Effects of Laser at 810 Nm on Wound Healing in Albino Mice

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Abstract

Many researches focused on laser therapy of wound healing in different animal models due to the lack of a standard protocol in the application of such phototherapy. Objective: To study the effects of 810nm laser at a constant irradiance of 41.63 mw/cm² and exposure (illumination) time of 5.15 minutes on wounds created on Albino mice (BALB/c).

Skin wound with elliptic shape and full thickness was created on the dorsal side of 45 mature male albino mice. Irradiated animals were divided into two main groups based on irradiation time, the first was irradiated for 5 min and the second for 15 min, each was subdivided into three subgroups (n=5) according to number of treatment days (3, 5 and 10 days). Both treated and respective control (n=15) subgroups were sacrificed on days 3, 5 and 10 posttreatment. Laser therapy was applied using a 810 nm diode laser with a continuous wave, an output power of 400 mw, and irradiance of 41.63. The 5 min dose was 12.5 J/cm², whereas the 15 min dose was 37.4 J/cm². The shape of the laser beam was fitted with a convex lens as ‘beam expander’ to irradiate a circular area of 3.4 cm diameter. Laser therapy was started after surgery and repeated for 3, 5 and 10 days, while its effects were examined by histological evaluation.

Results: At day 3 of treatment with near infrared 810nm laser at doses of 12.5J/cm² and 37.4J/cm², there was no evidence of wounds healing in irradiated groups which showed no differences with the respective control groups. At day 5 of treatment, the results showed an important increase in the scores of the parameters of wound healing (formation of granulation tissue and collagen deposition) in the irradiated groups. Near infrared 810nm laser had photobiostimulation effects on wound healing at irradiance of 41.63mW/cm² and doses of 12.5J/cm² for 5 minutes and 37.4J/cm² for 15 minutes exposure time. A complete picture of wound healing response appeared in all irradiated groups within 10 days of treatment, as expressed by complete ‘re-epithelialization’, moderate granulation tissue formation, and presence of collagen fibers, while incomplete wound healing response was observed in un-irradiated control groups within the same period. The study showed that 810 nm laser therapies had significant effects on wound healing, especially at a dose of 37.4J/cm².

Keywords: laser, wound healing

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The objective of the present study is to investigate the effects of 810nm laser at constant irradiance of 41.63 mw/cm² and exposure (illumination) times of 5 and 15 min on wounds created on Albino mice (BALB/c).

Subjects and methods

Study subjects

Forty five BALB/c mice with weight range of 18-32 grams were included in this research. Animals were kept in hygiene conditions in individual plastic cages, maintained at 22º in a day/night light cycle with wood chip bedding, fed with standard pelleted laboratory diet, and provided with water ad libitum. The study was conducted at the animal house of the National Centre for Drugs Control Researches / Iraq, and approved by the ethical committee in the centre.
Diode laser

During the study protocol, all equipment was calibrated in the beginning of the study to make sure of proper dose delivery. Before starting the experiments, the method of irradiation was standardized. Continuous wave, low energy semiconductor laser from ‘UK scientific Ltd’ was used in all experiments of irradiation. The parameters of lasers used in this study are listed in Table-1. Power meter (SOLOPE Genetc- EoInc, Canada) was used to measure the output laser power used. Laser was fitted with a beam expander at the distal end to irradiate a circular area, which integrated the wound and some surrounding intact skin. A convex lens was used to produce the homogenous laser spot which was approximately 3.4 cm in diameter for 810nm, and the distance between the lens and the surface of the mouse was kept fixed. This apparatus was kept constant for all irradiation regimens (Table-1, Figures-(1 and 2)).

![Laser diode and beam expander](image)

**Figure 1-Diode laser and beam expander.**

| Central wavelength(nm) | Operating mode | Output power (mW) | Output power with beam expander |
|------------------------|----------------|-------------------|--------------------------------|
| 810nm                  | cw             | 500mW             | 400 mW                         |

**Table 1-Characteristics of diode laser used in the study**

nm= nanometer  
cw = continuous wave
mw = milli watt  
Focal length of beam expander used was 50mm.

