials and methods

Selection of diabetic subjects

This study was conducted over a period of 6 months from April 2015 to September 2015 at a tertiary-level teaching hospital, Maharaja Yashwantrao Hospital, A.B. Road, Indore, after obtaining ethical clearance from the Institutional Scientific Review Committee. Type 2 DM patients with Meggitt-Wagner grade I DFUs of at least 6 weeks' duration were included. Those with clinical signs of ischaemia, fasting blood sugar (FBS) levels >200 mg/dL and signs of septicaemia were excluded from the study. Sample size was 30. Patients were randomized into two groups of 15 each (supplementary data, CONSORT diagram). All the patients in the LLLT group were explained the pros and cons of the procedure in understandable language through the Participant/Legally Acceptable Representative Information Document.

The procedure in the patients of LLLT group was started after taking informed written consent from the patients. The preliminary skin care started following review of the patient's medical history and physical examination and vascular evaluation. Ulcers were given conventional treatment in the form of debridement, slough excision and betadine solution dressings until the wound was healthy. Prior to each session of LLLT, the dressing was removed; the wound was thoroughly cleansed by normal saline to remove the remnant local applicant ointments, any pus or debris present; and then were gauze dried. Following these protocols, the wound was exposed to LLLT.

All patients were admitted to the surgical ward and were subjected to detailed evaluation. A complete haemogram and renal and liver function tests were carried out in all patients. Patients with FBS levels less than 200 mg/dL, measured on two occasions 24 h apart, were included. Ulcer area was calculated by digital analysis of the photographs taken on day 0, day 7 and day 15. Objective assessment of vascularity was done by careful palpation of peripheral pulses and calculation of Ankle brachial index. Colour Doppler imaging of the arterial circulation of lower limbs was performed in patients with feeble or absent pulsations.

Systemic antibiotics were administered for both the control based on culture sensitivity reports. Insulin/oral hypoglycaemic agents as advised by the physician/endocrinologist were used to maintain a good glycaemic control. Once adequate glycaemic and infection control had been achieved, LLLT was commenced.

LLLT protocol

All the patients included in the LLLT group were given sessions of LLLT through a handheld diode laser (660 ± 20 nm)-based source fabricated at RRCAT. It delivers a collimated beam of 20 mm diameter (Fig. 1a). The use of a collimated beam ensures a constant area of illumination. The diode laser source was held approximately 1 ft above the ulcer surface and the power density at the sample plane was measured to be ~50 mW/cm^2. Depending upon the wound area, light irradiation was carried out at 5–8 spatially separated points so that the entire wound area (the ulcer floor and edge) were irradiated. For each exposure, a fluence of ~3 J/cm^2 was delivered by keeping the irradiation time fixed to 60 s. The wounds were subjected to light exposure on a daily basis for 15 days. The image in Fig. 1b shows a photograph of the diode laser source in use for irradiating a diabetic foot ulcer. After each session of light exposure, the ulcer was covered with conventional moist dressing. It is pertinent to note here that the patients who had substantial amount of slough were sequentially debrided in multiple sittings before giving LLLT. Both the patients and

