

Stimulation burn healing using 790 nm diode laser in
rabbit's skin

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Summary

Burn is one of the most important conditions in veterinary and human medicine. The purpose of this study is to evaluate the enhancing therapeutic effect of 790 nm diode laser on the burn healing.

Twenty-five rabbits (mean weight was 2.3 ± 0.03 kg) were divided into 5 groups (A, B, F, H and control group), each group consists of 5 animals. All groups were inflicted with burn (scalds) by 99 degree of Celsius hot water applied for 30 seconds, on skin of the femoral region. Four groups were treated by laser and one group was without laser irradiation as a control group. The animals were treated by diode laser five times a week at different power density. The power density (PD) of 58.9 mW/cm^2 with exposure time 120 seconds was used for group (A), but in group (B), the same PD used with exposure time of 60 seconds, for group (F) the PD was 255 mW/cm^2 and for group (H) the PD was 888 mW/cm^2 and same exposure time of 20 seconds for both last 2 groups. Results of four groups were compared with control group clinically and histopathologically.

The study showed a good response in burn healing in (F) and (B) and (A) group, with no differences between group (H) and control group. The treatment of 5 times a week gave benefit in acceleration of the burn healing. The histological examinations revealed an increase in the proliferation of epithelial cells from the burn edge and decrease in the inflammatory cells infiltration in group F, B and A. The statistical results showed that the best results are in group (F) and group (B). The diode laser is effective in accelerating burn healing if used 5 times a week.

تحفيز التئام الحروق باستخدام ليزر الدايبود (ذي الطول الموجي ٧٩٠ نانومتر) في الارانب

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الهدف من هذه الدراسة هو تقييم تأثير ليزر دايبود ذي الطول الموجي ٧٩٠ نانومتر في تسريع شفاء الحروق استخدم في هذه الدراسة ٢٥ ارنب قسمت الى خمسة مجاميع كل مجموعة تضم خمسة ارنب. خضعت اربع مجاميع منها الى التشعيع بالليزر واطء القدرة وتركت مجموعة بدون اي علاج كمجموعة سيطرة.

تمت عملية احداث الحرق (سمط) في الحيوانات تحت التخدير العام باستخدام الماء الحار بدرجة حراره ٩٩ درجة مئوية على الجهة الظهرية من الفخذ وقد نتج عنه حرق من الدرجة الثانية. حيث تمت متابعة المجاميع من خلال المشاهدات اليومية وقياس مساحة الحرق واخذ عينات للفحص النسيجي في الايام خمسة و سبعة بعد التشعيع.

خضعت المجموعة A والمجموعة B الى نفس كثافة القدرة $٥٨٩,٠٠٥ \text{ mW/cm}^2$ واط/سم^٢ بينما كانت مدة التشعيع ١٢٠ ثانية في المجموعة A و ٦٠ ثانية في المجموعة B. اما المجموعة F فقد كانت كثافة القدرة $٢٢٥,٠٠٠ \text{ mW/cm}^2$ واط/سم^٢ وفي المجموعة H كانت كثافة القدرة $٨٨٨,٠٠٠ \text{ mW/cm}^2$ واط/سم^٢ مع مدة تشعيع ٢٠ ثانية في كلتا المجموعتين. وقد خضعت كل المجاميع للتشعيع بالليزر لمدة خمسة ايام في الاسبوع. اظهرت النتائج استجابة في تسريع شفاء الحروق في المجاميع F و B و A مقارنة بمجموعة السيطرة. كما اظهر الفحص النسيجي تكاثر الخلايا الظهارية في المجاميع F و B و A وارتشاح طفيف للخلاية الالتهابية في اليوم الخامس من التشعيع. اما المجموعة H فلم تظهر فروقات ملحوظة بالمقارنة مع مجموعة السيطرة.

نستنتج من هذه الدراسة امكانية تسريع شفاء الحروق باستخدام ليزر دايبود ذي الطول الموجي ٧٩٠ نانومتر وبقدرات واطئة من الطاقة.

