Kufa Journal for Veterinary Medical Sciences Vol.(7). No.(2) 2016

Kufa Journal for Veterinary Medical Sciences
vetmed@uoKufa.edu. iq

The effect of different doses of 660 nm laser therapy on open skin wound healing in Wistar rats

Muslim Fahim Diwan Thaier Alwan Abid
College of Vet. Medicine / AL-Qadisiyah University
email: Thaier.Abid@qu.edu.iq

Abstract

The study aim to evaluate the effect of 660nm wave length low level laser therapy (LLLT) at different doses on open wound healing in rat model. Twenty five adult male Wistar rats were randomly divided into five equal groups. Rats were anesthetized by IM injection of ketamine (50mg/kg B.W), xylazine (10mg/kg B.W) mixture. The back of animal was prepared for aseptic surgery, two parallel circular skin incisions 0.5 cm in diameter involving full thickness of the skin were made on both left and right sides of the back of each animal. Four groups were received a close-contact pulsed Gallium-Aluminum Arsenide (GaALAs) laser therapy on the left incision, with energy density of 2, 4, 6 and 8J/cm^2 of 660nm wave length, 50mw power output and 146Hz pulsing rat immediately after surgery then repeated the same dose daily for 7 successive days. Results declare that the laser treated group of 2J/cm^2 found slightly differences when compared with control group. At 4J/cm^2 laser treated group, the healing of wound was better than of control group by presence of narrow scar tissue and completely epithelization. The study found that 6J/cm^2 laser treated group was effective in open wounds, which showed faster keratinize layer formation and collagen fiber parallel oriented with the wound surface. However, energy density of 8J/cm^2 was more effective for healing process than other groups by presence of more collagen deposition, increase activity of myofibroblast, and presence of clear skin adnexa. *In conclusion*, the laser wave length 660 nm use in this study was effective enhance the healing process of open skin wound in rats in all the four energy density used; further to the 8J/cm^2 is seen the best in stimulation of wound healing.

Key words: Laser, LLLT, open wound, healing, rats.

تأثير العلاج بجرع مختلفة من الليزر nm660 على التئام الجروح المفتوحة في الجرذان

مسلم فاهم ديوان ثاير علوان عبد
كلية الطب البيطري/ جامعة القادسية

الخلاصة

تهدف الدراسة الى تقييم تأثير العلاج بالليزر واطئ الطاقة بطول موجي 660 نانوميتر بجرعات مختلفة على عملية شفاء الجروح المفتوحة للجرذان. أجريت الدراسة على خمس وعشرون جرذا (نوع Wistar) ذكر بالغ. تم تقسيم الحيوانات عشوائيا إلى خمس مجموعات, تم تخدير الجرذان بإعطائها جرعة عضلية من خلط الكيتامين (50 ملغم /كغم من وزن الجسم) و الزايلازين (10 ملغم /كغم من وزن الجسم) .وحضرت منطقة الجرح بطريقة معقمة, تم عمل جرحان دائريان متوازيان بقطر 0.5 سم يتضمنان كل طبقات الجلد على كلا الجانبين في ظهر كل حيوان . عرضت أربعة مجاميع

من الجرذان للعلاج بالليزر من نوع (Gallium-Aluminum Arsenide) بشكل ملامس لسطح الجرح الأيسر مباشرة بعد عملية الجرح وكانت مستمرة بشكل يومي بنفس الجرعة ولمدة سبعة أيام متتالية ، وبجرعات مختلفة لكل مجموعة (2، 4، 6، 8) جول /سم2 ، وبطول موجي ثابت (660 نانوميتر) وقدرة 50 مل واط ، وبتردد (146 هيرتز) . نسيجيا أظهرت نتائج مجوعة (2 جول /سم2) إن هناك اختلافات بسيطة عند مقارنتها مع مجموعة السيطرة. في المجموعة المعالجة بالليزر بقوة (4 جول /سم2) كان شفاء الجروح أفضل من مجموعة السيطرة وذلك لوجود طبقة خفيفة من نسيج الندبة وتكوين كامل لنسيج الظهارة. إما في مجموعة (6 جول / سم2) وجدنا أن الليزر واطئ الطاقة لدية تأثير على الجروح المفتوحة الذي أظهر تكوين سريع لطبقة الكيراتين وأن تكاثر الألياف الغروية متجه بشكل مواز لسطح الجرح. بالرغم من هذا فأن الليزر واطئ الطاقة بقوة (8 جول /سم2) كان أكثر تأثير على عملية شفاء الجروح من المجموعات الأخرى وذلك لوجود المزيد من الألياف الغروية وزيادة فعالية ألالياف العضلية وظهور ملحقات الجلد بصورة واضحة .وبالخلاصة فأن الليزر واطئ الطاقة بطول موجي 660 نانوميتر المستخدم في هذه الدراسة يؤثر في تعزيز عملية شفاء الجروح المفتوحة في الجرذان في جميع المجاميع الأربعة المعرضة لليزر, ولكن مجموعة (8 جول /سم2) وجدت أنها الأفضل في تحفيز شفاء الجروح.