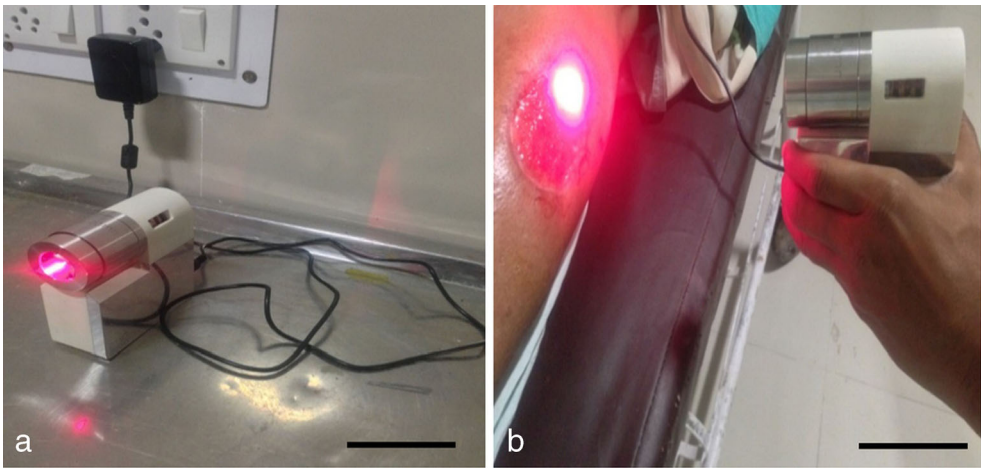

Fig. 1 **a** LLLT device with 20-mm probe. **b** LLLT being used on diabetic ulcer. *Scale bar*: 5 mm

administrators wore appropriate laser safety goggles as per safety regulation.

All the patients included in the control group were given conventional therapy in the form of daily wet saline or betadine dressings, antibiotic treatment, contact cast immobilization and slough excision as and when required. Pressure off-loading was carried out in patients with plantar ulcers. Healing or percent reduction in the size of the ulcer over a period of 15 days after commencement of LLLT was recorded as the end point of the study. Simultaneously, these patients were also educated about various aspects of DM including dietary restrictions, exercise and foot care in order to prevent recurrence.

Wound area and contraction analysis

The images were analysed using the ImageJ software (www.nih.org). The absolute wound area was calculated from the images. Wound contraction on day 15 was determined according to the following formula: [(area on day 7 or 15 − initial area)/initial area] × 100.

Statistical analysis

Student's *t* test was used for statistical comparison between two means. For comparison among more than two groups, one-way ANOVA followed by Fisher's LSD test was used. For assessing correlation between two parameters, Pearson's regression coefficient *R* was used. $p < 0.05$ was considered significant.

Results

In the 30 patients included in the study, male to female ratio was 2:1. Mean age of patients in the control group was 49 years as compared to 54 years in the LLLT group with *p* value 0.13. Average duration of ulcer at the time of enrollment in the study was 51 days in the control group and 56 days in the LLLT group ($p = 0.33$). The average duration of diabetes in the control and LLLT groups were ~5 and ~5.2 years, respectively ($p > 0.05$). The average FBS levels among controls were 158.33 mg/dL and 158.13 mg/dL in the LLLT group ($p = 0.5$). The demographic parameters, FBS levels and the measurements made on wound area at different time intervals for the two groups, control and the LLLT groups, are summarized in Tables 1 and 2, respectively. All ulcers in both groups belonged to Meggitt-Wagner grade I. The average initial area of the ulcers of the control and LLLT groups, at the start of experiment (day 0) were ~1352 and ~1484 mm^2, respectively. There was no significant difference ($p = 0.25$) between the average areas of two groups at this time point. The average area of the ulcer at day 7 was ~1180 mm^2 in the LLLT group and ~1250 mm^2 in the control group ($p = 0.34$). After completion of 15 days of therapy, the average final area of the ulcer at day 15 was ~930 mm^2 in the LLLT group and ~1146 mm^2 in the control group (Fig. 2, $p = 0.09$). The sequential images of ulcers of the control and LLLT groups are shown in Fig. 3. It can be observed that the ulcers of the LLLT group have more granulation tissue (red) compared to the control group which still has some amount of pus (yellow). The patients treated with the LLLT group showed significant reduction in percentage wound area, 37.3 ± 9% as compared to 15 ± 5% in control groups ($p < 0.001$). These results show significant benefit of patients treated with LLLT over patients not treated with LLLT (Fig. 4). Further, for ~75% of wounds of the treatment group wound area reduction of 30–50% was observed. In contrast, for the control group, ~80% of wounds showed a wound area reduction of <20% on day 15 (Fig. 5a, b). We did not observe any correlation between the FBS level and percent wound contraction for both the groups (Fig. 5c).

