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THE USE OF LASER IN FRACTURE HEALING

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ABSTRACT

To find the most suitable laser for fracture bio-modulation, seventy-five rats were subjected to experimental tibia diaphysial osteotomies then fixed with intramedullary pins. The rats were then exposed to either IR laser or red laser. The rats were divided into three equal groups. Group1 served as control group and left without laser exposure, group 2 and 3 were treated by infrared laser 1064nm and Red laser 650nm respectively. The dose was adjusted to 10 J/Cm^2 on daily basis for one week. Fracture healing was evaluated by x-ray on weekly basis for one month.

Key words:

Biomodulation, Laser, fracture, LLLT.

INTRODUCTION

The most common used light sources for fracture biomodulation are red diode lasers 650 nm, 660 nm, 670 nm and 690nm, infrared lasers (ND: Yag Laser 1064 nm,780 nm Diode Laser, Co^2 Laser 10600 nm, 830 diode laser) and LED 630nm. Previous studies showed that LLLT was effective for the stimulation of bone healing **(Yamada, 1991; Pyczek *et al.* 1994; Ozawa 1995; Horowitz, 1996; Yaakobi, 1996; Saito and Shimizu, 1997; Freitas *et al.*, 2000; Gerbi *et al,* 2003; Gerbi *et al,* 2004; Gerbi *et al,* 2005; Wen-Tynget *al.,* 2005)**. Acceleration in the metabolism and mineralization during early bone healing was evident **(Khadra *et al.,* 2004)**. The positive efficacy of low-level laser therapy for bone fracture repair is time and wavelength dependent **(Danillo *et al.,* 2012)**. Previous reports of laser biostimulation on rabbit bone fractures by **(Tang and Chai, 1986 and on mice by Trelles and Mayayo, 1987)** proved faster healing in the irradiated bones than in controls. Red (He-Ne) laser biomodulation of bone healing in rats showed a significant elevation in the maximal load to failure and the structural stiffness of the tibia in the irradiated group **(Luger *et al*, 1998)**. He-Ne laser irradiation biomodulated bone defect in Wister rats showed a significant increase in the area of neoformed trabeculae and concomitant invasion of osteoclasts during the first week, also hastened the organization of matrix collagen. Such phase was not recorded in non-irradiated bones at the same period. **(Garnavillo *et al*, 2003)** reached to the conclusion that active osteoclasts that invaded the regenerating site were responsible for the decrease in trabecular area by the fourteenth day of irradiation. The repair process of bone defects in rat femurs submitted to laser therapy in the red (660 nm) and infrared (780 nm) spectra was evaluated by **(Queiroga A. S. *et al.,* 2008)** . The bone defects submitted to laser therapy in the infrared spectrum had a more advanced repair compared to control group and red laser, characterized by a large amount of newly formed bone in the broken cortica as well as inside the defect and measured by morphometric analysis using the image segmentation method. A significant amount of newly formed bone within 15 days showed the biomodulated effect of laser therapy in the early stages of the repair process in which there was a large quantity of cells, mainly osteoblasts and undifferentiated cells. The infrared spectrum produced a positive biomodulation effect on the repair of bone defects in the femurs of rats **(Queiroga A. S. *et al.,* 2008)**. Studies of cell culture bone healing response to infrared light showed acceleration of osteoblast formation as well as calcium salt deposition under the influence of infrared light **(Steina *et al,* 2005; Fukuhara E. *et al.,* 2006)**. Infrared diode laser 830 nm, 40 mW, continuous wave (16 J/cm2 per session) LLLT was assessed on the repair of surgical defects created in the femur of the Westar Albinus rat. The animals in the irradiated group received 16 J/cm2 per session divided into four points around the defect (4J/cm2). Results showed increased amount of collagen fibers at early stages of the bone healing (15 days) and increased amount of well-organized bone trabeculae at the end of the experimental period (30 days) on irradiated animals compared to non-irradiated ones **(Gerbi *et al,*2008).** Infrared diode laser 830 nm biomodulation proved to have an effect on osteoblastic cells as they increased the mitochondrial activity and the population of osteoblastic cells. It caused an intense grouping of mitochondria in the perinuclea r region at 24 h and 48 hour following irradiation, while a change from a filamentous to a granular appearance in mitochondrial morphology and mitochondria distributed throughout the cytoplasm were observed 72 hour following proliferation **(Pires *et al,* 2008)**. LLLT accelerates bone repair in the initial phase independent of the wavelength used, and this effect remains for 14 days when using infrared laser **(Danillo *et al.,* 2012)**.