The laser used in the present study was a continuous wave, portable, semiconductor laser with Gallium Aluminium, Arsenide NIR (Ga-Al-As) and 810nm. Near infrared laser (810nm) was powered using a battery which was fully charged before the beginning of irradiation of wounds in each exposure.

| wavelengths | 810nm |
|-------------|-------|
| Output power (mW) | 400mW |
| *Beam spot size (irradiated area) (cm²) | 9.0cm² |
| Irradiance measured at the target area | 41.632 mW/cm² |
| Exposure time(min)/ day | 5 min 15 min |
| Dose J/cm² | 12.5 J/cm² 37.4 J/cm² |

**Table 2-Treatment parameters for diode laser used in the study**

Laser was organized in metal holders which fix the laser perpendicular to and at a fixed distance from the wound surface (Figure-2). Laser therapy was initiated directly after wounding and continued
over 3, 5, and 10 days. The protocol was selected according to the conventional clinical approach to laser therapy for wounds in 3 and 5 exposures per week for 24 hours [5, 6].

Wound model
Following sterilization with 70% alcohol, hair was shaved at the cervical to mid-lumbar dorsum of mice back and incisions were made, resulting in 1.5-3 cm long wounds with full-thickness and elliptic shape over the adjacent musculature and the thoracic spinal column. During the whole period of experiments, the wounds were left uncovered [5, 6].

Study design
Animals were divided into two principal groups; the control (15 animals) and the irradiated (30 animals) groups.

Irradiation treatment of wounds started at the beginning of day 1 and continued for 3, 5, and 10 days, after which the treated mice were sacrificed. This protocol was selected because it is commonly considered by previous studies of wound healing [5, 7, 8, 9]. The data of experimental groups are shown in Table-3.

Table 3-Experimental groups

| Groups                  | Exposure time 5 minutes | Exposure time 15 minutes | Control groups |
|-------------------------|-------------------------|--------------------------|----------------|
|                         | Number of animals       | Number of animals        | Groups         | Number of animals |
| Group irradiated for 3 days | 5                      | 5                        | Group sacrificed after 3 days | 5               |
| Group irradiated for 5 days | 5                      | 5                        | Group sacrificed after 5 days | 5               |
| Group irradiated for 10 days | 5                      | 5                        | Group sacrificed after 10 days | 5               |
| Total number of irradiated groups = 30 animals | | | Total number of control group =15 animals |

Histopathological estimation:
The tissue (whole skin) specimens were stained with hematoxlin and eosin and examined with a semi-quantitive method to evaluate histopathological parameters, including fibroblasts, granulation tissue formation, re-epithelialization, and polymorphic nuclear leucocytes [5, 7, 10].
Mice were randomly selected for each group at 3, 5 and 10 days after wounding and sacrificed by ether inhalation.

Glass slides were prepared and evaluated by two pathologists who were not aware of the sample codes. The sections were evaluated by two observers and estimated on a scale of 0-3 by using a light microscope (Olympus, Japan). Sections were graded for wound healing according to the following seven elements associated to acute inflammatory response and repair: formation of granulation tissue (angiogenesis and fibroblast), collagen deposition, inflammatory reaction (macrophages and leucocytes), and evidence of ‘re-epithelialization’. Each parameter was semi-quantitatively evaluated (from 3 = prominent or marked, to 0 = absent or no evidence) according to McMinn [10].

Wound healing process is described as complete healing, incomplete healing and no healing responses [7, 5, 10, 11].

- **Complete healing**
  Complete healing is characterized by moderate to marked granulation during tissue formation, complete re-epithelialization, presence of collagen fibres, and scattered to mild inflammatory cell infiltration.

- **Incomplete healing**
  It is characterized by incomplete re-epithelialization, mild to moderate granulation, presence of collagen fibres, and mild to moderate inflammatory cell infiltration.

- **No evidence of healing response**
  It is characterized by no evidence of re-epithelialization, no evidence of mild granulation during tissue formation, absence of collagen deposition and of moderate to marked inflammatory cell infiltration.

Statistical analysis

Data were analysed using the statistical package for social science (SPSS V.20) computer software. Histopathological parameters were correlated via chi-square test. The association between two categorical variables was assessed by chi-square test of independence.