Discussion

Among the various non-invasive treatment modalities for DFUs, LLLT is gaining increasing interest. The in vivo studies

Table 1 Demographic parameters, blood glucose level and ulcer area data of the subjects of the control group

S. no	Age	Gender	Duration of ulcer	Duration of DM-II	Location of ulcer	Ulcer size (mm^2)			FBS levels in mg/dL
						Initial	Day 7	Day 15	
1	42	M	12 days	1 year	Right lateral malleolus	521	520	517	154
2	32	M	1 month	3 years	Right foot dorsum	1100	1007	953	174
3	75	M	2 months	4 years	Right lateral malleolus	1256	1123	1000	111
4	40	M	15 days	1 year	Left foot dorsum	1450	1308	1180	123
5	61	M	13 days	2 years	Left foot dorsum	456	400	380	147
6	20	F	15 days	1 year	Right foot dorsum	894	800	703	178
7	36	F	25 days	2 years	Left foot dorsum	1243	1100	998	154
8	50	M	1 month	3 years	Left foot dorsum	789.5	735	681	139
9	55	M	2 months	7 years	Right foot dorsum	1678	1587	0	165
10	47	M	3 months	1 year	Left foot plantar	2346	2180.8	1901	137
11	48	M	1 month	5 years	Right foot plantar	2132	2071	1935	177
12	61	F	1 month	10 years	Right great toe	2766	2606	2401	197
13	49	M	2 months	6 years	Left leg	910	801	745	176
14	67	M	25 days	2 years	Right leg	1280	1160	1096	165
15	54	F	15 days	6 years	Left foot plantar	1400	1323	1200	178

have suggested that the healing enhancement properties of LLLT are likely to be not only due to photobiomodulation resulting in decrease in inflammation [34–36] but also increased granulation tissue, fibroblast proliferation, collagen synthesis [38, 39] and neovascularization [38, 40] in LLLT-treated wounds.

Results of the previous clinical studies have shown that LLLT can play a useful role in healing chronic diabetic ulcers resistant to conventional treatment [22–31]. In a randomized study on diabetic patients with foot ulcers, Landau et al. observed that compared to patients who received conventional treatment and placebo irradiation, the group which received conventional treatment and phototherapy with broadband visible light (400–800 nm) showed better healing [28]. Minatel et al. showed that combined irradiation with 660 and 890 nm delivered at fluence of 3 J/cm^2 promoted granulation and healing of diabetic ulcers that failed to respond to other forms of treatment such as 1% silver sulfadiazine cream [25].

Table 2 Demographic parameters, blood glucose level and ulcer area data of the subjects of the LLLT + conventional treatment group

S. no	Age	Gender	Duration of ulcer	Duration of DM-II	Location of ulcer	Ulcer area (mm^2)			FBS levels in mg/dL
						Initial	Day 7	Day 15	
1	45	M	2 years	5 years	Right foot plantar	1326	967	716	173
2	50	M	2 months	2 years	Right foot plantar	1560	1176	876	156
3	75	F	3 months	25 years	Right foot plantar	1256	850	650	170
4	45	M	45 days	2 months	Right foot dorsum	1440	1000	708	163
5	61	M	45 days	5 years	Right foot lateral malleolus	772	674	609	144
6	72	F	5 days	10 years	Right hand palmar	766	650	538	174
7	50	F	7 days	5 years	Left foot	1340	1224	991	193
8	32	M	3 months	1 year	Left leg medial	1350	958	803	127
9	70	M	3 months	2 years	Left foot medial	1567	1283	1008	156
10	40	M	5 months	2 years	Right great toe	987	761	621	121
11	60	M	1 month	10 years	Left foot plantar	2298	1877	1501.7	124
12	65	F	45 days	5 years	Left foot dorsum	1672	1324	1010	164
13	50	F	2 months	3 years	Left foot medial	1543	1399	1082	181
14	56	F	1 month	2 years	Right foot plantar	2998	2601	2108	199
15	40	M	1 month	3 years	Right foot dorsum	1377	902.8	735	127

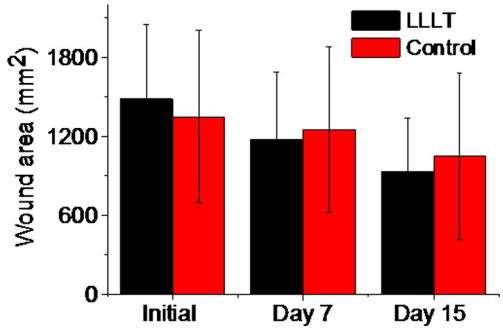

Fig. 2 Effect of LLLT on contraction of DFUs. The data represent means ± SD ($n = 15$) for each time point of each group. The comparison between means of different groups were made using one-way ANOVA followed by Fisher's LSD test. For the LLLT group, $p = 0.09$ for comparison of initial wound area and wound area on day 15

