

Effect of exposure time of laser He-Ne 632.8 nm, 1 m/watt on wound healing process in rabbits

Muhsin Abdul Jalil Falih¹, Abdalbari A. Alfaris¹ and
C.A.Emshary²

*1-Department of Surgery and Obstetric, College of Veterinary Medicine, 2-Department of Physics,
College of Education / Basrah University. Basrah, Iraq*

ISSN 1817 - 2695

((Received 23/3/2010 , Accepted 8/6/2010))

Abstract

The objective of this work was to assess a compare grossly and microscopically, the effect of exposure time of He-Ne 632.8 nm. 1 m/watt in wound healing in rabbits. For this purpose 15 adult male rabbits were used, divided into 3 groups, 5 rabbits in each group. Xylazine 20 mg/kg and ketamine 10mg/kg used I/m to induce general anesthesia. Surgical open wound was made and immediately. The 1st, 2nd and 3rd groups were exposure to 632.8 nm. 1 m/watts low level laser during 3,5 and 7minte. All the groups exposure was done on 1st, 3rd, 5th and 7th day. The spacement for histological studies taken on 3rd, 5th and 7th days and immediately kept in 10% buffer formaline for 24 hours. The result revealed that among three groups were the wound closed by fifth day under microscopic examination the slide show complete remodeling and natural skin layers of dermis, epidermis in comparison with groups first and 3rd. The 1st group shows fibroblast and collagen fibers fill the gap, while the third group shows mature normal structure at same stage.

Keywords: Laser ,injury, wound healing .

Introduction

Various researchers have studied the influence of chemicals (pharmaceutical) agents alone or in combination with physiotherapy (irradiation) on the mechanism of wound healing in comparing with healing take place under natural condition but in recent decade the researchers interest has been dramatically increase in studying the influence of various type of laser rays like Helium-cadimium (He-Cd) 442, Argon (670nm) ,Helium-neon (He-Ne)(632.8nm) Krepton (670 nm) and Galium Alomenium Arsinic (GA ALAS) 780 nm and 830 nm on wound healing. However some of the researchers shows in their study the efficacy of photon on wound healing while others tried to elucidate the mechanism of regarding its effect so they emphasized mainly on He-Ne(632.8 nm) efficacy on wound healing which considered to be the best among all type of irradiation[1].

Historically the first use of laser therapy for biostimulation in 1967 by Endremester in semmeliweis Budapest, Hungary. The high energy for healing has been used since earliest medical history, but it has gone out of favor in western medicine with of the exiting paradigm of more surgical and pharmacological basic, recently shift thinking has been emerging with an explosion of research, exploration and utilization of energy medicine modolatis [2]. After first work by Endre master the first demonstration of laser biostimulation done in 1967since then the medical treatment with coherent light sources (laser) has passed through its childhood and dolesence, currently low level laser (or light) therapy (LLLT) also known as, cold laser, soft laser, biostimulation or photobiomodulation which is practiced as part of physiotherapy in many part of the world [3].

The other names given to low level laser are low power laser irradiation (LPLI) low power laser therapy (LPLT) and low energy laser irradiation [4].

The first low level laser manufacturer to be given clearance to make low level laser irradiation machine in January 17.2002 is Rechonia [5].

The most type of laser irradiation used in treatment of wound healing accelerator was He-Ne 632.8 nm either alone or in combination with other irradiation and pharmacological agent [1], and other researchers mentioned about the efficacy of LLLT on wound healing are that the author [6], investigate the influence of low level rays intensity on skin wound healing saying that low level laser therapy at the proper wave length and dose can accelerate tissue repairs [6]. A wound healing successfully stimulated with low level irradiation [7], other say the low level laser therapy facilitates superficial wound healing in human so the wound treated with LLLT contracted more. Therefore LLLT may produce

Materials and Methods

The materials used in this study were: fifteen adult male rabbits brought out from the city local market and rayer on similar management condition. 20mg/kg xylazine and 10 mg/kg ketamine as anesthetic agent to induce general anaesthesia. Laser ray machine to produce He-Ne 632.8nm 1 m/watt and formalin 10% for spacement preservation.