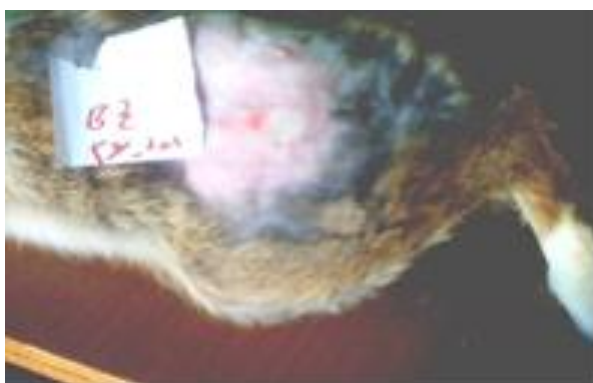
Introduction

For approximately forty years, light in form of low-level laser therapy (LLLT) has been used in treatment of a myriad of conditions. Low level laser therapy used in photobiostimulation, leading to biological and physical effects has been reported. Low level laser therapy has been found to modulate various biological processes (1), such as increasing mitochondrial respiration and ATP synthesis, facilitating wound healing (2) and promoting the process of regeneration (3). The LLLT action depends on wavelength, power density and exposure time. The most effective irradiation is that in the red and near infrared range of the spectrum. The most commonly used sources are the helium-neon laser and diode laser. In clinical practice LLLT could be considered for short-term relief of pain and treatment of many cases like wound injuries and burns, which were found to be highly, affected with LLLT (4). In this study, the effect of low level laser irradiation on burn healing in rabbits was investigated. The burns were irradiated by 790 nm diode laser in continuous mode. An understanding of the mechanism of laser tissue interaction is the basis for further development of diagnostic and therapeutic application. The purpose of this study was to evaluate enhancement effect of burn healing by using 790 nm diode lasers.

Materials and Methods:

Animal group: Rabbits were divided into five groups; each group consisted of five animals. Each group was caged separately, with standard animal pellets and diet, and was subjected to same environmental conditions. Hair plucking was made after using tranquillizer (acepromazine maleate 2) with 0.5 ml/10kg body weight dose. This was done one hour before animal exposure to experimental work. General anesthesia was used to prevent animal's pain and movement. A dose of 0.24 ml/kg body weight (b.w) of xylazin 2% and ketamine HCL (in dose 0.2 ml/kg b.w) were used to anaesthetize the animal by intramuscular injection. In 4 groups the experimental burn was treated with laser with different power densities .

Burning procedure: The experiment involved two preliminary studies to determine the optimum burn type (scalds) according to the temperature achieved and the time interval of the contact, with 99 degree of Celsius hot water applied for 30 seconds. Deep second degree burns were induced figure (1). Burn wound was inflicted on dorsal surface of femoral region, by using a plastic cylinder applied perpendicularly with a 1.2 cm diameter round end (area 1.13cm²). The hot water applied directly on prepared skin segment, thus the maximal size of the burn induced was (1.3-1.4cm) in diameters .



Figures (1) Type of burn

Diode laser system

Diode Laser "K laser 4" (Made in Italy, Eitech Sri, and 31100 Treviso) was used in this work. This laser system is (class IV) , wavelength 790±15%, 6 W max power: CW mode, Modulation frequency 1-20000Hz, Duty cycle 50%, Optical fiber laser 3mm diameter, Coupling spot size 8mm diameter, Beam laser divergence 60 milliradians ±20%, Treatment time calculated automatically with aiming beam of 2.5 mW max power, of 635-650 nm wavelength,.

Irradiation procedures: The rabbit was fastened on a special table by belts to prevent animal's movement during laser irradiation process and to ensure that laser light is incident perpendicular on the scalded surface and surrounding healthy skin. Burn wounds were exposed to first laser radiation after six hours postoperative, five times a week for four weeks. Thus, the healing of irradiated wounds was compared with that of non-irradiated burns in the control group. Power density and exposure time used in this work are listed in table (1).

Table (1). Laser irradiation power density and exposure time in all groups.

Animal group	Power density mW/cm ²	Exposure time in second
A	58.9	120
F	255	20
B	58.9	60
H	888	20

Measurement techniques:

Visual data were recorded every day by means of following criteria e.g., color, shape, and surface. Size index, (two-dimensional diameters of each wound) was measured by geometrical ruler each day as shown in figure (2).



Figures (2) Measurement technique

Specimens for histopathological examination were prepared by taking tissue samples from the burn site of all groups, on 5th and 7th day postoperatively

Results

Clinical results:

Clinical observations were followed and results are shown in table 2.