MATERIAL AND METHODS

Seventy-five rats were divided into three groups each of twenty-five rats then all of them was subjected to diaphysial osteotomy and fractures were fixed by intramedullary pins. The site of the induced fracture was then treated on daily basis as follow.

Group1: (Control group) the fractures were stabilized internally by intramedullary spinal needles and left without exposure to any light source.

Group2: (Infrared laser 1064 nm group) the fracture sites were exposed to 1064 nm diode laser source with energy density 10J/cm^2 on daily basis for one week.

Group3: (Red laser 650nm group) the fracture sites were exposed to red laser 650 nm with power density 10J/cm^2 on daily basis for one week.

Surgical operations for Bone osteotomies:

Atropine sulfate (0.05 mg / Kg) was injected subcutaneously to prevent the respiratory and circulatory failure, and then rats were anaesthetized by a combination of Ketamine 50mg / kg body weight, xylazine 5mg / kg of body weight and thiopental sodium 50 mg / kg of body weight **(Olson *et al*., 1993**). After surgical exposure of the tibia, complete mide-shaft diaphysial fracture was induced using mini drill Fig. (1). A spinal needle 25gauge was used as intramedullary pin which was introduced through the fracture proximal part then through the head of the tibia using manual mini drill Fig. (2). after penetrating the tibial head, the spinal needle was withdrawn till the distal part of the needle reaching to the distal part of tibia. The mini drill revolves the pin into the distal part of tibia till fixation was complete skin was then sutured.

Fig. (1): Sewing of the tibia of a rat using minidrill.

RESULTS

No radiographic changes were recorded in the fracture ends seven days' post-surgery in all the experimental groups except with the red laser group that showed early radiopaque callus Fig. (E). Exposure with red laser 650 nm resulted in indicative healing between the fracture ends one- week post-operatively Fig. (2, E, F, G and H). After 14 days' better results were recoded (E slide) as a periosteal callus was recorded in all groups Fig. (2, plates B, F, and L). A larger radiolucent area was seen in the periosteal calluses in the IR laser treated group compared with the control and Red Laser treated groups Fig. (2; B, F and L). At twenty-one days post-operatively there was complete osseous bridging over the fracture site in all groups Fig. (2: C, G and M). Four weeks post-operatively there was complete callus formation in all groups Fig. (2, D, H and N). IR laser group showed huge callus formed in one case Fig. (2, A, B, C and D).

Callus area percentage compared to the non-fractured area:

Seven days-post-operatively, the average callus area percentage in the control group was 115.8%±0.8%.Fourteen days' post-operative the callus area percentage became 120.1% ±1.1%. Radiographically, after twenty-first day it reached 121.3% ±2.8% and at 30 days' post operatively the observed callus area was 120.6% ±3.1% Fig. (3). With the use of red 650nm laser, the group showed at seven days' post operatively, the callus area percentage was 116.6%±0.7%. At 14 days' post operatively the callus area percentage became 123.2±2.8. At 21 days' post operatively, the area percentage became 121.1% ±1.0% and at 30 days post-operatively, it became120.4%±3.4% Fig. (3). In IR laser group, the callus area was 119.5% ±2.1% , 125.7% ±2.2%, 126.9% ±2.5% , 135.8% ±3.3% after 7,14,21 and 28 days' post operatively respectively. The callus area compared to the non-fractured area became larger. Fig. (2 plates A-D). The callus area percentage increased significantly compared to both the control and the red laser group Fig. (3).