Method: the rabbits are divided into three groups that is first, second and third. Every group having five rabbits, the power and dose to be given are fixed that is 632.8nm and 1 m/watt .While the time of exposure were for group the exposure time was for to 3 minutes on 1st ,3rd , 5th and 7th days. Group second received rays for 5 minutes on same days that is first (after wounding immediately), 3rd , 5th and 7th days, while group third received laser rays on first, 3rd

Results

After making a 5cm long open wound on the skin, the wound exposed to He-Ne laser of 632.8nm, 1 m/watt irradiation depending on the time were given for each group 3,5 and 7 minutes.

an indirect healing. Hence LLLT and an effective modality to facilitate wound contraction of partial thickness of wound [8].

Another one mentioned that He-Ne laser irradiation enhances wound healing at the cellular and molecular level [9]. In animal modulates studies have shown promise in improving a cut wound healing with laser therapy with reduced inflammation, faster progression through the phases of healing [10].In addition there are many authors reported the effect of laser therapy on burn wound and ulcer as given by [11, 12 ,13 and 14] were they mentioned that the efficacy of laser therapy in accelerate wound healing. Although most of the researchers have emphasized their work on wave length and dose of low level laser therapy a few of them studied the importance of exposure time in wound healing [15].

The objective of present study is to compare clinically and microscopically the effect of time of LLLT exposure on wound healing.

,5th and 7th days and time of exposure was for 7 minutes.

No antibiotic, or chemical agent either locally or parentally were used for dressing. After giving xylazine and ketamine at ratio of 1:0.5 I/M to induce general anaesthesia. The site cleaned and disinfected with weak tincture iodine lotion a 5cm cutaneous wound is made, and immediately exposed to laser irradiation.

The probes were positioned to contact the wound. Spacement from all the groups for histological studies were taken on day 3rd ,5th and 7th and immediately fixed in 10% formalin ph.7 for 24 hrs, then passed through increasing ethanol concentration (70 to 100 %) then cleared in xylene and embedded in paraffin to make block for tissue section cutting and slide making which at the end stained with Hematoxylin-eosin to be evaluated microscopically.

At the time of exposure all the bleeding capillaries stopped and the wound become dry and clean.

On next exposure that is in the 3rd day in all the groups the open wound were reduced in size

by one cm. the wound of first group shows slight mosaicized, while in second group, the wound shows healing tissue and the wound adages become more closed comparing to other 2 groups. Third group show more dryness and the gap between the adages enlarged and there were some sign of burn tissues.

Microscopically it was possible to notice the changes occur according to the time variation in all stages of wound healing. The slide taken in third day from the first groups shows epithelization filled the gap with new blood vessels and inflammatory cells (Fig-1). The 5th day slide shows inflammatory cells with increase collagen fibers (Fig-2). While after seven days reading indicate presence of inflammatory cells and the gap filled with collagen fibers (Fig-3).

In second group the changes in healing process are: on day third the space of the wound

filled with fibroblast with increased collagen fibers (Fig-4) In 5th day the histological finding is that inflammatory cells and immature collagen fiber (Fig-5) while the slide of 7th day shows the remodeling of healing process. Which consider to be good, there is also decrease in monocytes cells and collagen fiber. The dermis and epidermis shows normal skin structure (Fig-6).

Third group where the time of exposure is for 7 minutes with same power and dose that is He-Ne laser 632.8nm, 1 m/watt the process of healing on third day shows high amount of inflammatory cells (Fig-7) and on 5th day shows presence of inflammatory cells and an increase immature collagen fibers (Fig-8) but the slide of day 7th shows immature monocytes and normal structure of collagen fibers (Fig-9).

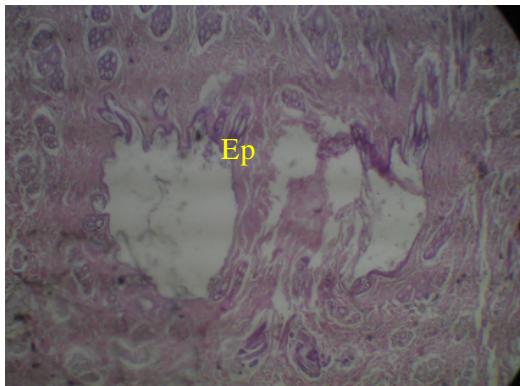


Figure-1:Cross-section in wound exposure for 3 minute of He-Ne laser in 3 days ,show epithelial tissue (EP) filled the gap of wound (H&E 20 X).