Table (2). Clinical results

group	Burn area mm2	Inflammatory zone(redzone) mm	Inflammatory zone disappearance	No. of day(s) crust fall down beforecomplete healing	Healing time
A	113-132	1-2	9th day	1	20 day
B	113-132	1-2	6th day	6	18 day
F	132-153	1	5th day	5	19 day
H	132-153	1-2	7th day	7	23 day
Control	132-176	3	th day9	2	26 day

The appearance of crust was in irradiated groups earlier than in control group. The thickness of this crust was more in control group than in irradiated groups. The crust fell down earlier in irradiated groups



.Figure (3) Features of the burn region



.Figure (4). Inflammation zone in group F and B



Figure (5). Crust formation after 5 days of laser therapy.



Figure (6). Crust fell down after two weeks from ..Laser therapy in group B

Microscopic results:

Control group: At 5th postoperative day the microscopic examination showed that epithelial cells and their nucleus are enlarged in size (see figure 7) with necrosis, destruction and sloughing of the epidermis in the burn region. The burn extended to the epidermis and dermis causing coagulation of the collagen fibers. Severe inflammatory cell infiltration (neutrophils and macrophages) are seen in the dermis and epidermis, with congestion in the blood vessels as shown in figure (8) with edema, proliferation of the fibroblasts, immature collagen fibers in dermis. The epithelialization process was began and showed layer of 1-2 epithelial cells. These cells are formed from the edge of the burn region. Epithelial cells and myofibroblasts extended to burn surface. At 7th day postoperatively, severe fibroblasts proliferation in different directions and shapes with immature collagen fiber production in the dermis are seen with severe inflammatory cells infiltrations (neutrophil, acidophil, macrophages and lymphocytes) in the dermis layer. Coagulation in collagen fibers under the epidermis with neutrophil cells infiltration is seen. Organization in the coagulated epidermis is noticed by large number of fibroblasts. The epithelial cells proliferated from the edge of healthy tissues to the burn region and formed layer of 2-3 cells with myofibroblsts.

Group F: At 5th day post laser therapy, congestion in the blood vessels and mild inflammatory cells infiltration (lymphocytes, neutrophil, and macrophages) in dermis layer are seen. The epithelial cells are proliferated under epidermis region and formed a layer of 6-7 cells with myofibroblsts (figure 9). The histological examination at 7th day of postoperative showed:-Normal appearance of the skin with complete healing of epidermis and dermis layers with all skin appendages is noticed. The hyalinization of the collagen fibers is not appearing.

Group H: At 5th day post laser therapy: Severe inflammatory cells infiltration (neutrophil, acidophil, macrophages) in dermis layer and hypodermis and coagulation in collagen fibers, with congestion in the

blood vessels are seen. The epidermal cells recognized by coagulation, necrosis, sloughing, and destruction and severe inflammatory cells infiltration (neutrophil, and macrophages) and invaded by fibroblasts and fibrous connective tissue production. Proliferation the epithelial cells from the burn edge and form a layer of 1-2 cells figure (10) supported on the fibrous connective tissue of burn surface. At 7th day postoperative:- The histological examination showed proliferation the epithelial cells from the burn edge and form layer of 2-3 cells and myofibroblasts. Mild inflammatory cells infiltration (lymphocytes, acidophil,) in dermis layer and hyalinization of the collagen fibers. Congestion of the blood vessels and proliferation of fibrous connective tissue are seen also.

Group B: At fifth day of the laser therapy, Normal appearance of the skin with mild inflammatory cells infiltration and hyalinization of the collagen fibers are observed. The epithelial cells are proliferated from the edge of healthy tissues to the burn region and formed layer of 3-5 cells thickness with myofibroblasts. At 7th day of begin experiment: The histological changes recognized by collagen fibers presentation with less fibroblast cells which are regular in their directions. -Decrease in the inflammatory cells infiltration in dermis layer.-Infiltration of large number of fibroblasts in burn region with fibrous connective tissue production. The epithelial cells and myofibroblasts are proliferated and consisted of a layer of 6-7 cells on the recognized burn surface (see fig 11).