الكلمات المفتاحية: الليزر واطئ الطاقة ، الجروح المفتوحة ، الالتئام ، الجرذان.

Introduction

Skin wound repair requires complex and highly coordinated interaction between different cells to restore the epidermal barrier and tissue architecture after injury (1). The wound healing is a multi-stage phenomenon that requires the activation, recruitment of numerous cell types as keratinocytes, endothelial cells, fibroblast, inflammatory cells, and macrophage which appear to be central to this process (2). Open wounds are extensive tissue damage and are contaminated or infected, they are allowed to heal completely by contraction and epithelialization (healing by second intention). Healing by second intention has the advantage of allowing progressive, gradual debridement of tissue in early stages of wound debridement. Open wounds provide optimal wound drainage (3). Healing is a complex process that can be divided into at least three continuous and overlapping processes: an inflammatory reaction, a proliferation process leading to tissue restoration, and eventually tissue remodeling (4). The inflammatory stage begins with platelet aggregation and formation of the fibrin clot, which reestablishes hemostasis and provides an extra cellular matrix for cell migration (5). This phase is triggered by a variety of mediators released from injured tissue cells and capillaries, activated platelets and their cytokines and the by–products of hemostasis (6). The subsequent proliferative phase lasts for about 2 to 3 weeks after the inflammatory phase, dominated by days the formation of granulation tissue and epithelization (**7**). Re-epithelization these phase also includes contraction of wound edges pull together to reduce the defect in the second step epithelial tissues are formed over wound site (8). Epithelization of open wound is different from that of closed wound. In open wounds epithelization occurs after a bed of granulation tissue has formed. There is a latent period of approximate 4 to 5 days before epithelization starts, (in closed wound epithelization may complete in 48 hours as the epithelium migrates through the fibrin clot) (3). The remodeling phase of wound healing is characterized by a decrease in cell population and an increase in collagen organization in granulation tissue which form a scar (9). During the remodeling phase myofibroblasts play an important role in wound contraction and scar formation (10). Low level laser therapy (LLLT) is a form of phototherapy used to promote wound healing in different clinical conditions. The LLLT at an adequate wavelength, intensity, and dose, can accelerate tissue repair (11). LLLT is effective in open wounds, which showed better regeneration and faster restoration of structural and functional integrity (12). The LLLT emission increased tissue oxygenation, morphofunctional activity, and substantial expansion of the microcirculation bed and in turn accelerated the restoration of the functional features of the injured area (13). Laser stimulation shortened the inflammatory phase as well as accelerated the proliferative and maturation phase, and positively stimulated the regeneration of injured epidermis and the reparation of injured striated muscle (14). It may reduce pain related to inflammation by lowering levels of prostaglandin E_2, TNF-α, cellular influx of neutrophil granulocytes oxidative stress, edema, and bleeding (15). LLLT accelerates wound healing by stimulating the biological activities and differentiation of fibroblast and by reducing the inflammatory process (16). All laser treatment led to marked improvements in dermal layer thickness and collagen fiber density, and increase in fibroblast number and hydroxyproline content. Hydroxyporline is a major component of collagen and rarely found outside collagen (17).