**Results**

- **Control groups**
  Nearly all histological sections of the control mice showed incomplete healing response. In addition, responses in mice sacrificed after 5 days of wounding were characterized by incomplete re-epithelialization, mild granulation tissue formation and absence of collagen fibres (Table-4, Figure-3), while those in mice sacrificed after 10 days were associated only with incomplete re-epithelialization (Tables-(4, 5), Figures-(3, 4, and 5)).

![Figure 3-Histological section of specimen (skin tissue) of days after wounding, with no evidence of healing (control group). (H&E staining, X10 magnification).](image-url)
Figure 4-Histological section of specimen (skin tissue) of 5 days after wounding. Incomplete healing with incomplete re-epithelialization, (H and E, X10)

Table 4-Main histological features of control groups

| Control groups | Day 3 | Day 5 | Day 10 |
|----------------|------|------|-------|
| Epithelialization | No evidence | Incomplete | Incomplete |
| Granulation tissue | Mild | Mild | granulation tissue : Moderate |
| Inflammation | Moderate | Mild | Inflammation: No evidence |
| Collagen | Absent | Absent | Collagen: Present |
| Necrosis | Present | Absent | Necrosis: Absent |

Figure 5-Histological section of a specimen of control mice 10 days after wounding. Incomplete healing response, granulation tissue formation (fibroblast and angiogenesis). (H and E X40)
Histological evaluation for groups treated with 810nm near-infrared laser

Irradiated groups were classified into two groups according to laser dose.

**Group 1**: irradiated for 5 minutes exposure time at a dose of 12.5J/cm².

**Group 2**: irradiated for 15 minutes exposure time at a dose of 37.4J/cm².

- **Histological assessment 3 days after treatment by NIR laser**

Three days after treatment, no evidence of healing response was seen in all irradiated groups and the respective control group (Table-5, Figure-6).

![Figure 6](image6)

* Significant (Chi-square=15, df= 2, P=0.001)

Irradiated groups for 3 days after treatment (NIR 810nm laser)

- **Histological assessment 5 days after treatment by NIR**

Five days after treatment, incomplete healing response was seen in irradiated groups and the respective control group, as expressed by incomplete re-epithelialization, enhancement in granulation tissue formation and collagen deposition. (Table-5, Figures-(7 and 8)).

Significant differences in granulation tissue and collagen formation was seen between irradiated and control groups (p=0.001) (Figure-7).

![Figure 7](image7)

* Significant (Chi-square=15, df=2, P=0.001)

Irradiated groups 5 days after treatment(NIR 810nm laser)

Figure 7-Semi-quantitative histopathological evaluation 5 days after wounding. Groups irradiated by 810nm NIR laser.
Figure 8-Histological section of the 5 days after wounding mice irradiated by NIR 810 nm (15 minutes exposure time, 37.4 J/cm² dose) showing incomplete healing with incomplete re-epithelialization. (H and E, X10).

- **Histological assessment 10 days after treatment by NIR laser**
  
  Examining wounds at day 10, irradiated groups showed complete healing responses with complete re-epithelialization. (Table-5, Figures-(9 and 10).

Groups exposed to 37.4J/cm² for 15 min showed marked granulation tissue formation, indicating better healing response, while groups exposed to 12.5J/cm² for 5 min showed moderate granulation tissues (p=0.001). The control group showed incomplete response of healing (Table-5).

**Table 5-Main histological features of groups irradiated by 810nm near infrared laser**

| Flow up period | groups irradiated by 810 nm | Control |
|----------------|----------------------------|---------|
|                | Irradiated groups          |         |
|                | Exposure time = 5 min      | Exposure time=15 min | Dose = 37.4 J/ cm² | Dose = 12.5 J/ cm² | no evidence of healing response | no evidence of healing response | no evidence of healing response |
| Day 3          | no evidence of healing     | no evidence of healing | complete healing response |
| Day 5          | incomplete healing         | Incomplete healing | response |
| Day 10         | Complete healing           | complete healing response | Incomplete healing response |

* Significant (Chi-square=15, df=2, P=0.001)

Irradiated group 10 days after treatment (NIR 810nm laser)

Figure 9-The Semi-quantitative histopathological evaluation 10days after wounding. Irradiated by NIR 810nm laser
Figure 10- Histological section of mouse wound, 10 days after wounding, irradiated by 810 nm NIR (5 minutes exposure time, 37.4 J/cm² dose), showing complete healing with complete re-epithelialization and presence of collagen deposition (H and E, X10).

