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LASER PROMOTED WOUND HEALING

By

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ABSTRACT

Recently, Laser therapy continues to be an invaluable modality in veterinary clinics all over the world. The aim of the present work is to investigate the effects of photobiomodulation (PBM) on healing of soft tissues and to evaluate the advantages of some light sources with different wave lengths in promotion of healing process of soft tissue injuries. Circular skin excisions were inflicted on sixty rats; those were divided into equal four groups. Control group was left without any treatment and the other three groups were subjected to su Red laser 650nm, Infrared laser 906nm group and red led group treated by 4-5 J/Cm^2 on daily basis for 7 sessions. A representing five rats of each group were euthanized at 3,7,15 days post-wounding. Results showed that Red laser was the best tool for soft tissue healing promotion followed by LED.

INTRODUCTION

Since 1967, medical treatment with coherent-light sources (lasers) or noncoherent light (light-emitting diodes, LEDs) has progressed greatly. Currently, low-level laser therapy (LLLT) known as cold laser, soft laser biostimulation or photobiomodulation was practiced as part of physical therapy in many parts of the world **(Roelandts, 2002)**. Recently, Laser therapy continued to be an invaluable modality in veterinary clinics all over the world, with documented benefits ranging from acute to chronic inflammation, soft tissue injuries and orthopedic fields **(Bryan Stephens, 2013)**. Low-level light therapy (LLLT) using red to near-infrared (NIR) (630 - 1000 nm) light has gained attention in recent years **(Mohamed Abdalla *et al,* 2012)**. Advancement in the basic science fields of photobiology has propelled LLLT into the therapeutic revolution. Biostimulation with different light sources and laser proved to be a rapid, noninvasive technique for stimulation of soft tissue healing in the living bodies **(Lucroy *et al,* 1999; Dragonea *et al.,* 2011; Simmons, 1985; Willenegger *et al.,* 1971; Cruess and Dumont, 1975; Brighton, 1984; Frost, 1989; Owen, 1970)**. The aim of the

present work was to investigate the effects of photobiomodulation (PBM) on healing of soft tissues in a rat animal model.

MATERIAL AND METHODS

The present work was carried out on sixty male rats at the National Institute of Laser Enhanced Sciences (NILES) in the Department of medical applications. Cairo University. Defects in rats were.

irradiated using different light sources which are:

-Red Laser Module 650nm.

-Infrared laser 906nm source

- Light emitting diode LED 630nm.

Soft tissue exposure:

Animal model:

The study was performed on 60 male Westar rats, 4 months old and of average weight 200 ± 30gr. They were cared according to the Guide for care and use of laboratory animals **(Ralph and Charles, 2011)**. Rats were acclimated for a period of three days, during which they were observed and examined for any sign of disease. All rats were subjected to a circular skin excision and divided randomly into four groups each of fifteen. In group1 (Control group) the induced tissue injuries in all rats were left without exposure to any light source for one week. In group2 the soft tissue injuries were exposed to biomodulation by 650nm red laser module using energy density 4-5J/Cm2 on daily basis for one week. In group3 are rats were exposed to infrared laser 906 nm. In group 4 the induced soft tissue injuries were exposed to biomodulation by 906 nm Infrared Laser using energy density 4-5J/Cm^2 on daily basis for one week. In group 4 soft tissue injuries were exposed to biomodulation by Red LED using energy density 4-5J/Cm^2 on daily basis for one week.

The surgical operations for rats were performed as follows:

Soft tissue injuries:

After aseptic preparation of dorsal skin, a 3 cm full-thickness circular excision was made 1.5 cm away from the dorsal midline including the panniculus carnosus.

Laser exposure:

Group 1 was left without exposure to any light source while Group 2 and 3 were submitted to seven hours sessions of radiation. The first dose was carried out immediately after surgery and the others were every 24 hours and applied transcutaneous around the wound edges.

The microscopic evaluation was applicable only with the experimental cases as follow:

-The soft tissue samples were taken from operated cases after 3, 7 and 15 days. Five rats from each group were sacrificed to provide histological evaluation. The sample that involved the wound with its surrounding tissues were fixed in 10% formalin, embedded in paraffin then sectioned. The sections were stained with hematoxylin-eosin (H&E) and Masson Trichrome for histological evaluation.

-Samples were examined for the following morphological findings:

-Collagen deposition and arrangement (poor collagen deposition type I collagen deposition arrangement parallel or hazardly arranged).