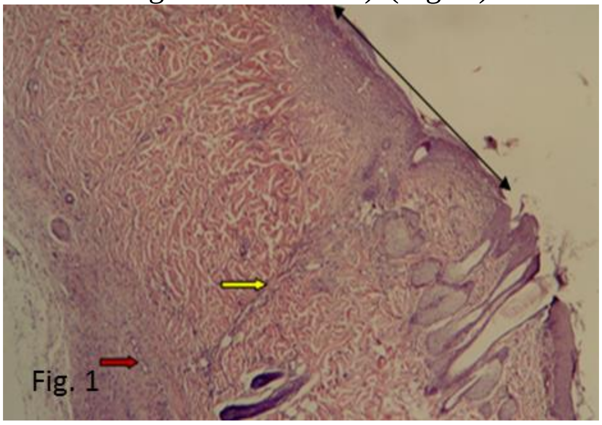
Materials and methods

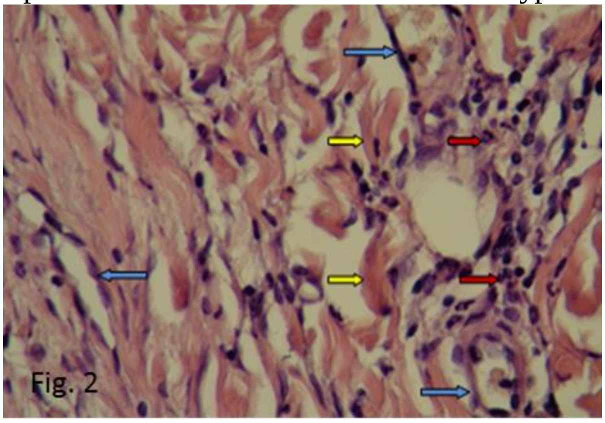
Twenty five adult male Wister rats weighing about (250± 1.45g) and aged between (10-12) months. Animals were randomly divided into five groups, five animals of each. The animals were kept in plastic cages with a metal top, in normal laboratory conditions (room temperature 20-24 ºC) and were fed a solid diet and water ad libitum. The laser device GaALAs in multi-wave length probes used was (Omega Laser Systems Limited, UK) emits a wave lengths under 1000nm utilizing red light (600nm to 780nm) and infrared (over 780nm). The power, pulsing frequency and programming is set via the control unit and the frequency laser pulse is varied (2.5Hz-10KHz). After general anesthesia utilizing IM injection of a mixture of ketamine 50mg/kg B.W and xylazine 10mg/kg B.W, the site of incision on the back of the animal was prepared for aseptic surgery (clipping, shaving and apply antiseptic), to induced (0.5cm in diameter) two open parallel circular full-thickness skin incision by using punch machine. The left incision was treated with laser therapy and the right incision left without treatment as control in the same animal. Treatment of the left incision only was applied immediately after creating the skin wound and repeated for 7 consecutive days. The diode laser used was (660 nm wave length), (50 mw power output), (146 Hz pulsing rate), and (0.5 cm spot size). The energy densities of 2, 4, 6, and 8J/ cm^2 were used in G_1, G_2, G_3 and G_4 respectively. The laser irradiation during the successive days was performed while the rat restrained with special restrainer and keeping the diode laser probe closely to the wound surface. Five male Wistar rats were used as control group in which make the skin incision without exposing to the laser. The skin specimens $1cm^3$ were collected after 14 days post wounding from the wound area involving all the skin wound, where prepared and sectioned for histolpathological examinations.

