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THE EFFECT OF DIFFERENT POWERS OF LOW-LEVEL LASER THERAPY ON WOUND HEALING OF DIABETIC RATS- A COMPARATIVE STUDY

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ABSTRACT:

Review: Tissue healing is a complex process involving local and systemic responses. Inadequate of inappropriate wound healing is a headache to many surgeons especially in compromised patients. Several technologies have been tested for their effect on improving and enhancing wound healing. The use of low-level laser therapy for example has been shown to be effective in modulating both local and systemic response healing responses.

Aim: This study aims to evaluate the effect of different powers of low-level laser therapy on wound healing in diabetic rats.

Methodology: Streptozotocin (45 mg/kg body weight) was intraperitoneally applied for diabetes induction. A full-thickness skin wound (2 × 2 cm2) was aseptically created with a scalpel in diabetic rats on the shaved back of the animals. The wounded diabetic rats were treated with low-level laser therapy (LLLT) for 14 days. The wound closure percent was calculated during the course of the experiment on days 1, 7, and 14.

Results: Clinical observation of skin lesion samples of the animals showed that skin lesions of the group (A) (control) exhibited an early-phase tissue repair pattern, with the formation of a whitish crust, with slightly elevated rims and a reddish core, group (B) and (C) wounds, which were showed complete tissue repair, showing scars with evident rims and a central portion slightly unleveled, These results suggest that LLLT is an efficacious method of tissue repair modulation, significantly contributing to more rapid and organized healing of tissues.

Keywords:LLLT, diabetic rats, wound healing

Review

Tissue healing is a complex process involving local and systemic responses [1,2]. Inadequate of inappropriate wound healing is a headache to many surgeons especially in compromised patients [3]. Diabetes mellitus is a very common disease and its detrimental effect on wound healing especially in uncontrolled patients is well documented [4]. Poor vascular network and increased infection risk are some of the reasons why wound healing is compromised [5]. Several technologies have been tested for their effect on improving and enhancing wound healing. Poor healing of oral wounds has also been reported in literature in depth [6]. Hyperglycemia causes vascular dysfunction and leads to insufficient blood supply reaching the wound surface and causing failure of wound healing [7]. It is therefore thought that enhancing angiogenesis in the wound bed improves the delivery of oxygen and nutrients at the wound and empirically improves wound healing [8]. It is therefore logical that therapies enhancing angiogenesis will accelerate and improve wound healing of diabetic patients [9,10]. The use of low-level laser therapy (LLLT) for example has been shown to be effective in modulating both local and systemic response healing responses [11-13]. It has been reported to improve the healing of cases such as diabetic foot and ulcers[14-15] . LLT includes wavelengths of 500-1100 nm and includes delivery of 1-4 J/cm^2 to the wound sites. It is reported to provide biological stimulation to the cells in the wound by light energy. The cells absorb the light energy and translate that by promoting cell function, regeneration and repair [16,17] without excessive temperature increases. Recently studies have concluded that LLLT moderates the effects of high glucose levels on vascular endothelial cells and cause the reduction of TNF- α concentration and so enhancing fibroblast [18]. On the other hand, the exact wavelengths and effects of different powers of LLLT are not yet concluded. The science behind the action of the different wavelengths support the idea that the effect on wound healing with lower wavelengths may be positive.

This study aims to evaluate the effect of different power of low level laser therapy on wound healing of diabetic rats.

