Effect of 1064-nm Pulsed High Intensity Nd: YAG Laser on Full Thickness Burn Wound: An Experimental Study

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ABSTRACT

Background: *Wound healing is a complex biologic and biochemical process that starts right after an injury and the repair of extensive burn wounds has long been a long term problem.* ***Purpose:*** *The aim of this study was to assess the effect of 1064-nm Pulsed High Intensity Nd: YAG Laser in the treatment of full thickness (3rd degree) burn wound on rats.* ***Methods:*** *Thirty two clinical healthy male Sprague-Dawley (SD) rats with induced 5 cm² dorsal full thickness burn wound, two groups of rats were studied; rats were randomized into two groups. Study group, fifty rats received 5 weeks pulsed high intensity Nd: YAG laser according to designed protocol, 10 minutes/session, 6 times per week and control group without laser treatment.* ***Results:*** *The result of this study revealed that there was statistical significant difference in wound surface area and percentage of healing for study group after 10,15,20,25 and 30 days as compared to control group. Also histological examination revealed rapid stimulation of fibroblast with collagen formation and complete wound closure in study group.* ***Conclusion:*** *The results have demonstrated the objective effect of pulsed high intensity Nd: YAG laser in treatment of full thickness burn wound and used as an effective, non invasive, non expensive and a new trend physical therapy modality in the treatment of full thickness burn wound.*

Key words: *Full Thickness Burn, Wound Healing, Pulsed High Intensity Laser.*

INTRODUCTION

Millions of people sustain different degrees of burn annually[1,2]. Burns often happen unexpectedly and have the potential to cause dysfunction, lifelong disfigurement and death. The repair of extensive burn wounds has long been a problem[3].

Severe burns cause extensive damage and are complicated by loss of body fluids, injury in the cutaneous vasculature and delayed wound healing[4].

Burn wound are characterized by central necrosis surrounded by an area of stasis with compromised perfusion. Secondary aggravation of the burn wound due to ischemia in the zone of stasis can also result in necrosis[5].

Full thickness burns cause damage to all layers of the skin and some subcutaneous tissues. It lacks viable epithelial elements, thus skin grafting for wound closure is often required, the burned skin is painless and appears dry, white and leathery or charred and cracked also the underlying fat may also be exposed[6].

LASERS provide a unique tool for medical therapy and surgery. The basic mechanism of laser application is its monochromaticity, which allows efficient coupling to the peak absorption of chromophores, enabling maximal photoactivation and stimulation of biological processes7.

The majority of studies conducted over the last thirty years in Laser therapy have been carried out with medium and low intensity Laser devices (Low Level Laser Therapy: LLLT), with wavelengths in the infrared and near infrared 600 - 900 nm . Within this spectrum the Laser beam is partially absorbed by the natural chromophores, like melanin, which withhold part of the energy irradiated. This study on the other hand is based on the use of Nd: YAG Pulsed High Intensity Laser Therapy (HILT), which characterized by a wavelength 1046 nm that allows it to penetrate and spread more easily through the tissue due to not having an endogenous chromophore[8,9].

Over 75 trillion cells in a human body need electrons to communicate with each other. Laser light delivers these required electrons directly to the cells and enhances both their ability to communicate with each other and their ability to produce ATP (energy).Additionally, Laser stimulate the release of healing enzymes, which were required for optimal functioning, as well as the production of endorphins, which are natural pain killers[10].

Biostimulation of wound healing process as reported by several investigators results stimulation of fibroblasts proliferation; significant increases in reepithelialization, collagen synthesis, granulation tissue formation; acceleration of wound closure, macrophage stimulation, and extracellular matrix production[12,13].

Over the last few years the High Intensity Laser Therapy has been making its mark with excellent results in sports traumatology and pain therapy[8,9,14,15], for this reason we decided to assess the possibility of transferring this method to the cure of different types of wounds as burn wound, therefore prepared an animal model with full thickness burn wound, therefore; the objective of this study was to assess the efficacy of Pulsed High Intensity Laser (HILT) on healing of full thickness burn wound.