Group A: At 5th day post-laser therapy: The blood vessels were congested with hemorrhage and appearance of the mature collagen fibers in dermis layer. Mild inflammatory cells infiltration (neutrophils, lymphocytes and macrophages) are seen in dermis. Epithelial cells proliferated at the burn edge and consisted of 2-4 immature cells layer. The organized burn region was less than that appeared in control group. At 7th day postoperative, the histological changes are the same at day 5th with maturation of the organized region that leads to decrease in the thickness of burn region. Proliferation of the epithelial cells and a layer of 3-5 cells extend to the burn region are shown (see fig 12).

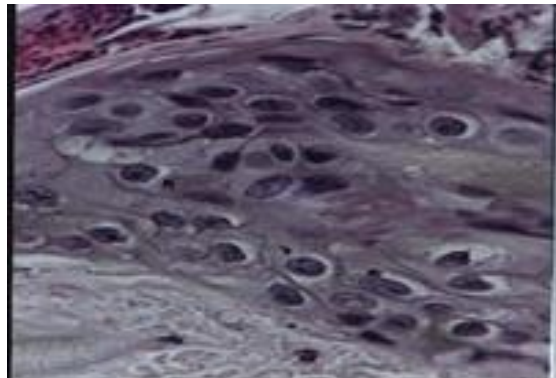


Figure (7) Enlargement of the epithelial cells and their nucleus in size, at day five in control group. X 400, H&E.

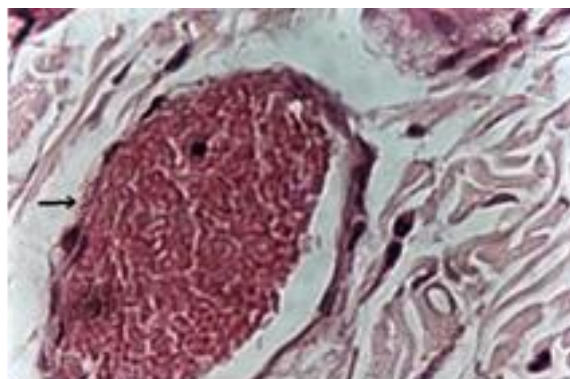


Figure (8) Congestion the blood vessels, Coagulation of the collagen fibers and immature fibroblasts at 5th day postoperative in control group. X 400, H&E.

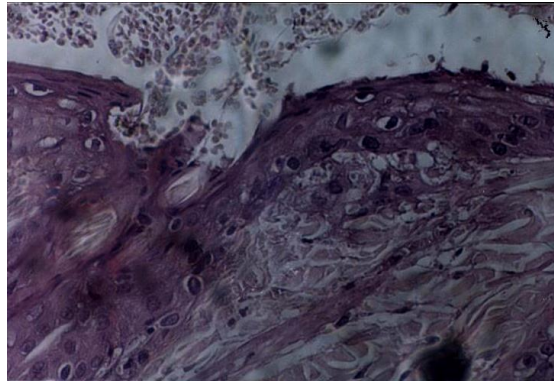


Figure (9) Group F- The epithelial cells are proliferated under epidermis region and formed layer of 6-7 cells with myofibroblasts, at day 5 post laser therapy. X 200, H&E.

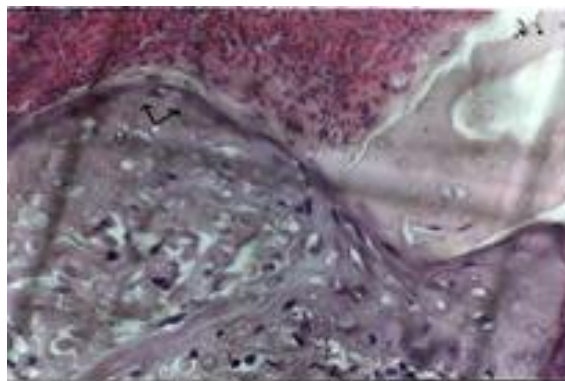


Figure (10) Proliferation of the epithelial cells from the burn edge and form a layer of 1-2 of cells and myofibroblasts at 5 day post laser therapy in group H.X 200, H&E.