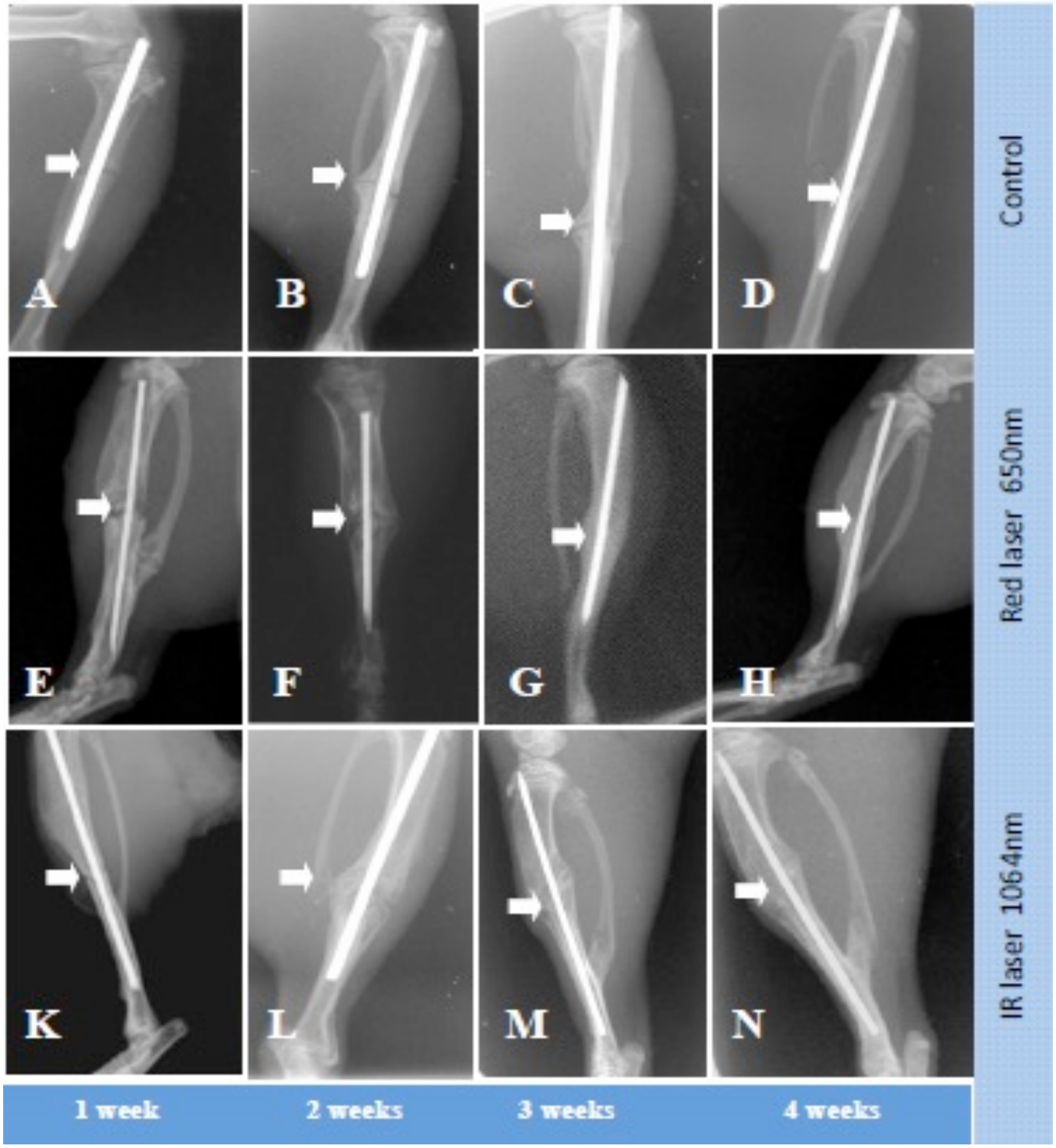

Fig. (2): is showing x-fay of tibiae for each group at 1, 2, 3, and 4 weeks.

Notice: both laser and LR laser biostimulated showed callus formation at the 14th day with better healing than control. LR laser showed larger callus formation compared to the control and red laser groups.