Materials and methods

60 male albino rats weighting 80–100 g were obtained from the National Research animal house and used in the present investigation. The rats were maintained at a temperature of 22–25 °C with a 12-hours light/dark cycle and allowed free access to water and standard pelleted diet in the Animal House of National Research Centre, Dokki, Giza, Egypt

Diabetes induction

Streptozotocin (STZ) was obtained from Sigma Chemical Company, St. Louis, MO, USA. All other used chemicals were of analytical grade. Induction of Diabetes Mellitus by STZ was dissolved in cold citrate buffer (pH 4.5) and was freshly prepared for immediate use within a few minutes. STZ was intraperitoneally injected as a single dose of 45 mg/kg body weight (b. wt) [1] [2] to rats deprived of food and water for sixteen hours (hrs) .The dose of STZ was administered at a volume of 1 ml/kg b. wt rats. Ten days after STZ injection, rats were deprived of food overnight (10–12 h) and then were orally administered glucose at dose level of 3 g/kg (b. wt) after 2 h of oral glucose gavage (3 g/kg b. wt). Blood drops were obtained from the lateral tail vein of each rat and blood glucose concentration was measured by Glucometer (Model Bionime Rightest GM 100). The rats that have blood glucose concentrations ranging from180 to 300 mg/ dl were considered as mild diabetic rats and were included in the experiment while others were excluded.

Surgical wound

Rats were anesthetized by intraperitoneal administration of ketamine at a dose level of 60 mg/kg b. wt. [3] The dorsal fur of the anesthetized rats was shaved with an electric clipper and the area of the wound was outlined on the back of the rats with a marker pen. An excision wound of size 2 cm2 was made by cutting out a 2 × 2 cm piece of skin from the shaved area. The wounds were of the same full thickness type extending up to the subcutaneous tissue [1] [2] [3][4] {figure 1}. The animals were given antibiotics immediately after surgery.

Fig. (1) Showing wound made in diabetic rat

Low-level laser therapy (LLLT)

Rats were subjected to low-intensity diode laser three times per week for two weeks .using a therapeutic laser apparatus"soft-laser-SL-202" manufactured by scientific-and-production allegation "PERTO LASER"** {figure 2} with the following technical features:

Laser radiation power	1-100mW
Emitted wavelength	870 nm
Type of radiation	continuous modulated mode
Exposure time range	1-99 sec. [5]

Animal grouping:

After induction of diabetes mellitus (10 days post-STZ injection) and wounding. Twenty animals for each group:

Group A: Rats included in this group were diabetic wounded rats receiving no treatment as a control.

Group B: Rats included in this group were diabetic wounded rats subjected to LLLT for 14 days. They were treated by low-intensity diode laser application with power 100 mW

Group C: Rats included in this group were diabetic wounded rats subjected to LLLT for 14 days. They were treated by low-intensity diode laser application with power 50 mW

The method of irradiation is scanning uniformly over the surface of the wound. the spot diameter of 2mm. with the intensity of 1591 mW/cm2 and the dosage of 24 J/cm2, which corresponds to action by continuously modulated radiation (CW mode) and exposure time of 60 sec [5]

Fig. (2) Showing PERTO LASER device

(A) (B) (C)

Fig.(3)
(A) Wound on the 7th day in a diabetic controlled rat
(B) Wound on the 7th day in diabetic rat treated with LLLT with power 100 Mw
(C) Wound on the 7th day in diabetic rat treated with LLLT with power 50 Mw

<u>method of assessment:</u>

. Measurement of wound diameter and closure

The wound diameter was measured on days 1, 7, and 14 after incision and wound closure {figure 3}. Percentage healing was calculated using the following equation: wound closure rate on day X (%) = [(wound diameter on day 0 – wound diameter on day X)/(wound diameter on day 0)] × 100 [1] [2] [3]

Results:

Wound closure

In controlled wounded diabetic rats, the wound closure was more severely affected. the wound closure percent reached 0% on day 1 and increased to 18.3% on day 7 and to 36.7% on day 14 after injury. In diabetic rats treated with LLLT with power 100 MW the wound closure percent of the wound was 0% at day 1 after injury, 63.3% at day 7 after injury, and 83.3% at day 14 after injury. {Table 1}

In diabetic rats treated with LLLT with power 50 MW the wound closure percent was 0% at day 1 after injury and increased to 79.2% at day 7 after and to 98.3% at day 14 after injury.