- Type, degree of inflammation and cellular infiltration (neutrophils, macrophages, and fibroblasts).

- Epithelialization

- Vascularization

RESULTS

In control group at day 3rd day, the healing of the induced wounds showed partly epithelial coverage of the wound center.The area was completely covered by polymophonuclear leucocytes (Fig.1 and 2). At 7th day, severe inflammation with complete epithelial coverage was marked and predominant leucocytic infiltration composed of polymophonuclear leucocytes and moderate number of fibroblasts and macrophages were seen. Lymphocytes in a very small number were seen at that time Fig. (2, 3and 4) and thin collagen fibers arranged in delicate interlaced bundles were observed within a loose connective tissue Fig. (4 A).

At day 15th day, the intensity of the inflammation was mild with abundant mononuclear cells and high number of fibroblast cells Fig. (3 and 4).

In Red 650 nm diode laser group at 3rd day inflammatory changes appeared in the form of low number of fibroblasts, some macrophages and lymphocytes. Almost complete epithelial coverage Fig. (1). at 7th day, a well differentiated epithelium completely covered the wound was seen Fig.(2). Moderate number of haphazardly arranged fibroblasts was observed. However, three cases showed severe inflammation with marked edema and high number of inflammatory cells. Seven cases showed moderate inflammation indicated by oedema and inflammatory cells. At day 15, the intensity of the inflammation was mild with abundant

mononuclear cells Fig (3 and 4). Collagen fibers became thicker and densely arranged in paralleled manner.

In 906 nm IR diode laser group, the wound showed delayed healing, absence of epithelial proliferation, vasodilatation and a mild inflammatory cell infiltration in comparison with the control group Fig. (1). at 7th day, inflammation was severe indicated by edema and inflammatory cells in all wounds with fibrin clot and infiltration with macrophages Fig. (2). the wounds showed delay in healing process represented by presence of mild vasodilatation poor epithelial coverage of the wound Fig. (2). The IR laser group showed loosely arranged collagen at 7th day (plate E). At 15th day, the intensity of the inflammation was moderate represented by the presence of inflammatory cells with abundant mononuclear cells Fig. (3, 4). In the irradiated IR laser group, there was a mixture of collagen fiber arrangement, with well packed densely arranged fibers. Thirty percent of the samples presented thine fibers loosely arranged close to the edges of the lesion and densely compacted gross fibers in the central region Fig. (4 plates: E, G).

In LED group at 3rd day, the wounds showed macrophages and polymophonuclear leucocytes and early appearance of fibroblasts Fig. (1). at 7th day the wounds of the LED 632 nm irradiated rats showed complete epithelial coverage of the wound. Presence of fibroblasts was moderate in most samples Fig. (2). in one case there was severe inflammation indicated by high number of inflammatory cells and moderate in the rest of rates. Macrophages and lymphocytes were few in number. Collagen fibers were dense and loosely arranged Fig. (4). At day 15th, the intensity of the inflammation was mild with abundant mononuclear cells. Collagen fibers were arranged parallel to each other Fig. (4).Collagen fibers were thicker in both Red LED and Red laser groups and arranged in the wound area in organized parallel manner Fig. (4) (plate F and H). Collagen deposition had persisted over thicker collagen fibers that were arranged densely in the whole area of the wound. Both red laser group 650 nm and Red LED group showed, densely arranged parallel thick collagen fibers Fig. (4 plate B and D).

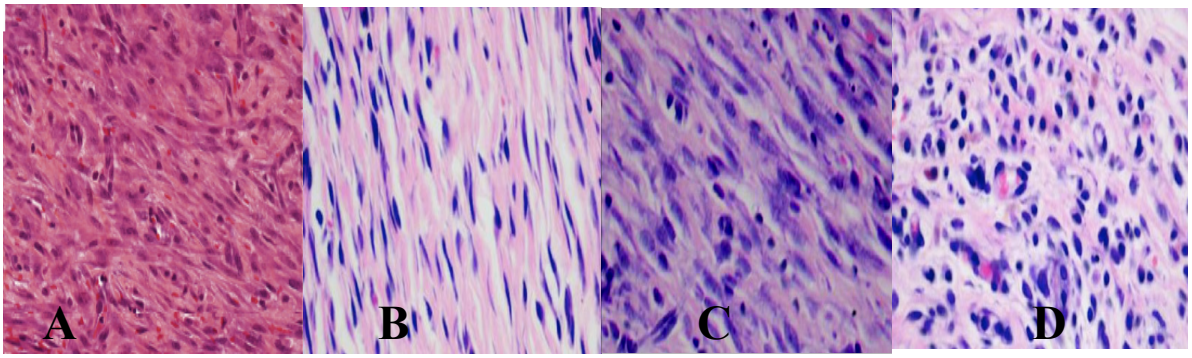

Fig. (1): showing the inflammatory cell infiltrates in wounds of all groups after 3 days post-wounding **(H&E stain)** and optical magnification **40 X.**

(A) : Control wound maPolymorphnuclear leukocytes infiltrating the wound area.