MATERIALS AND METHODS

Animal preparation

Thirty one clinical healthy male Sprague-Dawley (SD) rats' weights ranging from 280–320 g (mean 300.7±12.05 g) and aged about 16 weeks were used in the study (supplied from Biochemistry Department, Faculty of Medicine). The animals were caged individually in a controlled environment at 23-25°C and 50% humidity with a 12 hours artificial light cycle. Animals were housed in solid bottomed cages; food and water were allowed on an ad libitum schedule. The animals have been divided in two groups: study group 15 rats and control group 16 rats. A mixture of Xylazine and ketamine at ratio of 1:0.5 was injected IM (intramuscularly) to induce general anesthesia (Xylazine, Sanofi; Sante Nutrition, Laballasrere-3301, Libonne Codex, France; Ketamine, Rotex-medicr GMBH, Germany). The site for experiment prepped with 10% betadine solution, after anesthesia area of the back to which the burn would be inflicted were prepared (fixed part for all rats) using an electric hair shaver. Plastic jelly cups with a hole of 2.6cm in diameter cut at the bottom were put around the neck of the rats as collars to prevent them from licking their wounds. Animal protocols were reviewed and approved by the Animal Care and Use Committee and the study approved by the Research Advisory Committee (RAC).

Experiment protocol

All animal were subjected to a preheated rounded metal probe measuring 5 cm^2 (2.5 x2.5 cm) and weighing 300 g, an electric soldering iron, was set at 180 °C. The metal probe applied in contact with the shaved flank of the rat for 20 s, there was no additional pressure applied to the rod while in contact with the skin except the natural gravity. One round burn wound measuring 5 cm^2 were created aseptically at the middle of the spinal column on the back of each animal. The model of the burn wound was produced according to Hoekstra standard [16]. Weight variations of the rats over the course of treatment and abnormal signs and behaviors observed on the rats after the burn injury was detected along the study period.

In each rat, the wound in control group (untreated) was irrigated with normal saline and left without treatment, while the wound in study group (treated group) was exposed for 5 weeks, 10 minutes 6 days/week consecutively to a Pulsed High Intensity Laser High intensity laser machine by ASAsrl company, Hilterapia, HIRO 3.0, Italy. High peak power produced is 3 KW, energy content 150-350 mJ, brief duration 120-150 µ. Sec., frequency 10-30 HZ, duty cycle 0.1%, wavelength 1064 nm, irradiated spot diameter 0.5 cm, energy density 360-710 J/cm2 .The laser irradiation process was performed by keeping the head of the laser device 0.5 cm perpendicular to the wound and at a 1 cm distance from the wound surface. The irradiation was performed without contact with the wound and done in three phases (initial, intermediate and final phase) according to designed protocol for laser application (Table 1).

Table (1): Treatment protocol of pulsed high intensity 1064 Nd: YAG laser for wound healing.

Phase	Mode of application	Frequency (Hz)	Time (minute)
Initial	Fast Scanning (Vertical and horizontal on wound area)	15	3
Intermediate	Applied at the periphery of the wound (Fixed points)	15	4
Final	Slow Scanning (Vertical and horizontal on wound area)	15	3

Evaluation methods

The efficacy of pulsed high intensity laser has been evaluated by: Wound Size Measurement and Healing Percentage Calculation and Histological examination.

Wound size measurement and healing percentage calculation

The wound sizes of the rats were measured on days 10, 15, 20, 25 and 30. Each rat was transferred to a transparent, air tight plastic box with diethyl ether soaked cotton wools for general anesthetization by inhalation. The small volume of the box limited the movements of the rat and saved the drug; yet there was sufficient air space to prevent suffocation when the lid was closed. As soon as light anesthetization was achieved (when the rat stopped moving), the rat was taken out for weighing, photographing and tracing of the wound size. A 75% alcohol cleaned transparency was laid on the wound surface while the wound size on the transparency was outlined with a permanent marker.

The wound size was later measured by counting the number of squares on a standard graph paper that were included by the wound outlines on the transparency. The healing percentage of the wounds was calculated by comparing the wound size on day 30 to that of first day of burn for both groups.