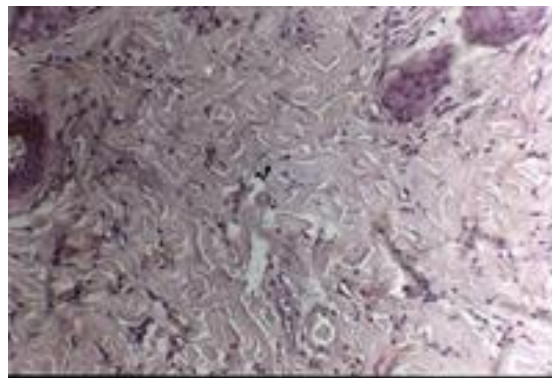


Figure (11) Coagulation of the collagen fibers at day 7 post-irradiation in group B. X 200, H&E.

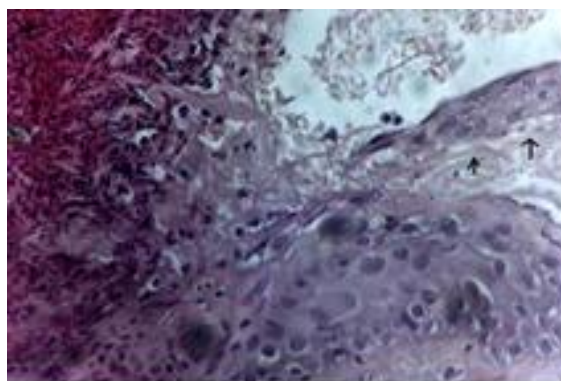


Figure (12) Proliferation the epithelial cells which consist of a layer of 3-5 cells, 7 day post-irradiation in Group A.X 200, H&E.

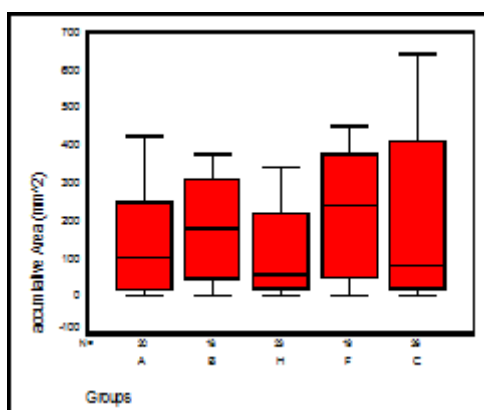


Figure (13) Maximum burns wound area, range of mean and median of burns area.

The statistical results in all groups can be summarized in table (3) and Figures (14, 15,16,17,18,19,20,21, and 22) which represent the autoregressive mode according to different power density and exposure time.

Table (3). Statistical results

Group	R	R ²	F	SIG	Time B	SIG
A	0.931	0.867	117.71	.0000	-8.29	.0000
B	0.990	0.980	810.03	.0000	-24.6	.0000
F	0.983	0.966	492.47	.0000	-29.7	.0000
H	0.928	0.861	130.41	.0000	-16.61	.0000
Control	0.915	0.839	125.09	.0000	-25.9	.0000

Constant BO	SIG	t-value	SIG
385.8	.000	-10.84	.0000
414.51	.000	-28.46	.0000
520.017	.000	-22.19	.0000
315.7	.000	-11.42	.0000
545.99	.000	-11.185	.0000

where R means correlation coefficient, R² means determination coefficient, F means goodness of fit of mathematical models, SIG means significant degree, time B means the effect of the time in healing process, t-value means test statistical grad which null by standardized value of parameter estimation by the effect of time.

The method used in statistical results was autoregressive AR (1)-lag (1) (table3). It shows that: Correlation coefficient (R) which refers to the evaluation of the effect of time in the healing processes. And the best result was in group B, F and A respectively. And there are no differences between group H and control group.

Determination coefficient (R²) was the best result in group B and F respectively and there were no differences between group A, H and control group.

T-value was highest in group B and F, but there were no differences between group A, H and control group.