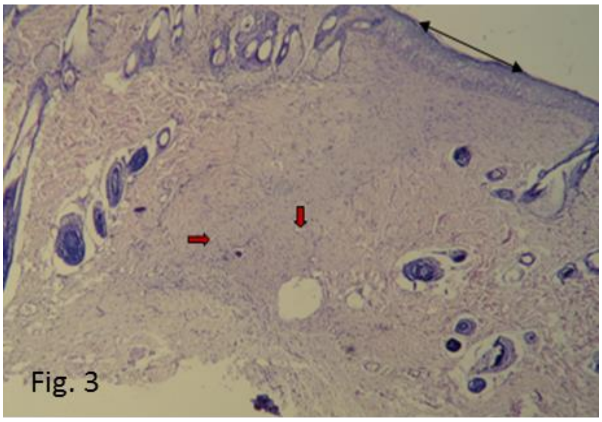
Results

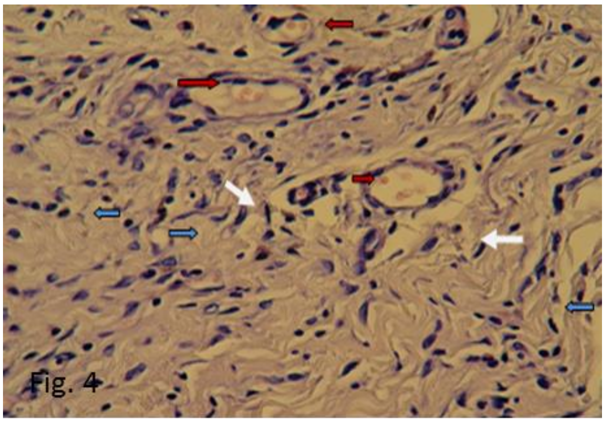
Clinically during the fourteen days post wounding (PW) observation period, animals were seen healthy, active, with normal healing aspect of the wound observed on the operated site, without clinical evidence of infection.

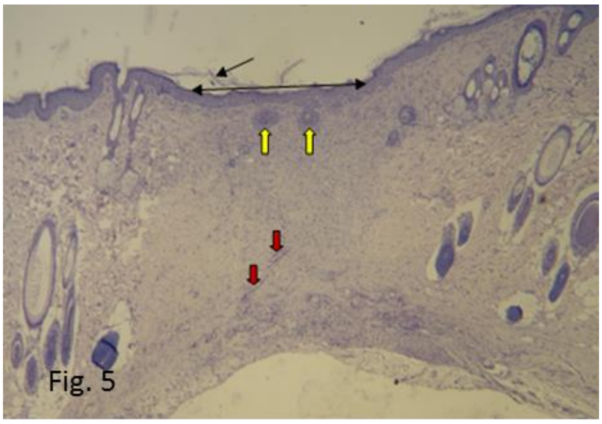
Histologically after 14^{th} day PW in G1 ($2J/cm^2$ laser exposure dose), there were wide immature scar tissues observed (Fig. 1), typified abundant of collagen fibers achieved proliferation of fibroblast, and new blood vessels formation, occasionally absence of keratinized, with infiltration of inflammatory cells (Fig. 2). In G2 ($4J/cm^2$ laser exposure dose) There were narrow scar tissue, with complete epithelialization was seen (Fig. 3). Also presence of granulation tissue below the epithelial layer, and stamping abundant collagen with formation of new blood vessels, and infiltration of inflammatory cells (Fig. 4). In G3 (6J/ cm^2 laser exposure dose). There were narrow scar tissue, presence of thin keratinized layer, presence of granulation tissue which characterized the wound and formation of fibrous connective tissue with collagen fibers parallely oriented with the wound surface (Fig. 5). Also presence of collagen, and presence of few skin adnexa (primitive, stray hair follicles and sweat gland were seen) (Fig. 6). In G4 ($8J/cm^2$ laser exposure dose), there were narrow scar tissues, thick of keratinized layer, and thick epidermis with upward proliferation of stratum basal. Also a typified