Wound closure percent in a day (1, 7, 14) in a controlled group, LLLT with power 100 mW and LLLT with power 50 mW

Groups	**Day 1**	**Day 7**	**Day 14**
Group A	0%	18.3%	36.7%
Group B	0%	63.3%	83.3%
Group C	0%	79.2%	98.3

Histological examination:

After sacrifice and decapitation, rats were dissected and skin in the regions of wounds was rapidly excised and fixed in 10% neutral buffered formalin for 48 h. The fixed skin of each rat was then processed for wax blocking and sectioning. The sections were taken at 5 µm thickness using a microtome, processed in xylene alcohol-series, and stained with alum hematoxylin and eosin (H&E) [9]. The sections were examined microscopically for the evaluation of histopathological changes.

Results showed in the group (A) in the control group, abutting the margin of the surgical wound, a discrete epithelial proliferation was noted, and along practically the whole extension there was the presence of tissue with a wide area of ulceration and fibrin necrotic tissue over the granulation tissue

In group (B) increased neovascularization and fibroblast proliferation, as well as decreased quantity of inflammatory infiltrate in the surgical lesions (Figure 5). Conversely, in group (C), the histopathologic study showed material with an intact epidermis lining well-developed granulation tissue with a connective tissue rich in collagen fibers parallel to the surface of the wound, characterizing a better-organized tissue repair process (Figure 6).

Clinical observation of skin lesion samples of the animals showed that skin lesions of the group (A) (control) exhibited an early-phase tissue repair pattern, with the formation of a whitish crust, with slightly elevated rims and a reddish core due to accentuated presence of blood irrigation in that area, indicating presence of granulation tissue. On the other hand, group (B) and (C) wounds, which were showed complete tissue repair, showing scars with evident rims and a central portion slightly unleveled, but presenting advanced morphological and functional recovery of involved tissues (Figure 5). These results suggest that LLLT is an efficacious method of tissue repair modulation, significantly contributing to more rapid and organized healing of tissues.

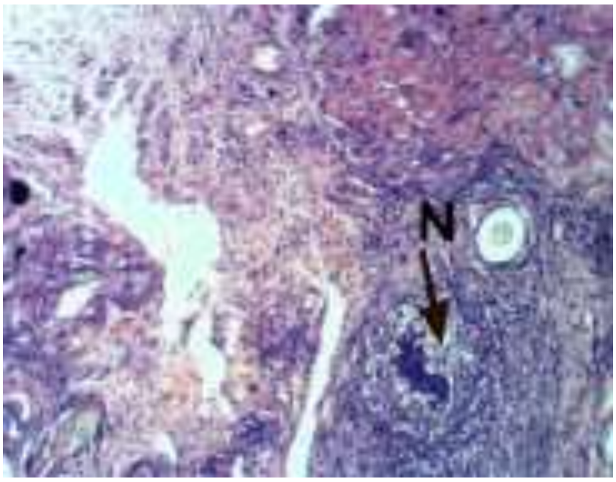

Figure (4): Photomicrograph of vertical section at day 14 after wounding .in skin of diabetic wounded rat (control group): group (A) showing necrosis associated with massive inflammatory cells infiltration (N)

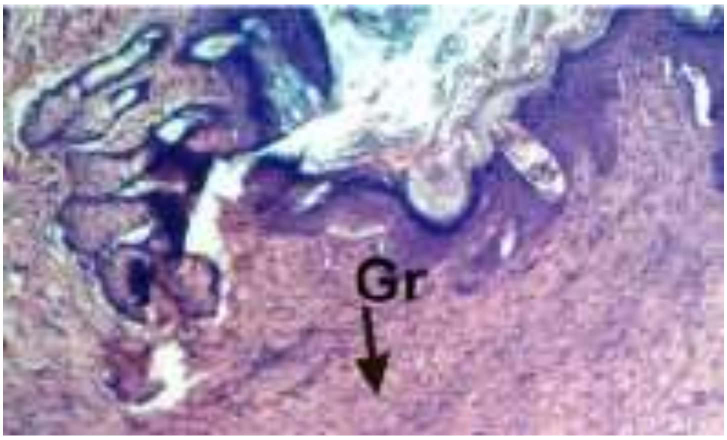

Figure (5): Photomicrograph of vertical section on day 14 after wounding .in skin of diabetic wounded rat with LT (group B) showing dermal granulation tissue formation