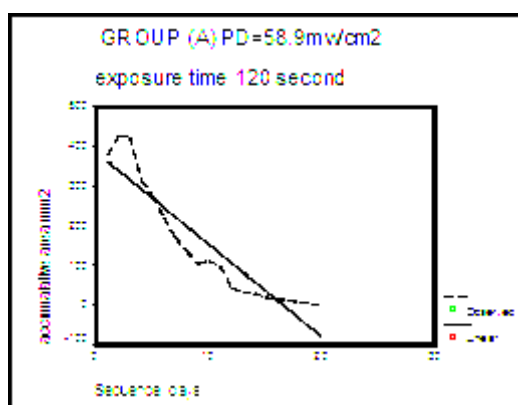


Figure (14) Relation between burn area in group (A) and time in days and shows the long linear trend of linear autoregressive mode

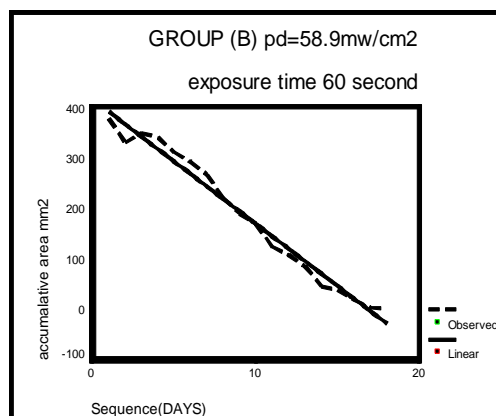


Figure (15) Relation between burn area in group (B) and time in days and shows the long linear trend of linear autoregressive mode.

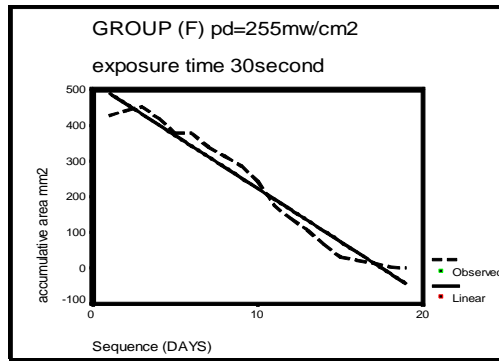


Figure (16) Relation between burn area in group (F) and time in days and shows the long linear trend of linear autoregressive mode.

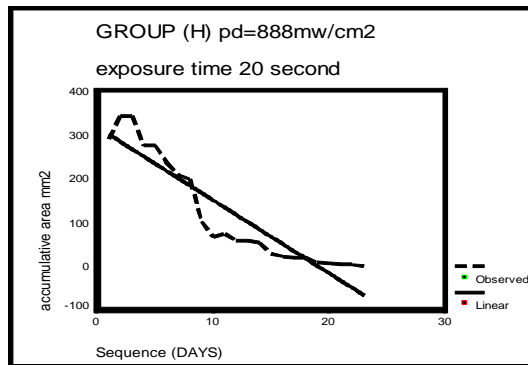


Figure (17) Relation between burn area in group (H) and time in days and shows the long linear trend of linear autoregressive mode

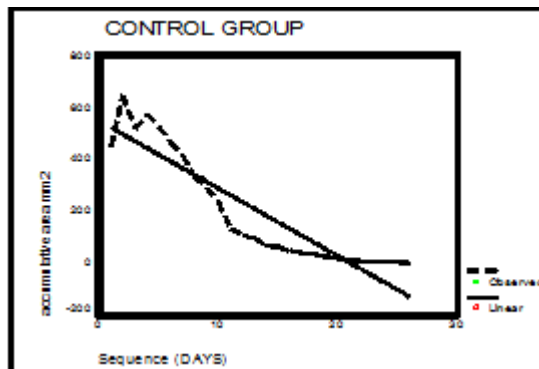


Figure (18) Relation between burn area in control group and time in days and show the long linear trend of linear autoregressive mode

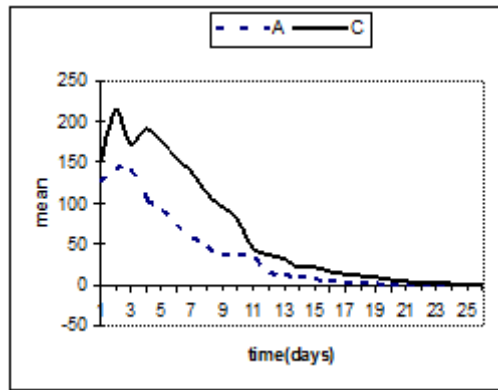


Figure (19) Relation between mean value and time (days) in control group and group (A, PD=58.9mW/cm² with exposure time 120 second) through the different healing stages.