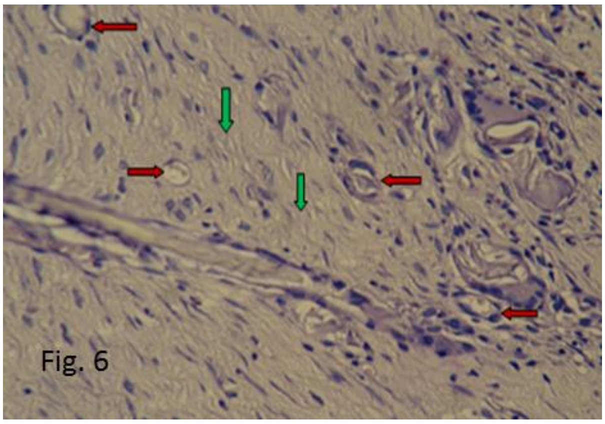






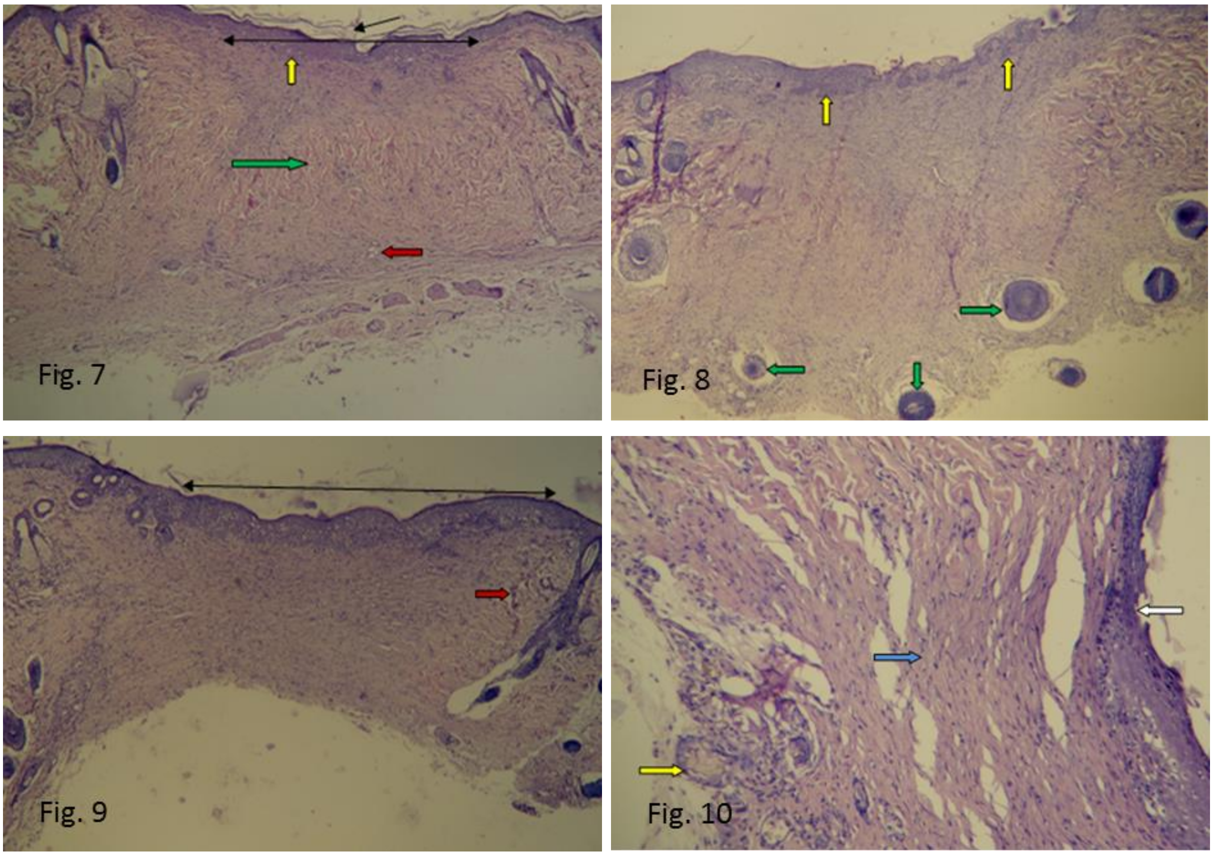






Histological sections (H&E Stain) in open wound of rat skin 14 days PW; (Fig 1, and 2, $2J/cm^2$ laser treated). Fig. (1): Show a wide immature scar tissue (double head arrow), abundant of collagen (red arrow), with absence of keratinized layer (yellow arrow)(X50). Fig. (2): Show abundant collagen fibers (yellow arrows) and new blood vessels formation (blue arrows), with infiltration of inflammatory cells (red arrows) (X200). (Fig. 3 and 4, $4J/cm^2$ laser treated). Fig. (3): Show narrow scar tissue (double head arrow) and complete epithelialization. There was presence of granulation tissue below the epithelial layer (red arrow) (X50). Fig.(4): Magnified field (X200) show granulation tissue characterized by proliferation of endothelial cells, new blood vessels (red arrows) and abundant of collagen (white arrow), also there was infiltration of inflammatory cells (blue arrows). (Fig. 5 and 6, $6J/cm^2$ laser treated). Fig.(5): Show narrow scar tissue (double head arrow), thin keratinized layer (thin arrow), more compacted and paralled-oriented collagen fibers on the surface of the wound and formation of new blood vessels vertically on the

surface of wound (red arrow), and mild presence of skin adnexa (primitive and stray hair follicles) (yellow arrow) (X20). Fig.(6): Observe granulation tissue, typified by fibrous connective tissue (green arrow) and formation of new blood vessels vertically on the surface of wound (red arrow) (X200).