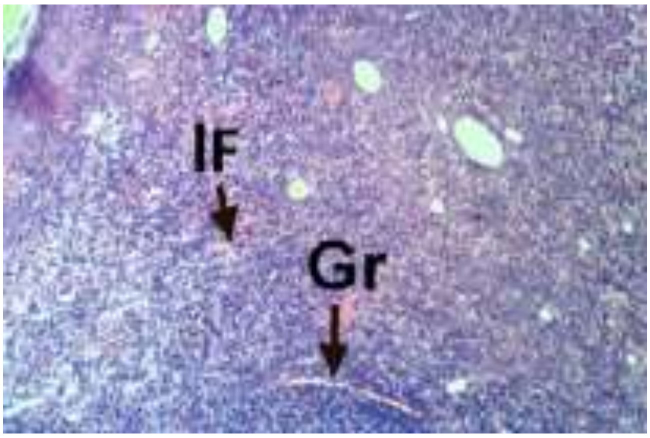

Figure (6): Photomicrograph of vertical section at day 14 after wounding .in skin of diabetic wounded rat with LLLT group (C)) showing granular tissue formation, inflammatory cells infiltration, and collagen fibers production.

Discussion

Low-level laser therapy has been reported to improve wound healing in different regions of the body[19]. the effects of LLLT in the dental field extraorally have also been reported briefly in the literature [20,21] .Medically, LLLT was mainly used to reduce inflammation, reduce pain, and help in tissue repair and augmentation [22.] The low levels of laser and infrared lights produced by LLLT directed to the cells are lower than that used with ablative or cutting lasers. The energy absorption is then directed to the mitochondria which leads to biostimulation of various processes including improved enzyme activity, electron transport, and ATP production [23,24]. It also alters the cellular redox state inducing intracellular signaling pathways and changing the affinity of transcription factors necessary for cell proliferation, survival, tissue repair, and regeneration [25]. The lower wavelengths are thought to cause less heat production and so less detrimental effects on the tissue. Our study aimed to compare the use of different LLLT wavelengths on the healing of diabetic wound models in rats. The results showed improved overall healing in Group C (Diabetic rats, wounds subjected to 50 mW LLLT). Although Group B (diabetic rats, wounds subjected to 100 mW LLLT) also showed better healing than the control group; the percentage of wound healing was lower than that in Group C. That may be attributed to the effects of higher wavelengths which damage some cells whilst improving healing. This result agrees in principle with what others have reported in the literature of 50 mW improving tissue healing 26 and also with the results of 50 mW on its own on improving gingival healing. [27].

The results of our in vivo study can be used to help in clinical trials by applying LLLT at 50mW to enhance wound healing, especially in cases of medically compromised cases with poor healing such as diabetic patients.

In this sense applying energy density at the rate of 4J/cm2 in which studying with HeNe laser showed better effects in the production of collagen type III, but in another dose between 7 and 9 J/cm2 make an opposite effect, decreasing the production of collagen fibers [28,29]

It is known that specific frequencies or doses of light may work on photostimulation systems to enhance collagen formation. Regulating cellular division and raising the level of growth factors for fibroblasts. Yet another explanation for this would be the case, according to the authors above, if improved mitochondrial uptake of such energy and higher ATP and nucleic acid synthesis as a result acid, leading to an increase in collagen synthesis increased development of and hastened epithelial repair granulation tissue[30]

Zanotti et al. claim that excitatory dosages (up to 8J/cm2) are mentioned when the intervention's objective consists of improving the sodium/potassium pump; accelerating ATP synthesis and reestablishing the potential of the membrane; enhancing metabolism; and cell proliferation.[31]

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