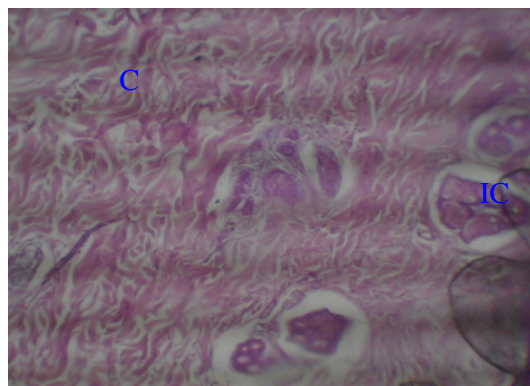


Figure-2:Cross-section in wound exposure for 3 minute of He-Ne laser in 5 days ,show inflammatory cells(IC) and collagen fiber (C) (H&E 20 X).

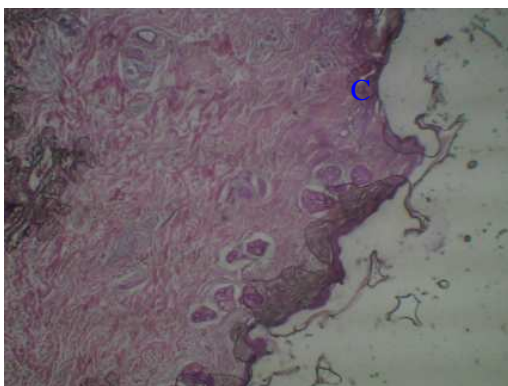


Figure-3:Cross-section in wound exposure for 3 minute of He-Ne laser in 7 days, show collagen fiber(C) and epithelial tissue similar normal structure (H&E 20 X).

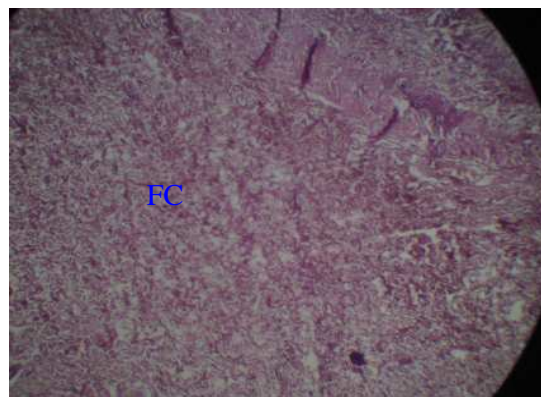


Figure-4:Cross-section in wound exposure for 5 minute of He-Ne laser in 3 days, show fibroblast and collagen fiber filled(FC) the gap of wound (H&E 20 X).

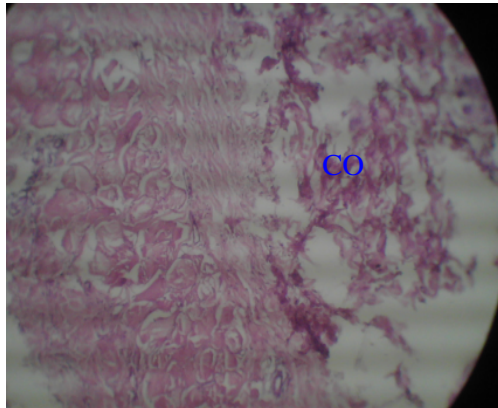


Figure-5:Cross-section in wound exposure for 5 minute of He-Ne laser in 5 days, show immature of collagen fiber filled (CO) the gap of wound (H&E 20 X).

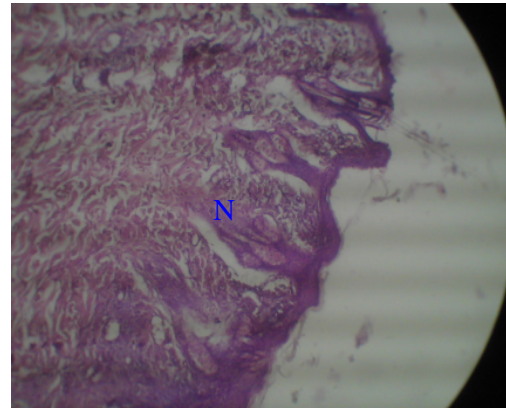


Figure-6:Cross-section in wound exposure for 5 minute of He-Ne laser in 7 days ,show normal structure (N) of skin (H&E 20 X).