Histological sections (H&E Stain) in open wound of rat skin 14 days PW; (Fig. 7 and 8, 8J/cm2 laser treated). Fig.(7): Show narrow scar tissue (double head arrow), with thick keratinized layer (thin arrow), and thick epidermis with upward proliferation of stratum basale (yellow arrow), a typified abundant of collagen fibers (green arrow), and adipose tissue in sub dermal layer (red arrow) (X20). Fig.(8): Show narrow scar tissue (yellow arrows), with formation of skin adnexa (development of solitary and primary hair follicles) in sub dermal layer (green arrow) (X20). (Fig. 9 and 10 control group). Fig. (9): Show wide scar tissue (double head arrow), with profuse fibrous connective tissue (proliferation of fibroblast), with entity of scant collagen (red arrow) (X20). Fig. (10): Show wide scar tissue, with epithelial invagination (white arrow). Entity of granulation tissue which involved profuse fibrous connective tissue (proliferation of fibroblast). Absence of skin adnexa. There was hemorrhage in the dermis (yellow arrow) (X50).

of abundant collagen with presence of adipose tissue in sub dermal layer (Fig. 7). There was presence of skin adnexa (development of solitary primary hair follicles, invasion with smooth muscles) (Fig. 8). In G5 (control group without laser treatment), there were wide scar tissue, lack of keratinized layer, with few up ward proliferation of stratum basal, presence of epithelial invagination and also presence of thin epidermis layer (Fig. 9). Entity of granulation tissue which involved profuse fibrous connective tissue (proliferation of fibroblast) and proliferation of endothelial, and absence of skin adnexa. There was hemorrhage in epidermis (Fig. 10).

Discussion

The aim of this investigation is to demonstrate the role of the LLLT on the open wound healing processes following the use of different energy parameters, and also to make critical suggestions for the application of LLLT on the skin of rats. The result revealed that the $2J/cm^2$ laser exposure observed a wide immature scar tissue, typified abundant of collagen fibers achieved proliferation of fibroblast, and new blood vessels formation. This result is in agreement with (18) who found that LLLT at energy density $2J/cm^2$ is capable of enhancing collagen synthesis, his study showed a significant increase of both fibroblast in experiment group and endothelium of blood vessels at the maturation of the wound healing process. But this dose is still much little to enhance of healing and minimization the size of the open wound. This result is in agreement with (19) found that there is a little advantage in using low-level laser irradiation therapy at $2J/cm^2$ for the treatment of full thickness skin wounds. With $4J/cm^2$ laser treated group, there is complete epithelization, with the presence of granulation tissue stamping abundant collagen, with formation of new blood vessels, these results corresponded with (20) who found that the use of LLLT for treatment of wounds result in a thick epithelial layer that completely covered the wound tissue, the granulation tissue is thick and composed primarily of fibroblast and collagen, and a moderate of neovascularization, acceleration of the wound healing process with laser can be explain by a higher collagen synthesis from fibroblast, and numerous vascular proliferation in the connective tissue, coupled with higher mitotic activity of the epithelial cells. Also (21) report that use of laser result in re-epithelialization of the dermis. Also this result is consistent with (22) who found that this specific energy density ($4J/cm^2$) has been found to increase fibroblast proliferation, which may be the mechanism by which photobiomodulation (PBM) capable of altering wound healing. The use of GaALAS laser at lower energy densities had a significant effect on the cicatrization of tissue, acceleration of tissue repair through decrease edema, improve granulation tissue and fibroblast proliferation stimulation. We also find abundant collagen and this confirmed with (23) who find that LLLT contributed to a larger expression of collagen and elastic fibers during early phase of wound healing process, the use of $4J/cm^2$ is more effective in the synthesis of collagen, and mainly in speeding up the rate of wound closing. The healing process appeared accelerated in the treated group with a thinner resulting scar in comparison to the control group. In the $6J/cm^2$ laser treated group, we found the presence of granulation tissue characterizing the wound with formation of fibrous connective tissue and collagen parallel oriented with the wound surface. This result is corresponded with (20) who found the wounds in the $6J/cm^2$ laser treatment are characterized by thick granulation tissue dominating by fibroblast, and extensive deposition of collagen and moderate amount of neovascularization. The fibroblast and collagen fibers seen minimal parallel orientation below the epithelial layer. The LLLT is increasing the ATP production, cellular metabolism and DNA synthesis, thus increasing the fibroblast proliferation and causing earlier formation of granulation tissue (24). The Cytochrome c oxidase is the terminal enzyme of mitochondrial respiratory chain, functions of cytochrome c oxidase as a signal generator as well as a signal transducer in irradiated cell. Two mechanisms occurring in cytochrome **c** oxidase under irradiation, these are an increase of electron flow inside of cytochrome **c** oxidase and relieve of nitric oxidase NO block in the catalytic center of cytochrome **c** oxidase (25). Also Presence of the new blood vessels vertically on the surface of the wound was found in comparison to those of the control group, this result is consistent with (26) who found that the proliferation of blood vessels in irradiated group was superior. In the 8J/ cm^2 laser treated group, there is a narrow scar tissue, presence of thick keratinized layer, and thick epidermis with upward proliferation of stratum basal. This result is corresponded with (14) who found that the thickness of the keratin layer in the laser-stimulated wound is similar to that of the intact epidermis, so regeneration of the epidermis is completely finished. Also our result is agreed with (27) who report that the increase in number of dividing cells and in the expression level of inducible nitric oxide synthase, keratinocyte growth factor, and keratinocyte growth factor receptor may facilitate earlier and thicker re epithelization in laser wounds. In this dose of laser ($8J/cm^2$) we found presence of skin adnexa like development of solitary primary hair follicles and presence of adipose tissue in sub dermal layer, invasion with smooth muscles, same result also reported by (28) who found the low energy laser produce complete healing of the skin with hair follicles incorporated into the healed tissue. Also it is consistent with (22) who found the most significant characteristic aspect of wound was the presence of adipocytes distributed within the dermis.