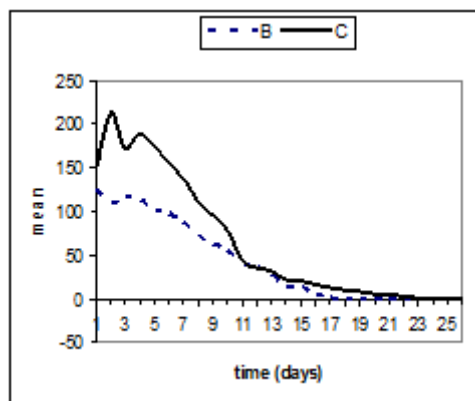


Figure (20) Relation between mean value and time (days) in Control group and group (B, PD=58.9mW/cm² with exposure time 60second) through the different healing stages.

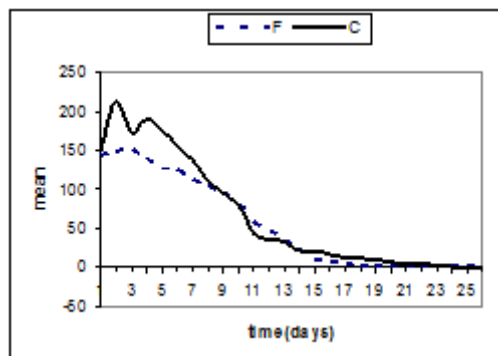


Figure (21) Relation between mean value and time (days) in control group and. group (F, PD=255mW/cm² with exposure time 30second) through the different healing stages.

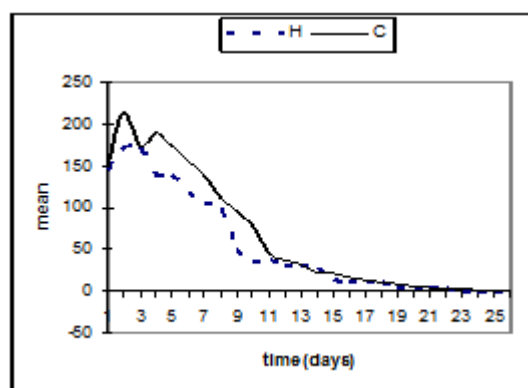


Figure (22) Relation between mean value and time (days) in control group and group (H) PD=888mW/cm² with exposure time 20 second) through the different healing stages.

DISCUSSION AND CONCLUSIONS

In this study the stimulation of burn healing by laser irradiation showed acceleration healing in different stages and there is rapid healing of the burn in group F, B, A and there was no differences between group H and control group. The cause of enhancement of healing, first of all, is the increase of the proliferation of the epithelial cells, fibroblasts and new vessels formation following laser irradiation⁽⁹⁾. This study revealed that burn healing in group F, B and A is vastly enhanced and takes dramatically less time to complete. Also stimulation is the function of immune cells and the lymphatic and vascular systems. Where Yu⁽³⁾ revealed in histological evaluations that laser irradiation improved wound epithelialization, cellular content, granulation tissue formation, and collagen deposition in laser-treated wounds and this agrees with Thamer⁽⁶⁾, Schindl⁽⁷⁾, Bisht⁽⁸⁾ and Reddy⁽⁹⁾.

In the present work the histopathological examination revealed that the proliferation of the epithelial cells increased in group (F), group (B) and group (A) more than in group H and control group as show in figure(9),(10) and (12).these results were confirmed with Koutn study⁽¹⁰⁾ where revealed that the laser light of the near-infrared region (830 nm) stimulates cell proliferation to a varying degree according to the irradiation doses and revealed at 96 hours the proliferation activity was significantly higher in the treated populations than in the non-treated cells.

Mary Dyson⁽¹¹⁾ revealed that LLLT can help wounds healing. Acute inflammation is resolved more rapidly and the proliferative phase of healing begins earlier. So the low level laser therapy decreased the inflammatory reaction of wound healing and this agrees with the result of this study that indicates good response of laser therapy in group (B), (F) and confirmed the statistical results as shown in values of correlation and determination coefficient and t-value, which were more in group F and B than another groups. The good response of group F, B may be related to stimulation of the inflammatory cell or activation of the chemotactic factor by irradiation with these doses. Where Petrova⁽¹²⁾ observed that under LLLT the high phagocytic activity of macrophages was observed as early as 6 hours after trauma. Bisht et al (8) showed an increased leukocytic infiltration and neovascularisation was seen in the laser irradiated wounds. These results participated in rapid debridement of the wound and prepared conditions for the proliferation phase. Kesava et al.⁽¹³⁾ showed that good response may be related to changes the permeability of membrane. These physiological changes affect a variety of cells which produce the various cell types including macrophages, fibroblasts, endothelial cells and mast cells. So increase in the permeability of the cell membrane to calcium (Ca⁺²) ion lead to Ca⁺² ion entering the cell, activating variety of enzyme molecules and can produce a cascade