(B): Red laser 650nm with few inflammatory cells infiltrates.

(C): LED treated wound showing polymorphonuclear leucocytes infiltrating wound area.

(D): IR laser treated wound with inflammatory cells which exceeds those of the LED and the red 650nm laser.

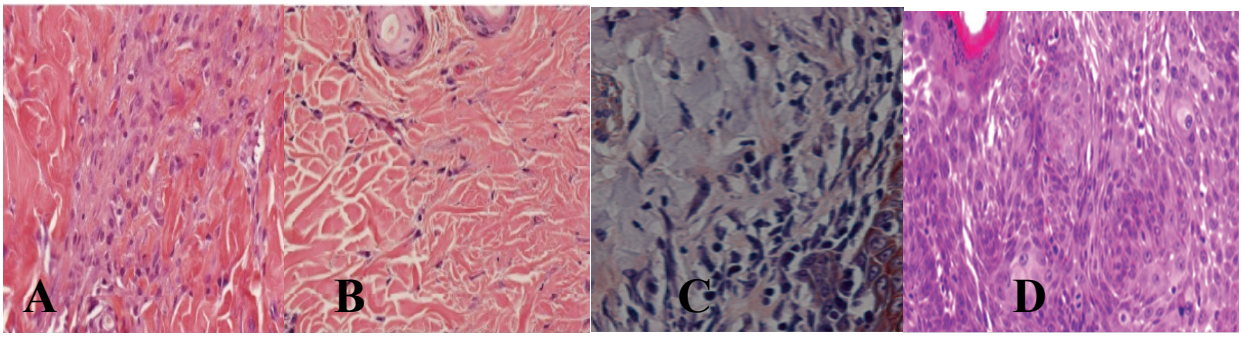

Fig. (2): is showing histological section at day 7 in all groups stained with **(H&E)** and optical magnification **(40 X).**

(A): Represents control wound showing fibrin clot with moderate number of fibroblasts and leukocyte infiltrate.

(B): Represents 650 nm diode laser groups showing moderate inflammation, number of fibroblasts and leukocytes.

(C): Represents LED treated wound showing moderate inflammation, leukocyte number and fibroblast cell number.

(D): Represents IR laser treated wound showing sever inflammation with fibrin clot leukocyte infiltrate with massive fibroplasia.

There is an increased cellular infiltration, obvious increased collagen deposition and organization in comparison to control **(Slide A).**

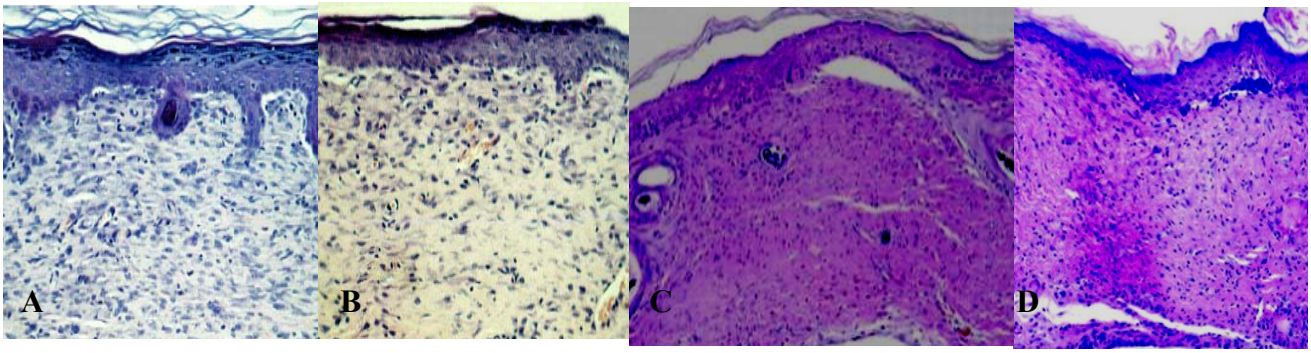

Fig. (3): showing healing of wounds15 days post-surgery.