References

1-McGee, H. M.; Schmidt, B. A.; Booth, C. J.; Yancopoulos, G. D.; Valenzuela, D. M.; Murphy, A. J.; Steven, S.; Flaveell, R. A. and Horsley, V. (2012). IL-22 Promote fibroblast–mediated wound repair in the skin. J. of Investig. Dermatol. Adv. Public. 463: 1ooo – 1038.

2-Rodero, M. P. and khosrotehrani, K. (2010). Skin wound healing modulation by macrophage. J. of Inter. Clin and Exp. Patho. 3 : 643- 653.

3-Swaim, S. F. and Henderson, J. R. R. A (1997) Small Animal Wound Management (2nd ed.) Williams and Wilkins. pp: 27 – 29.

4-Li, J.; chen, J. and Kirsner, R. (2007). Pathophysiology of acute wound healing. J. of Clin. Dermatolo. 25: 9- 18.

5-Digelmann, R. F. and Evans, M. C. (2004). An over view of acute fibrotic and delayed. J. of Front Bio. Sci. 9: 283 – 289.

6-Strodtbeck, F. (2001). Physiology of wound healing. J. of Newborn and Infant Nursing Rev. 1: 43 – 52.

7-Alam, G.; Singh, M. P. and Singh, A. (2011). Wound healing potential of some medicinal plants. J. of Inter. Pharmaceutical Sci. Rev. and Res. 9: 136 – 145.

8-Stadelmann, W. K.; Digenis, A. and Tobin, G. (1998). Impediments to wound healing. J. of Am. Surg. 176: 39 – 47.

9-Kumar, V.; Cortran, R. and Robbins, S. (eds.) (2003). Basic pathology. 7th ed. Saunders, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo. Pp. 873 – 891.

10-Li, B. and Wang, J. (2011). Fibroblast and myofibroblasts in wound healing: force generation and measurement. J. of Tissue Viability. 20: 108 – 120.

11-Hawkins, D. and Abrahamse, H. (2006). Effect of multiple exposure of low-level laser therapy on the cellular responses of wounded human skin fibroblasts. J. of Photomed. and Laser Surg. 24: 705 – 714.

12-Hussein, A. J.; Alfars, A. A.; Falih, M. A. J. and Hassan, A. N. A. (2011). Effects of a low level laser on the acceleration of wound healing in rabbits. J. of Med. Sci. North Am. 3: 193 – 197.