of intracellular signals that initiate acceleration or inhibition of biological processes⁽¹⁰⁾⁽¹⁵⁾. A cascade of reactions connected with alteration in cellular homeostasis parameters (pH, Ca⁺², cAMP, ATP and some others) is considered as a photosignal transduction and amplification chain in the cell⁽¹⁾ and so that Cytochrome c oxidase is considered as a photoacceptor when cells are irradiated with monochromatic red to near-IR radiation where cytochrome c oxidase becomes more oxidized which means that the oxidative

metabolism is increased due to irradiation at laser irradiation 633, 670, and 820 nm wavelength. The evidence showed that changes in the redox properties of the respiratory chain components following photoexcitation of their electronic states ⁽¹⁶⁾ and this supports the study of Karu ⁽¹⁾ that revealed the mechanism of low-power laser therapy at the cellular level is based on the electronic excitation of chromophores in cytochrome c oxidase which modulates a redox status of the molecule and enhances its functional activity. Chukuka ⁽¹⁷⁾ and Bill ⁽¹⁸⁾ have shown that light energy is absorbed by endogenous chromophores in the mitochondria and used to synthesize ATP. The resulting ATP is then used to power metabolic processes; synthesize DNA, RNA, proteins, enzymes, and other biological materials needed to repair or regenerate cell and tissue components, rapid mitosis or cell proliferation and restore homeostasis. The clinical benefits resulting from these demonstrated effects are pain control and tissue repair in the multiple of circumstances.

The dose in group H of laser therapy causes prolong time in the inflammation stage, may be due to inhibition in the biological processes of the cell ⁽¹⁹⁾. Lagan et al ⁽²⁰⁾ revealed that LLLT provides no advantages in the management of minor postoperative wounds over current practice, in this study five times laser therapy every day in group B and F revealed that the hyalinization is reduced. This results enhancement of the acceleration of wound healing in those two groups before group A, H and control group and rapid complete burns healing. The histopathological examination in group A, H indicated that the hyalinization of the collagen fibers still appear, which is related to fibroblasts proliferation. There are many studies that revealed with use of LLLT the proliferations of the fibroblast increased. Kreisler ⁽²¹⁾, Vinck ⁽²²⁾ revealed that when the cells are irradiated by 809-nm diode laser, a considerably higher proliferation activity will appear than the controls, where Asencio-Arana ⁽⁵⁾ showed that The differences were significantly up to 72 hours after irradiation, but Pereira ⁽²³⁾ revealed that the Irradiation with 3 and 4 J/cm² lead to increased procollagen synthesis of cultured fibroblasts by a low-power laser. Almeida-Lopes ⁽²⁴⁾ revealed that the LLLT acts by improving in vitro fibroblast proliferation. Where Efendiev et al ⁽²⁵⁾ recorded the same result with LLLT, but with increasing the power of radiation, the reparative processes in a wound are slowed and disturbed. The rate of wound contraction was proportional to cell number, that begin from the healthy tissue surrounding the wound and often increasing its surface area ⁽²⁶⁾. There are many studies showed that Diode laser enhancement healing process ^{(27), (5) (28)}. Where Rodriguez ^{(29) (30)} revealed that when patients whose submitted to low-intensity infrared laser beam 830 nm the healing process was faster with reduction of sore area and immediate relief of pain following first irradiation

- 1- The diode laser is good therapy for enhancement of tissue regeneration and burn healing.
- 2- The best parameter of diode laser (790 nm) enhancing burn healing was Power density 0.255 W /cm² with exposure time 20 second and Power density 0.0589 W /cm² with exposure time 60 second.
- 3- Increase the Power density 0.888 W /cm² with exposure time 20 didn't show any improving to burn wound healing.
- 4- The histological examination revealed increase the proliferation of the epithelial cells

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