Discussion
The fundamental aim of this study is to examine the impact of 810nm laser radiation on wound healing in mice.

The results showed a significant improvement in histological parameters of healing (granulation tissue formation, re-epithelialization, and collagen deposition) in the irradiated wounds, with the effect being dose dependent.

Epithelialization is an essential structure of wound healing that is used as an important parameter of wound healing development, and without epithelialization, wound cannot be considered as healed. For these reasons, this research concentrated on re-epithelialization as an essential parameter for histopathological assessment of wound healing, in addition to the formation of granulation tissue, collagen deposition and inflammatory cells infiltration [12].

The irradiated groups showed a significant enhancement in the percentage of wound closure and histopathological parameters when assessed at day 5 after treatment, indicating that cold laser was efficient in healing wounds. This finding is in conformity with the results reported by Chung et al. [13], Al-watban et al. [8], Lee et al. [14], Ferreira et al. [15], and Fekrazad et al. [16]. Previous reports showed that various types of cells respond differently to laser irradiation, depending upon wavelength, power density (irradiance) and dose. Different types of cells are involved in the wound repair and tissue regeneration, i.e. fibroblasts and macrophages, (essential cells for granulation tissue formation and angiogenesis) and keratinocytes (fundamental cells for skin re-epithelialization [11].

In the field of wound research, fibroblasts are correlated to the granulation tissue formation which is the most essential component of extracellular matrix responsible for wound tensile strength. Macrophages are attracted to such circumstances with the general essential growth factors, resulting in energetic angiogenesis and augmentation of fibroblasts at wound margins [17] [18].

As an essential explanation for the stimulation of wound healing in mice by using cold laser in this study, such an effect is possibly due to absorption of laser light with specific wavelength by the target tissue, leading to improvement of fibroblast proliferation (increased fibroblastic activity) and the subsequent progress in the formation of granulation tissue and extracellular matrix along with the progress of collagen metabolism. This explanation is in accordance with the results of previous studies which proposed an increased fibroblast activity and proliferation in irradiated groups when compared with non-irradiated subjects. This indicates an increased proliferation of fibroblast in in vivo conditions [19] [20].

In this research, the laser beam was delivered through a beam expander to cover the whole area of the wound, and this method was used in many previous studies [8, 7, 5, 20, 21].
Positive results were also obtained using 810nm near infrared laser at two doses of 12.5J/cm² and 37.4J/cm². These doses lie in the range generally used with near infrared wavelengths, and its advantage is in agreement with the study of Castano et al who found a positive result using an 810nm laser with a beam expander at a dose of 30J/cm² in the treatment of inflammatory arthritis in rats [21]. Several studies proposed that the enzyme cytochrome c oxidase is among the important chromophores located in the mitochondria, representing a unit in the respiratory chain located in the inner membrane of mitochondria. Absorption bands of this protein lie in the visible to near-infrared regions; incident photons absorbed by cytochrome c oxidase increase the activity of this enzyme [22] [23]. The impact of cold laser therapy depends on the stimulation action on cytochrome c oxidase, while the increase in the strength of the response depends on the availability of enough time to have actual influences on the cells. Cold laser therapy depends on improving and recognizing the reactivity of cytochrome c oxidase [22]. Some previous reports showed that irradiance and dose (exposure time) were important in establishing the effect of cold laser therapy.

Conclusions
1. At day 3 of treatment with 810nm near infrared laser at doses of 12.5J/cm² and 37.4J/cm², there was no evidence of wounds healing in irradiated and control groups.
2. At day 5 of treatment, the experiments showed a significant increase in wound healing parameters (granulation tissue formation, re-epithelialization and collagen deposition) in irradiated groups.
3. Near infrared 810nm laser had photobiostimulation effects on wound healing at irradiance of 41.63mW/cm² and doses of 12.5J/cm² for 5 minutes and 37.4J/cm² for 15 minutes exposure time.
4. A complete picture of wound healing response appeared in all irradiated groups within 10 days of treatment, as expressed by complete re-epithelialization, moderate granulation tissue formation and presence of collagen fibers, while incomplete wound healing responses were observed in un-irradiated control groups within the same period.

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