13-Ihasn, F. R. (2005). Low level laser therapy accelerates collateral circulation and enhances microcirculation. J. of Photomed. and Laser Surg. 23: 289 – 294.

14-Gal, P.; Vidinsky, B.; TopoRcer, T.; Mokry, M.; Mozes, S.; Longauer, F. and Sabo, J. (2006). Histological assessment of effect of laser irradiation on skin wound healing. J. of Laser Med. Sci. 24: 539- 547.

15-Bjordal, J. M.; Johanson, M. L.; Iversen, V.; Aimbire, F. and Lopes-martins, R. A. (2006). Low-level laser therapy in acute pain: a systematic review of possible mechanisms of action and clinical effects in randomized placebo-controlled trials. J. of Photomed. and Laser Surg. 24: 158 – 167.

16-Araujo, C. E. N.; Ribeiro, S.; Favaro, R.; Zezell, D. M. and Zorn, T. M. (2007). Ultra structural and auto radio graphical analysis show a faster skin repair in He-Ne laser-treated wound. J. of Photochem. and photobio. Bio. 86: 87 – 96.

17-Liu, H.; Dang, Y.; Wang, Z. ;Chai, X. and Ren, Q. (2008). Laser induced collagen remodeling a comparative study in vivo on mouse model . J. of Laser in Surg. and Med. 40: 13 – 19.

18-Bayat, M.; Azari, A. and Gol Mohammadi. (2010). Effect of 780 nm low – level laser therapy with pulsed Gallium Aluminum Arsenide laser on the healing of surgically induced open skin wound of rat. Photomed. and Laser Surg. 28: 465 – 470.

19-Peterson, S. L.; Botes, C.; Olivier, A. and Guthris, A. J. (1999). The effect of low level laser therapy (LLLT) on wound healing in horses. J. Equine Vet. 31: 228 – 231.

20-Byrens, K. R.; Barna, L.; Chenault, M.; Waynant, R. W.; Ileva, I. K.; Longo, L.; Miracco, C.; Johnson, B. and Anders, J. J. (2004). Photobiomodulation improve cutaneous wound healing in an animal model of type diabetes. Photomedicine and Laser Surgery. 4 : 281 – 290.

21-doNascimento, P. M.; Pinheiro, A. L.; Salgado, M. A. and Ramalhol, L. M. (2004). A preliminary report on the effect of laser therapy on the healing of cutaneous surgical wounds as a consequence of an inversely proportional relationship between wave length and intensity histological study in study in rats. J. of Photomed. and Laser Surg. 22: 513 – 518.

22-Webb, C.; Dyson, M. and Lewis, A. V. H. (1998). Stimulatory effect of 660 nm low level laser energy on hyper trophic scar – derived fibroblasts : Possible mechanisms for increase in cell counts. Lasers Surg. Med. 22: 294 – 301.

23-Pugliese, L. S.; Medrado, A. P.; Reis, S. R. and Andrade, Z. A. (2003). The influence of low – level laser therapy on biomodulation of collagen and elastic fibers. J. of Pesqui Odontol Bras. 4: 307 – 313.

24-Gracia, V. G.; Macarini, V. C.; deAlmeida, J. M.; Bosco, A. F.; Naguta, M. J.; Okamoto, T.; Iongo, M. and Theodoro, L. (2012). Influence of low level laser therapy on wound healing in nicotine treated animals. J. of Lasers Med. Sci. 27: 437 – 443.

25-Karu TI. (2008). Mitochondrial signaling in mammalian cells activated by red and near-IR radiation. J. of Photochem. and Photobio. 84 : 1091 – 1099.

26-Corazza, A. V.; Jorge, J.; Kurachi, C. and Bagnto, V. S. (2007). Photobiomodulation on the angiogenesis of skin wounds in rats using different light sources. J. of Photomed. Laser Surg. 25 : 102 – 106.

27-Fukuda, Y.; Ito, Y. and Azumi, H. (2002). Cell death and proliferation in Nd: YAG lasers electrocautory and scalped wound on mice skin. J. of Dermatol. Sci. 28: 106 – 118.

28-Romanos, B. E.; Dpelekanos, S. and Strub, J. (1995). A comparative histological study of wound healing following Nd: YAG laser with different energy parameters and conventional surgical incision in rat skin. J. of Clin. Laser Med. & Surg. 13 : 11 – 16.