Histological evaluation

Histological analysis was used to determine the true burn depth. The state of viable adnexal structures (hair follicles) provided helpful information of the level of burn depth. Necrosis observed in the surrounding tissues. On day 1 after the infliction of burn injury, histological samples taken from both groups showed that the burn depth had reached the deep dermal layer that was equivalent to third degree burn injury. The epidermis layers of both groups were completely destroyed leaving behind a few hair follicles with the hair shafts gone. The deep dermal structures and the fibroadipose tissues were destroyed, indicating (full thickness) third degree burn.

Also histological evaluation was collected from the wound area including a part of the skin edge on the 6th and 15th days post burn. The specimen was preserved in 10% formalin solution for slide preparation, which was later stained with hematoxlin eosin stain.

RESULTS

Data were collected and statistically analyzed using repeated measures ANOVA to test hypothesis and to control both within and between variabilities at level of significance of 0.05 by using SPSS version 16.0. At the beginning of the experiment, thirty six rats were available for assessment. However, three rats died on day 7 and two on day 10, possibly from pulmonary, gastrointestinal and/or renal complications.

Weight Variations of the rats over the course of treatment

The average weight of the thirty one rats (excluding five rats that died on days 7 and 10 respectively) used was 300.7g. There was a sharp decrease of 35g, from 300.7g to 265.7g, five days after the induced burn injury (Figure 1). From day 6 onwards, the rats gradually gained weight to 275.1g, 289.2g, 295.6g, 302.2 and 310.5g on days 10, 15, 20, 25 and 30 respectively.

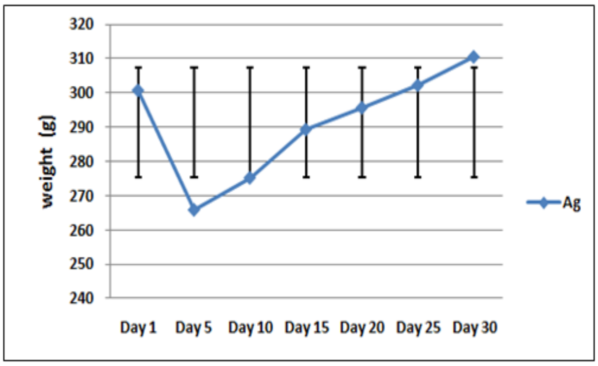

Fig. (1): The average weight of the rats over the 30 days post burn period.

Abnormal Signs and Behaviors Observed on the Rats after the Burn Injury

Immediately after the burn injury, all of the rats arched their backs after the thermal trauma until around day 5 post burn. Also, nose bled was found in seven of the rats on day 3 post burn, which might be due to nasal mucosal capillary damage from dehydration after the burn injury. Occasionally, diarrhea was observed in some of the rats. Hair loss was another major sign noticed from the animals.

Degree of wound healing

The percentages of wound healing of the study and control groups on days 10, 15, 20, 25 and 30 were shown in (Figure 2).The wound healing percentage of day 10 which was obtained by comparing the wound size difference between first day after burn and day 10 was 24% for the experimental group and 0.8 % for the control group. The wound healing percentage increased further to 41%, 57% and 74% for the experimental group and 1.8 %, 2.2 % and 2.6 %for the control group on days 15, 20 and 25 respectively. Finally, on day 30, the wound healing percentage of the experimental group reached 97% while the control group attained 3.6 %. There is significant difference was found in the wound healing percentage between the experimental and control groups in all five assessment days (days 10, 15, 20, 25 and 30) (Table 2).

Table (2): The mean values (Mean ±SD) of wound surface area on days 10, 15, 20, 25 and 30 and percentages of wound healing (%) of the study and control Groups.