Although in these previous studies different wavelengths (in the spectral range 600 to 900 nm) and fluence have been used, it has emerged that a wavelength around 660 nm [26, 30, 31] at a fluence of ~2–4 J/cm^2 is effective for chronic ulcers [25, 29–31]. Guided by these studies, we have investigated the effect of 660 nm light source at a fluence of ~3 J/cm^2 on the absolute and relative wound area reduction compared to baseline on day 15 post treatment. In the results presented in Fig. 2, we have plotted the actual wound area for the two groups as a function of time. Although the initial mean wound area for the LLLT group was slightly more compared to the control group on day 15, it is lower than the control group. This advantage of LLLT becomes more clear from Fig. 4 where the data is normalized with respect to the initial wound area. Compared to the control group, for the LLLT group, there is a factor of 2.5

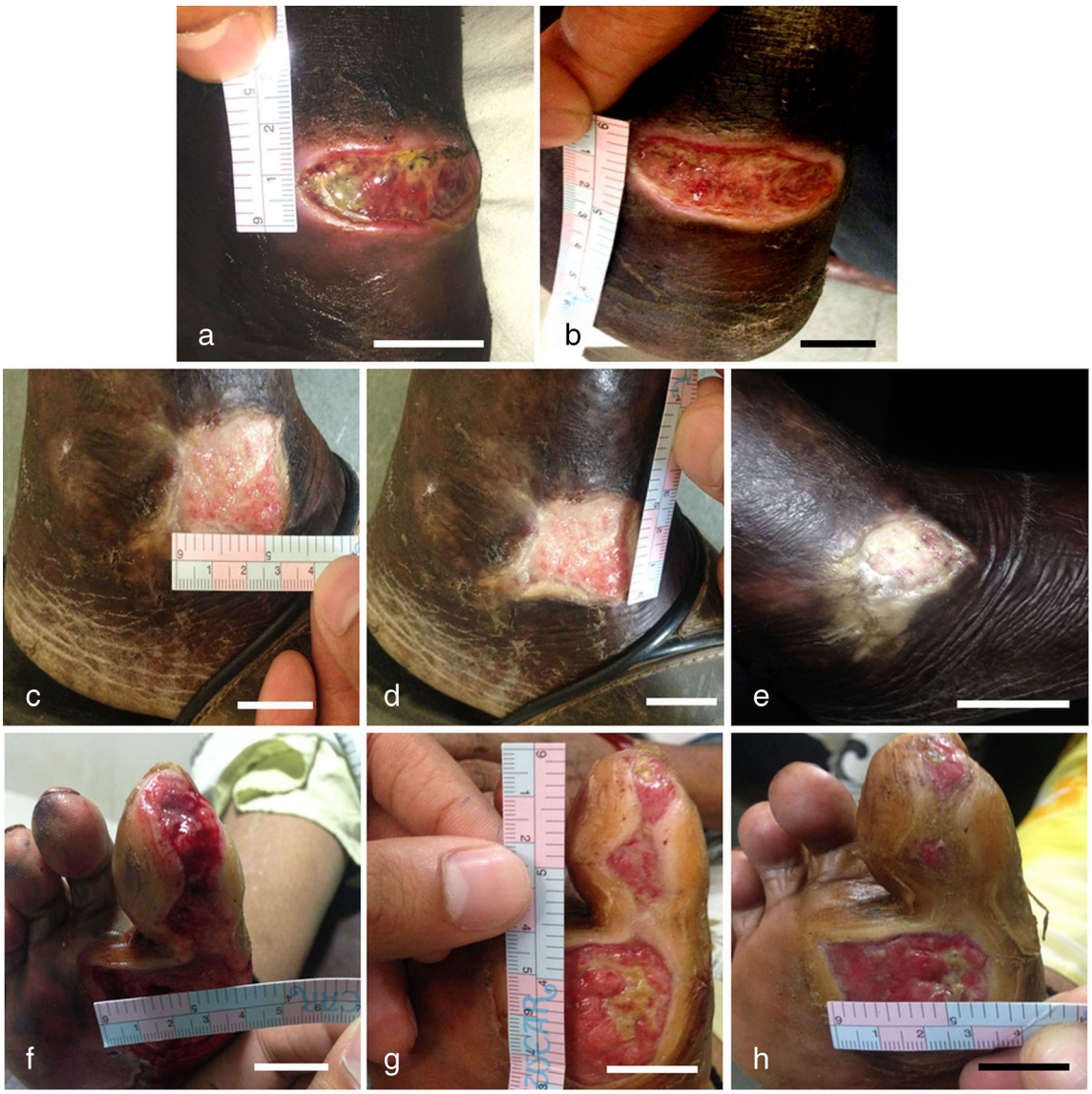

Fig. 3 Representative photomicrograph of wound ulcer of back of ankle of a patient of the control group on day 0 (**a**) and day 15 (**b**). Wound reduction in the ankle of a patient of the LLLT group on day 0 (**c**), day 7 (**d**) and day 15 (**e**). The *lower panel* shows representative photomicrographs of wound reduction of toe ulcer of a patient of the LLLT group on day 0 (**f**), day 7 (**g**) and day 15 (**h**). *Scale bar*: 2 mm

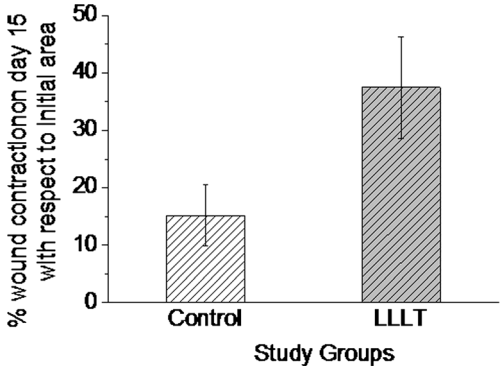

Fig. 4 Effect of LLLT on mean wound area contraction of the control and LLLT groups on day 15. The data here represent means ± SD ($n = 15$) for each group. A Student's t test for comparison of means of these two groups yielded a p value of <0.001, suggesting a considerable difference between two means