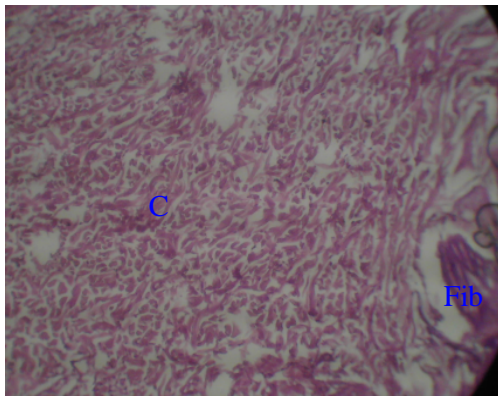


Figure-7:Cross-section in wound exposure for 7 minute of He-Ne laser in 3 days ,show fibroblast (Fib) and collagen fiber(C) filled the gap of wound (H&E 20 X).

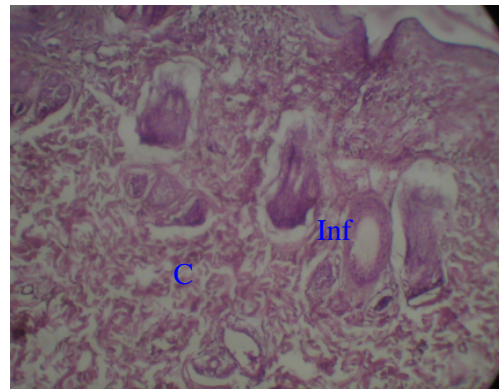


Figure-8:Cross-section in wound exposure for 7 minute of He-Ne laser in 5 days ,show collagen fiber (C) and inflammatory cells(Inf) are present (H&E 40 X)

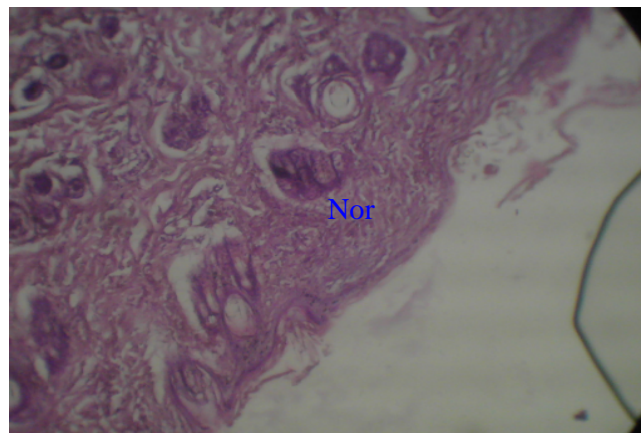


Figure-9:Cross-section in wound exposure for 7 minute of He-Ne laser in 7 days ,show began normal structure(Nor) are present (H&E 40 X).

Discussion

From clinical findings, the low level laser therapy treated wound grossly appear reduced in size from 5cm long to 4cm by 3rd day this indicate that LLLT enhance wound healing by the same way as in [8], when he mentioned that the LLLT resulted enhanced healing as measured by wound contraction when wound subjected to LLLT treatment comparing to unexposed wound which shows less contraction, so LLLT effective modality to facilitate wound contraction of partial thickness wound. The wound of first group also shows moisture and gap between wound edges but there were no inflammation in all wound of the three groups. This is similar to the finding as of [10]. Animal studies have shown promise in improving cut wound healing with laser therapy with reduced inflammation and further progression through the phases of healing. Another finding noticed that all the treated wounds are infection free, this may support the use of LLLT in tuberculoses patient who has faster clearance of tubercle bacilli from the sputum [17]. In group 3 where the time of exposure with LLLT was 7minute a sign of burn and necrosis were noticed this could delay healing process that have been proved by [11], when stated that LL He-Ne LT induced the destruction of skin by making a third degree burns of rats. In group one that is some humid wound and clot blood, that is similar to the finding reported by [15], according to the report that clinical inspection of skin lesion samples shows amount of humid clot on the surface.