		Study group (n=15)	Control group (n=16)
Wound Surface Area (cm^2) (Mean ±SD)	Day 10	3.78 ± 0.27	4.96 ± 0.63
	Day 15	2.95 ± 0.30	4.91 ± 0.09
	Day 20	2.11 ± 0.18	4.89 ± 0.11
	Day 25	1.29 ± 0.28	4.87 ± 0.11
	Day 30	0.15 ± 0.18	4.82 ± 0.11
Percentages of wound healing (%)	Day 10	24%	0.8 %
	Day 15	41%	1.8 %
	Day 20	57%	2.2 %
	Day 25	74%	2.6 %
	Day 30	97%	3.6 %

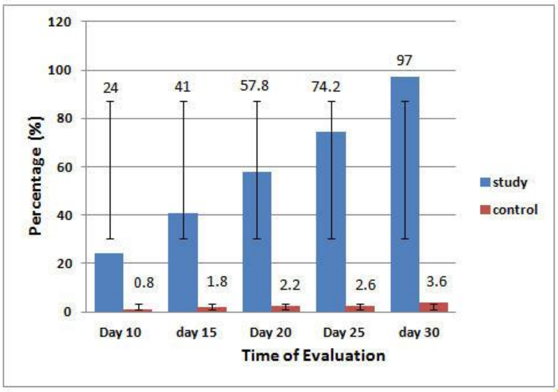

Fig. (2): The Percentage (%) of wound healing of the study and control groups on days 10, 15, 20, 25 and 30 compared to the wound Size on first day (Mean ± SE).

Histological analysis

In control group (untreated), the histopathological readings of the prepared slides of the 6th day post burn showed hemorrhage with inflammatory cells infiltration, which are mainly neutrophils, in addition to congested blood vessels in the gap (Figure 3). In the 15th day slide findings showed irregular fibrous connective tissue in proliferation with congested blood vessels in the gap with infiltration of mononuclear cells. There was also moderate dense cellular fibrous connective tissue with a few mononuclear cells replacing the gap and covered by thick, larger cellular epidermis (Figures 4).

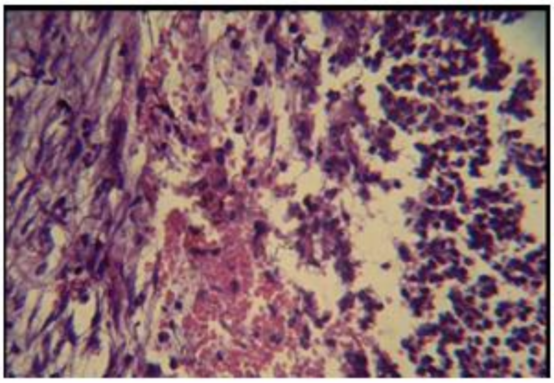

Fig 3. Control group at 6th day post burn shows the gap contains necrotized neutrophils with hemolysis RBC.

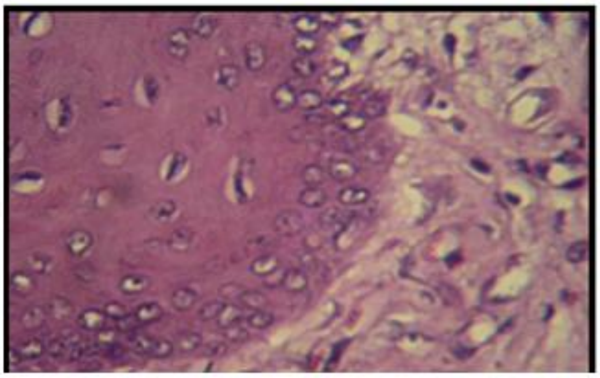

Fig 4. Control group at 15 days shows dense moderates cellular fibrous connective tissue with few mononuclear replaced the gap and cover by very thickness cellular

In study group (treated), the 6th day post burn slide indicated severe infiltration of inflammatory cells, mainly neutrophils with proliferation of fibroblasts forming a few fibrous connective tissues (Figure 5). The section prepared on the 15th day revealed debris material surrounded by a thick layer of connective tissue and dense collage, but less cellular fibrous connective tissue was present in the dermis covered by a thick epidermal layer (Figures 6).

In this group, the wound was completely closed and a skin layer was formed with less scar formation and shrinking of the size of the wound at day 30. When compared to control group, the wound was not yet completely closed and scar formation was noted and there was minimal shrinkage in the size of the wound.