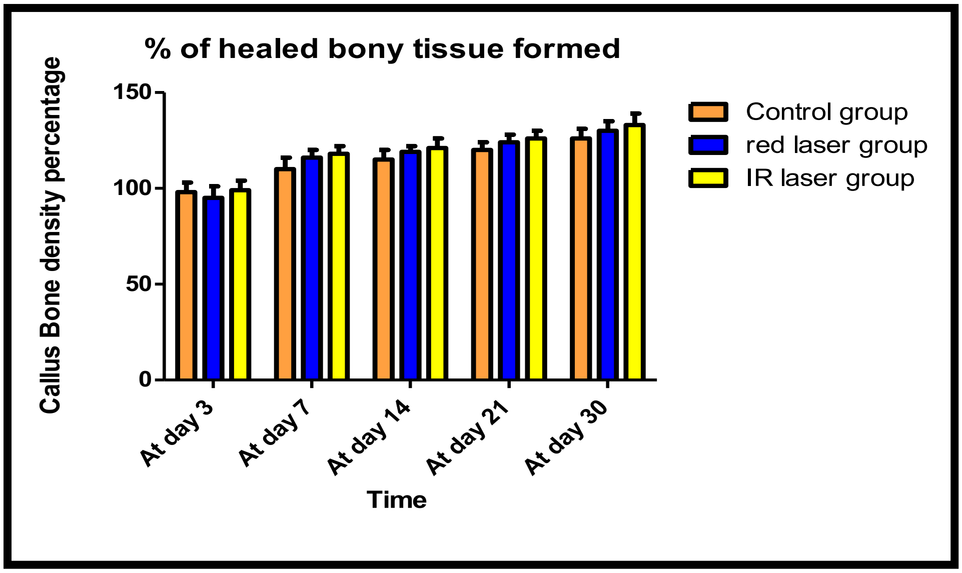

Fig.(3): Is showing the Bone density for various groups as a percentage tothe original radiographs before fracture induction.