higher reduction in wound area ($p < 0.001$). At the same time, results in Fig. 5 show that, compared to LLLT, only one fifth of the patients showed ≥20% ulcer area reduction. In a study by Kajagar et al., 34 ulcers treated with LLLT showed significant reduction in percentage wound area, that is, 40 ± 6% compared to 12 ± 4.3% in control groups [27]. Although a direct comparison cannot be made because of use of different wavelengths and fluence, our results are similar to that reported by Kaviani et al. [26] who used 685 nm and ~10 J/cm^2 fluence twice a week for 2 weeks. An earlier study by Schindle et al. [22] suggests that the initial wound size also influences the LLLT outcome. To check this aspect, the 15 patients of the LLLT group were subdivided in three subgroups based on the area of the initial wound (<1000, 1000–2000 and 2000–3000 mm^2). While the average wound contraction for wounds of area 1000–2000 mm^2 is ~41%, for the wounds of area >2000 mm^2, it was ~32% ($p = 0.056$, ANOVA followed by Fisher's LSD test).

Apart from the reduction in wound area, the presence of granulation and pus on wound bed was also monitored. It was observed that the majority of the wounds of LLLT groups was devoid of pus and exhibited granulation. In contrast, the wound that received conventional treatment showed more pus and lesser granulation and required more debridement and dressing changes. It is also pertinent to note that the patients of the LLLT groups did not feel any discomfort with the procedure and were satisfied with the reduction of pain during this duration. The LLLT procedure is therefore a good adjuvant for the treatment of diabetic foot ulcers. At the same time, it would be desirable to carry out studies on more dosages and wavelengths for longer durations. However, based on available information, we made a choice of 660 nm and fluence of ~3 J/cm^2 and this has shown good results. This result, we hope, will motivate further studies.