Microscopically, slid obtained on 3rd day from group first showed blood vessels within the inflammatory cells filling the gap with new epithelization . Similar was obtained by [15], when he reported that irregular amount of humid clot on the surface and the presence of many blood vessels in the wound area re-epithelization and granulation has along with inflammatory

cells. While in second group where the exposure was to 5 minutes, the changes in 3rd day is inflammatory cells (fibroblast) filled the space of the wound and increase collagen fibers. This shows by [17], when laser used singly or in combination promote biochemical and morphological induces healing by agument collagen synthesis [17 and 18],montioned the properties of laser treated wound revealed that the amount of total collagen was significantly increase and this promotes the tissue repair by accelerating collagen.

In third group where the time of exposure was for 7 minutes, 3rd day slide shows necrotic cells also with the amount of inflammatory cells (fibroplast). Similar findings were reported by [11], when he stated that He-Ne therapy causes the distruction of skin by making 3rd degree burn.

5th day spacement 1st group spacement shows on 5th day shows presence of inflammatory cells and increase collagen fibers . While filled the gap of the wound with slight remodeling , comparing with that of 2nd group which shows excellent healing by day 7th where there is decrease of monocytes and collagen fibers with formation of dermis, epidermis and normal structure of skin, while in group 3 the slide shows monocytes, collagen fibers as well as immature normal structure [8 and 9].

There for the conclusion driven from above findings that group second where the time of exposure to He-Ne 5minutes is excellent and the wound closed before the natural time that at 5-6 days compare to 1st and 3 group where the healing took little longer time. This findings agreed with [14] conclusion when he concluded that low level light therapy (LLLT) can have positive effect on the repair of coetaneous wound.

References:

1. A.H.Farouk, ;A.Al-watban and L. Bernard. Therapetic photons in wound healing. Reference library. Business Brifing Global Health care. pp: 1-6. (2002).
2. M. Jeffrey, and P. Karen . Healed by the light. <http://www.spiediscrebab.com/Laser.htm>. (2009).

3. R.H.Michael and N. D. Tatiana .Mechanisms of low level light therapy. Proc of SPIE Vol. 6140 pp.: 1-9. (2006).
4. L. Jones. Laser and led therapy is phototherapy. Low level light therapy. pp.: 1-5. (2009).
5. Rechonia world leader in low level therapy Healing at light speed. <http://www.spinediscrehab.com/laser.htm>. (2009).
6. R.T Simoes ; D. Dasilvea. and N .D. Araujo . Effect of low-intensity polarized visible laser radiation on skin burn light microscopic study (Abst.). journal of clinical laser Medicine and Surgery. 22(1): 59-66. (2004).
7. A. Lomeli-Rivas; E Krotzsch and A. Michtchenkor. Dosis de uso clinico, sobre la prolifera on de fibroblastos humanos cultivados (English abstract). <http://www.laser.nu/lllt/science.htm>. (2004).
8. J. T. Hopkins; T. A Mcloda.; J.G .gmiller and D. Daxter. Low level lasertherapy faciities superficial wounf healing in humans. A triple-Blind, Sham-controlled study Journal of Athletic Training. 39(3) :pp.223-229 . www.journalathleticctraining.sry . (2004).
9. K.I.Chang; H.S. Yu; J.w. Chen ; C.I .Yu and J.r. Chon.. Low energy helium neon laser irradiation stimulates interleukin-1 Alpha and interleukin-8 release from cultuiured human keratinocytes. Pub.Med. pp.:1-2. (2003).
10. R. Cordrey. Commentary: light therapy and advanced wound care on neuropathic plantar ulcer on a charcot foot. Journal of wound, Ostomy and continence nursing. 35: pp.116-117.<http://www.nursingcenter.com/bibrary/journal/Article.asp&Article ID:767848>. (2008).
11. M. Bayat; M.M. Vasheghani. N. and Rasvi. Effect of low level helium-neon laser the therapy on the healing burns in rats. (Abstract). J.Photochom Photobial B. 83(2): pp.87-93. (2006).
12. V. Schubert. Effects of phototherapy (LLLT) on pressure ulcer HE after a falling trauma. (Abstract). Photodermcefol photoimmunol photomed. 17(1): pp.32-38. (2001).
13. M.E .Sugrue; J. Carolan and T.M. Feeley. The use of infared laser therapy in the treatment of ulceration. Ann. Vasc.Surg. 4: pp.179-181. (1999)..
14. T.P. Mendez; A. Pinherio; M.N. Pacheco and L. Ramalho. Dose and wavelength of laser light have influence on repaire of cutaneous wound. Journal of clinical laser medicine and surgery. 22(1): pp.19-25 (Abstract). (2004)
15. W.L .Concalves; F.M. Souza; C.I. Conti; J.P. Cirqueria; W. N. Rocha ; J.G. Barros and M.R. Moyses. Influence of He-Ne laser therapy on the dynamics of wound healing in mice treated with anti-inflammatory drug. Brazilian Journal of Medical and Biological research. 40:pp.877-887. (2007).
16. H.F.Hans B.U. Van and B.Dop . Power density and exposure time of He-Ne laser irradiation more important than total energy Dose in photo-Biomodulation in human fibroblast *in Vitro* (2009).
17. S. E.Chukuka. and G.K. Raaddy.The biological effects of laser therapy and other physical modalites on connective tissue repair processes. Laser therapy, Vol. 12 special Millenum edition. <http://www.spinediScrehab.com/laser.htm>.(2000).
18. G.K. Reddy; L. Stehno-Bitttd and C.S. Enwemaka. Laser photostimulation accelerates wound healing wound repair and regeneration. 9(3): pp.218-255 (Abstract). (2001).