A: Control group with complete epithelial coverage of the wound, presence of moderate number of fibroblasts close to macrophages and lymphocytes. Various newly formed blood vessels were present. **(H&E X 100).**

B: Red 650 nm laser diode treated rat group. Wounds are showing complete epithelium and moderate fibroplasia in a defined arrangement. **(H&E 100X).**

C: LED wound healing stimulated by showing neovascularization and mild inflammation **(H&E 100X).**

D: showing wound healing after IR laser 15 days post-operative stained with **(H&E 100X).**

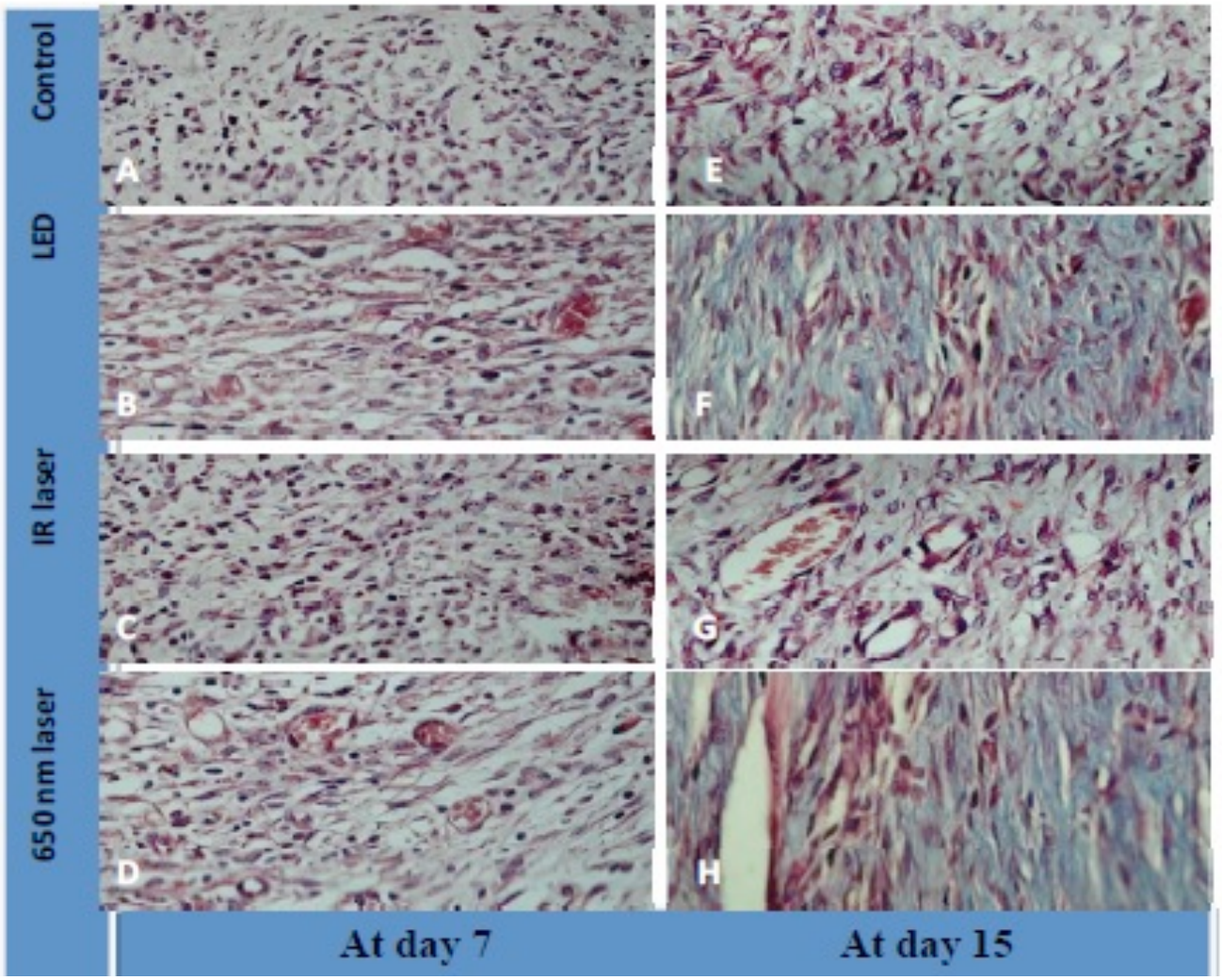

Fig. (51): Collagen distribution of animals sacrificed at 7 days.