DISCUSSION

The objective of the present study was to evaluate the repair process of standardized bone fracture in tibiae of rats submitted to biomodulated laser therapy. The used experimental model in the study was similar to that applied by **(Elchanan *et al.,* 1998 and Ralph Meyer *et al*, 2001)**. As the proper time for normal healing in 6 weeks old rats is about one month **(Ralph Meyer *et al*, 2001)** this is why we set the time interval for the experiment 4 weeks similar to the earlier work of. In the red (650 nm) and infrared (1064 nm) spectra, the bone osteotomies submitted to laser therapy in the infrared spectrum showed larger amount of newly formed bone compared to red laser and control group. Such result is similar to the previous studies in which laser therapy in the invisible spectrum was also used **(Freitas *et al.,* 2000; Gerbi *et al.,* 2003; Gerbi *et al.,* 2005; Khadra, 2004)**. The results proved that, the group treated with laser therapy in the red spectrum showed bio- stimulatory effect. Such result was similar to earlier studies that recorded the same positive effect **(Luger *et al,* 1998; Rochkind S *et al.,* 2004)**. On the other hand, contradicting results to those of the present study were recorded by previous studies as it recorded no effects of the biomodulation with the red laser in bone repair process **(Stein *et al.,* 2005; David *et al.,* 1996; Queiroga *et al.*, 2008).** In the present study, to reach to suitable wave length for fracture repair, higher penetrating wave lengths than earlier studies were used to be suitable for transcutaneous application. It was concluded that Red laser and IR laser applied by other researchers were not suitable. As it was found that lower wavelengths were less resistant to the dispersion and do not penetrate deeply into the tissues. The red light (632.8 nm) penetrates 0.5-1.0 mm before losing 37% of its intensity, while the infrared wavelengths penetrate 2 mm before losing the same percentage of energy. **(Kucerova *et al*, 2000)**. Systemic effects may not be disregarded when visible laser light is used **(Rockhind S *et al.,* 1989)**. Infrared light between the wavelengths of 700 and 950 nanometers has a relatively low absorption in tissue and the light of those wavelengths is able to penetrate several centimeters into tissue **(McCarthy, 2012)**. Water ,which is largely present in most tissues, is a strong absorber in the IR regions above 1300 nm **(Hale and Querry, 1973; Prahl, 1998)**. The wavelength defines the depth of penetration in the target tissue. The radiation emitted in the ultraviolet region and in the medium infrared region showed a high coefficient of skin absorption, resulting in radiation absorption on the surface, whereas in the region of the near infrared (820-840 nm), there was a low coefficient of absorption, resulting in a maximum penetration in the tissue **(Basford, 1995)**. These factors justify the use of infrared laser to biomodulate bone tissue taking into account other parameters as the fluency and power density. In the meantime, a lot of literature proved that, the biomodulatory effects of laser therapy depend upon the dose used **(Karu, 1987; Albertini *et al.,* 2004; Grossman, 1998; Kana *et al.,* 1981; Kipshidze *et al.*, 2001; Kreisler *et al,* 2002)**. It worthy to mention that in the present study, the energy applied was 10 J/cm^2 for the Red laser 650nm, 5 J/cm^2 for the IR laser system. Very close energy level was used in other protocols dealing with clinical and experimental studies in which positive biomodulation effects of the laser in both soft and bone tissues were observed in the range 1-4 J **(Silva Junior *et al*, 2002; Gerbi *et al.,* 2004; Saito S. and Shimizu, 1997**). **Ebrahimi *et al* (2012)** stated earlier parameters used in similar researches.The influence of the laser therapy also depends on the stage of cell growth **(Silva Junior *et al*, 2002; Khadra *et al.,*2005; Kreisler *et al.,*2002**; **Yu *et al*,1997**), the wavelength **(Karu and Pyatibr *et al*, 1995; Mi XQ *et al*, 2004)**, as well as the frequency and number of laser sessions **(Silva Junior *et al,* 2002, Limeira Junior *et al.,*2003)**, once a single application of the laser irradiation is not sufficient to obtain cellular effects **(Schlager *et al.,*2000).** As the frequency and number of sessions influence the final result of LLLT **(Silva Júnior AN *et al.,* 2002**), it was planned in the present work to use continuous laser sources and it is advisable to use pulsed laser system to avoid the thermal impact for IR laser sources. For evaluation of bone healing, X-ray digora was used in the present work. However, the method of evaluation was used by earlier researchers as (**Ricardo *et al*, 2004**). In the meantime, the present results showed that, the control group healed completely within 4 weeks' interval. In all groups, periosteal callus formation became visible by 9^{Th} day after fracture induction. In the control group, it was found that, the smallest fracture area percentage was at 7^{th} day post-operative (115.8±0.8%) while at the 14^{th} day the area percentage increased to (120.1±1.1%). Radiographs at the 21^{st} day showed almost stable callus (121.3±2.8%) and at 30 days' post-operatively a minor reduction in the fracture area was observed (120.6±3.1%). As seen in the control, the callus area is linearly increasing till the third week. In the 4^{th} week an insignificant regression in the callus area was noted indicating presence of early remodeling. Using IR laser at 7^{th}day post-operative the fracture area was 119.5±2.1% and at the 14^{th} day the size continued to increase and became 125.7±2.2%. The progress ceased at the 21^{st} day post-operativelyasitbecame126.9±2.5%.After 30days'post-operatively it reached 135.8±3.3% representing a great increase in the size of the callus. The results regarding the callus area agree with those of **Piret Hussar and Ülo Hussar (2003)**. At Twenty-first day the calluses were fully formed and began to resorb in the process of remodeling. The present findings allied with their counterpart Hua **Long *et al.* (2010)**. The process of remodeling is responsible for the decrease of callus area in the 4^{th} week of fracture healing. The mean of red laser group callus area was at 7^{th} day 116.6±0.7 showing slight increase in callus area compared to the control and the IR groups. At 14^{th} day post-operative the area reached 123.2±2.8. A major decrease in the area percentage as it reached 121.1±1.0 and at 30^{th} day post-operative it became 120.4±3.4%. Such results indicate earlier fracture remodeling of the callus during Red laser biostimulation. A larger radiopaque area was seen in the periosteal calluses in the IR laser treated group relative to the control and Red Laser treated groups, suggesting that Red laser treatment enhanced bone formation more than control group while IR laser enhanced both healing and callus size formation in relation to other groups. Formation of osseous bridging over the fracture site was completed by day 21^{st} day.

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