أهمية وقت تعرض الجروح لأشعة ليزر هليوم-نيوم 632.8 انفراد لأشعة
0.14 جول/سم² في تحفيز عملية الالتئام في الأرانب

محسن عبدالجليل فالح¹، عبدالباري الفارس¹، جاسب عبدالحسين مشاري²
1 فرع الطب الباطني والجراحة والتوليد- كلية الطب البيطري، 2-قسم الفيزياء- كلية التربية جامعة البصرة،
البصرة، العراق

الخلاصة

ان الهدف من هذه الدراسة هو مقارنة تأثير وقت التعرض لأشعة الهليوم-نيوم 632.8 انفراد 1 m/watt عيانياً ومجهرياً للجروح المستحدثة في الارانب. في هذه الدراسة استخدمت خمسة عشر ارنباً (ذكر) قسمت الى ثلاث مجاميع، كل مجموعة تحتوي على خمسة ارناب. استخدم الزايلازين 20ملغم/كغم والكيثامين 10ملغم/كغم كمخدر عام وتم التحدير بعد ان نظفت المنطقة وعقت بمحلول صيغة الايودين ومن ثم عمل جرح بطول 5سم ومباشرة عرضت الى الاشعة الليزرية كل حسب الوقت المحدد ففي المجموعة الاولى كان التعريض لمدة ثلاثة دقائق والمجموعة الثانية لمدة خمسة دقائق والثالثة لمدة 7دقائق. كان التعريض مباشرة في اليوم الاول واليوم الثالث والخامس والسابع وكان اخذ العينات في اليوم الثالث والخامس والسابع بعد اخذ العينات قمنا بوضعها في محلول 10% فورمالين لمدة 24 ساعة ثم مررت في محلول الايثانول وبعدها بالبرافين لتحضير القوالب المعدة للتقطيع النسيجي وصبغها بصبغة هيماتوكسلين-ايوسين. وقد لوحظ ان طول الجرح المعمول قل بعد التعريض في جميع المجاميع من 5 الى 4 سم اما المجموعة الثالثة لوحظ اضافة الى ذلك حرق في الجرح وحوافه. اما في المجموعة الاولى لوحظ رطوبة الجرح وطراوة الحواف. وان جميع المجاميع كانت خالية من الالتهابات والتقرحات كما اثبت ذلك من الفحص المجهري للشرائح المحضرة واستنتج من البحث ان المجموعة الثانية كانت المثالية بسبب الالتئام السريع واستكمال النمو النسيجي واغلاق الجرح بصورة طبيعية وكاملة حيث ظهرت طبقات الجلد متميزة الى طبقة الأدمة وتحت الأدمة. مقارنة مع ماوجد في المجموعة الاولى اذ كانت لفايبروبلاست والكولاجين يملأن جوف الجرح بينما في الثالثة هنالك اعادة للتنظيم والتميز الا انه لازال غير ناضج بصورة غير متكاملة.