(A) : Control group: collagen, with fibers arranged loosely.

(B) : RED LED group: The collagen is thick with densely arranged fibers.

(C) : IR laser group showed loosely arranged collagen.

(D) : Red laser 650 nm group: collagen is thick with densely arranged fibers.

Collagen distribution of animals sacrificed at 15 days.

(E) : Control group collagen was thicker and loosely arranged throughout the wound area.

(F) Red LED group collagen fibers became thicker and arranged parallel to each other.

(G) : The IR laser group loosely arranged collagen.

(H) : Red 650 nm group showed thicker collagen fibers arranged throughout the wound area in organized parallel manner **(Trichrome, x 400).**

DISCUSSION

At 3rd day, control group revealed the presence of blood clot and incomplete epithelial coverage throughout the wound. Polymorphonuclear cells were absent after the 3rd day following wound induction. While both red laser group and LED group showed almost complete epithelial coverage beneath the scab with inflammatory changes appeared in the form of some macrophages and lymphocytes and presence of some early fibroblasts. Infrared laser group showed a delay in healing, absence of epithelial proliferation, vasodilatation and a higher number of inflammatory infiltrate cells with degenerating lymphocytes and polymophonuclear leukocytes. Such results were in agreement with those recorded with the use of Red laser by Tatiana and her **collages (2004) and PeterGál *et al.*, (2006) (Tatiana Machneva *et al.*, 2008; PeterGál *et al.*, 2006)**. Also a similar result was recoded with the use of infrared laser group, as delay in healing could be attributed to the incompatibility of the IR laser wave length to the corresponding chromophores in the intracellular mitochondria. Moreover, this could be attributed to the thermal effect of the deeply penetrating photons. Although Red laser photons penetration is deep (about 0.5 cm) still the absorbance is high by these chromophores reducing the dissipation of heat and preventing the thermal injury in case of red laser **(Karu, 1999)**. A visible light probe (630 -780 nm) has a penetration depth of a few millimetres (0.5-50 mm) and is used for wound healing and superficial skin conditions **(Olsen *et al,* 1980; Moore *et al,* 2002)**.In the same time, biostimulating the proliferation of fibroblasts, increasing their number at early stage and producing collagen earlier than other groups. But collagen was not detected in all samples of all groups in the first 3 days including the Red Laser group and LED group. The biostimulation of collagen was supported by the earlier research of **Pinar Avci *et al.*, (2013).** Also **Peter Bjerring *et al.* (2001)** found that collagen production was increased after biomodulation with laser **(Pinar Avci *et al.*, 2013; Peter Bjerring *et al.*, 2001)**.At day 7 the inflammation was severe in all the samples of the control group with complete epithelial coverage with leukocyte infiltrate and macrophage. Lymphocytes were very small in number at that time. While both red laser group and LED group, showed well differentiated epithelium that completely covering the wounds.This result indicates biostimulation of wound healing through increasing the epithelial and fibroblast cell proliferation. In red laser group 650 nm, collagen was thick with densely arranged fibers, in the 7th day and after 14 days collagen was thicker with thicker fibers arranged throughout the