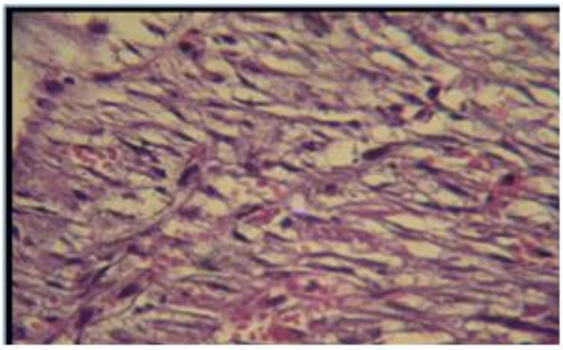

Fig 5. Study group at 6th days post burn shows severs granulation tissue with mononuclear cell infiltrated

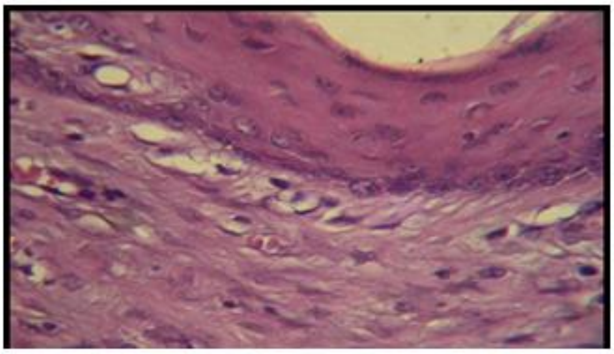

Fig 6. Study group at 15 days shows dense collagens, less cellular fibrous connective tissue present in the dermis covered by thick epidermal layer

DISCUSSION

Lasers currently enjoy wide application in physiotherapy practice, typically for the promotion of wound healing, and currently in burn healing, despite the lack of evidence to support its use. Previous researchers have found evidence of the release of growth factors by irradiated white blood cells, providing a biological basis of this modality in enhancing wound healing. Other investigations reported increased epithelialization and improved tensile strength of scars[17].

The important point is that the stimulatory effects of laser appear to be related to specific events during the first two phases of wound healing: the inflammatory phase and the proliferative phase, indicating that the period of intervention may be critical[13]. For this reason, in this study, in the study group the treatment was performed at days 1 and 3 aiming the inflammatory phase and at days 8 and 10 aiming the proliferative phase. According to Medrado and collaborators[18] laser treatment would reduce the intensity and duration of the inflammatory phase.

The results showed clear promotion of healing in the study group in comparison with the control group. The histopathological examination observed that the good response of this group may be related to stimulation of inflammatory cell or activation of the chemotactic factor by irradiation in the first day after burn. This observation agreed with a previous study[19], in which by using Laser, the high phagocytic activity of macrophages was observed as early as 6 hours.

Laser can facilitate wound healing, which may be due to acute inflammation is resolved more rapidly and the proliferation phase of healing begins earlier[20], therefore, the pulsed high intensity laser decreased the inflammatory reaction of wound healing.

During the healing stage, some studies revealed the presence of neutrophils at the site of injury at 48-96 hours and reached a peak in 72 hours[21,22]. These cells have an important role in healing during phagocytes; they then lyse and enter the cement material[21,22]. The results of these studies are in agreement with the current study.

Fibroblasts are known to be essential in the healing of tissue injuries including surgical wounds, the epithelialization and granulation tissue formation was created in the repair stage; fibroblasts begin to synthesize collagen and ground substances. Also the laser-treated group in comparison with the control group showed higher numbers of fibroblast proliferation in the early stage. These observations confirm the results achieved by a previous study[11] that indicated a possibility of laser-induced fibroblast proliferation during healing mechanism.

On day 15, the process continued and the faster epithelization on study group continued when compared to control group showing even more fibroblast proliferation.

Laser stimulates fibroblasts on wound regeneration by maintenance of a high mitotic activity of the fibroblast in the later healing period[24].The macroscopic and microscopic results in study group could be due to the increased collagen production by fibroblasts.

In the study group there was greater wound contraction on day 30 than the control group. Several theories may help explain the enhanced wound contraction observed in this study. In vitro studies have shown an increase in fibroblast proliferation after irradiation, suggesting that laser therapy may facilitate fibroplasia during the repair phase of tissue healing[25].