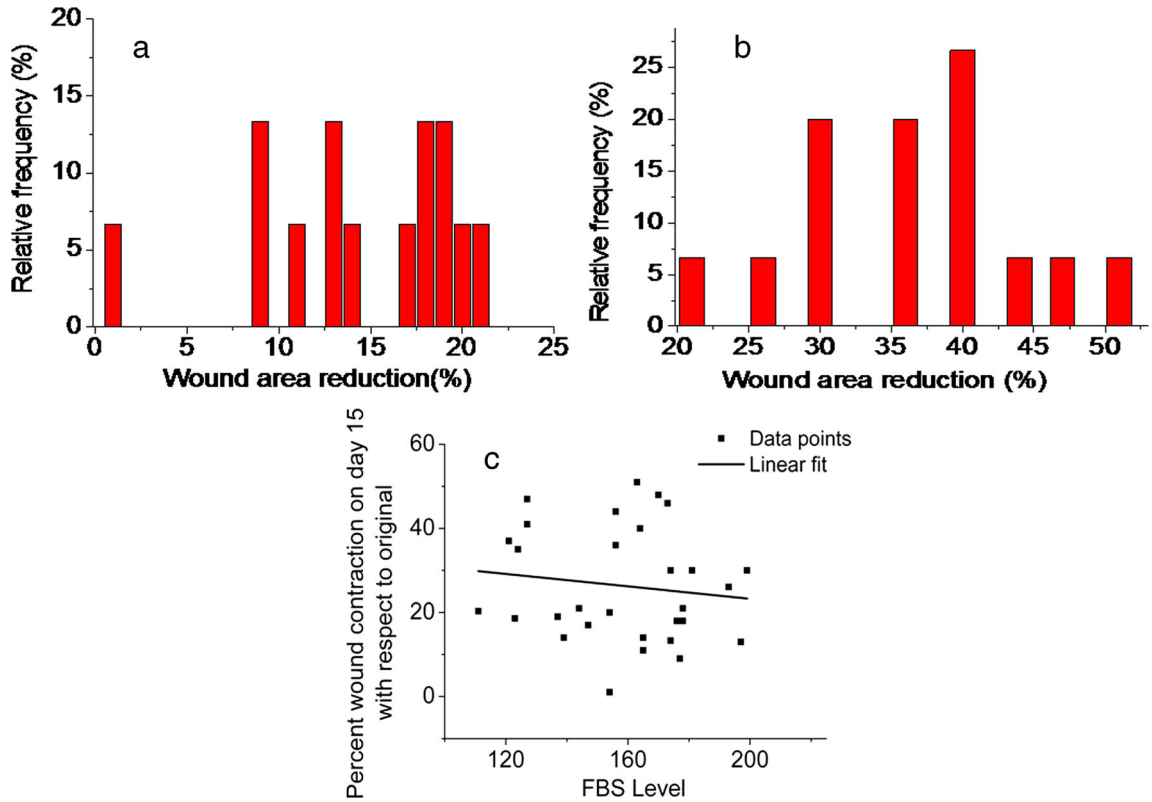

Fig. 5 The relative frequency of different percent wound contraction of the control (**a**) and LLLT groups (**b**). The *red columns* give the relative frequency of different percent wound contraction of the control (**a**) and LLLT groups (**b**). Width of the bands is 5%. The LLLT group can be seen to have larger reduction in wound area. Correlation between percentage wound contraction and mean FBS level of patients (**c**)

Conclusion

In conclusion, the wounds in subjects treated with LLLT contracted significantly more than the wounds in the non-treated group (37.2% for the LLLT group versus 15.12% for the control group, $p < 0.001$), which indicates that LLLT is an effective modality to facilitate wound contraction in patients suffering from diabetes and can be used as an adjunct to conventional mode of treatment (dressings and debridement) for healing of diabetic wounds. Due to its stimulatory effect and no reported side effects, LLLT can be used to treat chronic wounds, including diabetic ulcers.

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Compliance with ethical standards

Ethics statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All the patients in the LLLT group were explained the pros and cons of the procedure in understandable language through the Participant/Legally Acceptable Representative Information Document. The procedure in the patients of the LLLT group was started after obtaining a written informed consent.

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Conflict of interest The authors declare that they have no conflict of interest.

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