wound area in organized parallel manner. These results agreed with the previously published work of **Pinar Avci *et al.*(2013)** as they found that Red laser and LED are suitable for biomodulation of skin wound healing **(Pinar Avci *et al.*,2013)**. While, in the Infrared laser group the inflammation was severe in all wounds with fibrin clot infiltrated by macrophages. The wounds showed delay in healing represented by presence of mild vasodilatation due to the thermal effect and delayed epithelial proliferation, low numbers of lymphocytes and macrophages. Indicating, the withdrawal of the immune mediating cells and beginning of the end of delayed inflammatory stage. Our finding goes with the findings of **Ruthinéia *et al.* (2010)** as they stated that Low level-laser enhances the phagocytic and chemotactic activity of leukocytes in vitro. Also they found that in the process of wound repair, activation of lymphocytes by laser radiation can make them more responsive to stimulatory mediators present in injured tissues. He-Ne and Ga-As lasers increase collagen production **(Ruthinéia *et al.*, 2010)**. The IR laser group showed loosely arranged collagen at day 7, and after 14 days still loosely arranged, mixture of collagen fibers arrangement, sometimes thicker, with well packed densely arranged fibers, sometimes presenting thinner fibers loosely arranged close to the edges of the lesion, but densely compacted gross fibers in the central region in 30% of the samples. Collagen fibers appeared to be less abundant and thicker in red laser group than in IR laser group by the end of the wound healing. While Infrared Laser group caused excessive hyperplastic fibroplasia. This could be explained through the thermal injury caused by the Infrared laser. These findings agree with the earlier published work of **(Ruthinéia *et al.* 2010)** as well as **(Peter Bjerring *et al.*, 2001)** in regard to collagen production biostimultion **(Ruthinéia *et al.*, 2010; Peter Bjerring *et al.*, 2001)**.At day 14, the intensity of the inflammation in the control group was mild with abundant mononuclear cells. The Red laser group and LED group showed complete healing with absence of inflammatory cells. While, Infrared laser group intensity of inflammation was moderate with abundant mononuclear cells. This result was supported by the earlier red laser results of Karu and the LED results of Pinar **(Karu, 1999**; **Pinar Avci *et al.*, 2013)**.The maturation and remodeling phase in rats started on the sixth day and agreed with **Tatiana Mendez *et al.*, (2004)** who compared histologically the effect of infrared laser (wave length 830 nm, power 35 mW) and red laser (wave length 685 nm, power 35 mW) lasers, with Energy density of 20 or 50 J/cm^2 on cutaneous wounds in the dorsum of the Westar rat. Their work agreed with our results except they used higher power densities which explain the inflammation seen in all groups in their

results. Their combined IR-Red lasers showed the best result and this disagree with us since we found that IR laser caused a delay in healing **(Tatiana Mendez *et al.,* 2004)**.Concerning the IR laser group as compared to control group, although difference in the time of healing was insignificant, it was delayed than in the control group. This could be attributed to a bioinhibitory effect produced by the infrared wavelength spectrum since mitochondria possess cytochromes (mitochondrial proteins responsible for cellular respiration) with little affinity to IR stimulation and may be denaturized by the IR thermal effect. In a similar work performed by others they found that, infrared laser 904 nm positively promoted the healing of surgical wounds **(Aragão *et al.,* 2011)**. They disagree with the results obtained in this work since we found that IR laser has either negative result or adverse impact on healing of skin by increasing the degree of inflammation. Their work agrees with us only in the following; the increased deposition of collagen and the newly formed blood vessels and the high degree of inflammation observed with IR laser **(Aragão *et al.,* 2011)**. Differences between the effect of both Red diode laser and LED on wound area and its area percentages were insignificant in the favor of Red diode laser. This could be attributed to the intensity of laser compared to the divergent LED light as seen in previous study where they found to be efficient promoter to cell proliferation **(Myung *et al*, 2015)**. Evaluation of soft tissue healing microscopically indicated that, light biostimulation by Red Laser 650nm and Red LED shortened the time of wound healing. Although, Infrared laser stimulated the growth of granulation tissue, exaggerated fibrosis and cicatrisation were evident. While these results agree with **Bin Shu *et al*. (2012)** it disagree with **Aragão *et al.,* (2011)** this could be upraised due to difference in the frequency of the IR laser used (**Bin Shu *et al.,* 2012; Aragão *et al.,* 2011)**.The continuous laser can lead to thermal damage while the fractionated dose showed allowed the tissues to dissipate the heat and relax. This is why we used frequency pulsed laser during skin biostimulation. Treatment sessions and the 4 Joel /cm^2 dose were day after day similar to earlier work of other researchers **(Silvia *et al.,* 2012)**.Red laser 660 nm accelerated angiogenesis reaching its peak on day 15 compared with Control group.The same as our result **Silvia *et al.,* (2012)** investigated single dose irradiation group versus fractionated dose group of a red laser (λ 660 nm) the lesions were irradiated with 4 J/cm² Results confirm that both protocols used accelerated angiogenesis and stimulated leukocyte chemotaxis on burn treatment. In addition, single-dose LLLT accelerated the inflammatory phase of skin repair. Their work agree with us in the dose and the results although their evaluation methods are

different their results are similar in the inflammation modulation by red laser **(Silvia *et al.*, 2012)**. We can conclude that wound healing was promoted by red laser and red light emitting diode while it was hindered by IR laser.

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