In the present study, the histopathological examination revealed that the proliferation of the epithelial cells appeared in fifth days post burn in study group, which was faster than the control group. These results are in agreement with a study that reported that laser induced faster epithelialization that culminated in better formation of the epidermis with formation of normal epidermis and disappearance of the scar[26].

The enhancement of collagen syntheses that may be due to light energy was observed by endogenous chromophores in the mitochondria and used to synthesize adenosine triphosphatase. The resulting ATP was 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[20,27].

A myofibroblast is a modified fibroblast with ultrastructural and functional properties of fibroblasts and muscle cells .Facilitated wound contraction may also be supported by work from researchers who reported that laser irradiation transforms fibroblasts into myofibroblasts. Myofibroblasts are directly involved in granulation tissue contraction and increased numbers could lead to facilitated wound contraction. Cytoplasmic fibrils of actomyosin allow for contraction of myofibroblasts, pulling on the borders of the wound and reducing the size during the repair phase of soft tissue healing. Because our data provided support that pulsed high intensity laser enhanced wound contraction but did not necessarily enhance other variables associated with superficial wound healing, myofibroblast stimulation may be a viable explanation.

The exact mechanism by which laser facilitates wound healing is largely unknown. However, several theories may help explain the enhanced wound contraction observed in this study. In vitro studies have shown an increase in fibroblast proliferation after irradiation, suggesting that laser therapy may facilitate fibroplasia during the repair phase of tissue healing. Our data support this suggestion.

So the observed differences between the study and control groups from day 10, when wounds would have been well into the repair phase of soft tissue healing. However, it should also be noted that other investigators found no in vitro changes in fibroblast proliferation after LLLT. The disparity could be due to changes in specific laser irradiation settings, such as wavelength, duration, power, and intensity.

The pulsed high intensity laser is an effective treatment for enhancing wound contraction of full thickness burn wound. Therefore these data focused on enhanced healing of full thickness burn wounds on rats, and believethat, this is the first step to use pulsed high intensity laser for application on humans.

Conclusion

The results of the present study showing better regeneration and faster healing with restoration of structural and functional integrity as compared to the control group and indicate the favorable beneficial effects of pulsed high intensity Nd: YAG laser photostimulation for the healing process after full thickness burn (Third degree burn). Accordingly, this form of treatment, laser photostimulation, may be of value after thermal burn injury.

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الملخص العربى

دراسة تأثير الليزر النابض عالى الشدة على التئام جروح حروق الدرجة الثالثة فى الفئران : دراسة تجريبية

شفاء الجروح هى عملية معقدة بيولوجيا وكيميائيا وتبدأ مباشرة بعدالاصابة : الهدف من هذه الدراسة هو تقييم تأثير الليزر النابض عالى الشدة على التئام جروح حروق الدرجة الثالثة فى الفئران . تم اختيار اثنين وثلاثين من الذكور الأصحاء من فصيلة (سبراغ داولي السريرية) تم احداث حرق من الدرجة الثالثة مساحتة خمسة سنتيميتر فى الجزء العلوى من الظهر وتم تقسيم الفئران بصورة عشوائية الى مجموعتين . مجموعة الدراسة تلقت الفئران فى هذة المجموعة الليزر النابض عالى الشدة لمدة خمسة أسابيع وفقا لبروتوكول مصمم عشرة دقائق ست مرات في الأسبوع والمجموعة الضابطة بدون استخدام الليزر وكشفت نتائج هذه الدراسة أن هناك فرقا كبيرا ذو دلالة إحصائية في مساحة الجرح ونسبة الالتئام لمجموعة الدراسة بعد عشرة،خمسة عشر،عشرون ،خمسة وعشرون وثلاثون يوما بالمقارنة مع المجموعة الضابطة كما كشف الفحص النسيجي سرعة تشكيل شبكة الكولاجين واستكمال اغلاق الجروح في مجموعة الدراسة . الخلاصة : لقد أظهرت النتائج تأثير الليزر النابض عالى الشدة في علاج جروح حروق الدرجة الثالثة ويمثل اتجاها جديدا فى طرق العلاج الطبيعي في علاج الحروق .
الكلمات الدالة : الليزر النابض عالى الشدة، التئام الجروح ،حروق الدرجة الثالثة .