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Repair Tibial Chronic Defect by Using 810 ± 10 nm Continuous Diode Laser in Rabbits

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Abstract

The present study was designed to Study the effect of low level laser therapy of continuous diode laser at 810 ± 10 nm on repair Tibia chronic defect in rabbits. Eight adult rabbits were employed to induce 3 mm in diameter chronic defect in Tibia, by insert the stainless steel screw in the induced hole for one and half month post operation, then the stainless steel device removed to create the chronic bone defect. The animals were divided to two equal group, the control group of 4 rabbits were followed up for normal healing processing without any treatment, while the treatment group was exposed daily to single dose of continuous diode laser at 810 ± 10 nm, 500 Ma for 10 minutes at 72 hours interval for 14th days at the medial aspect of the Tibia. The results of the clinical observation were same in both groups which revealed body depression with loss appetite, immediately after surgical operation, which retained to normal after few days, and the inflammatory signs at the surgical wound, disappear with satisfactory wound healing after 5 to 7 days post operation, the radiographic finding at the end of the first and second week post irradiation in the treatment group shows increase sclerotic area around the chronic defect margin more dense and wide with decrease in the bone defect diameter compare with the control group, while the macroscopic examination at the end of the experimental period were same in both group which show heavy fibrous connective tissues fill the chronic defect, while the microscopic examination revealed profuse and more prominent osteoblast cells blood vessels angiogenesis and lamellar bone formation more obvious and clear in the treatment group compare with the control group at the end of the experimental period. The conclusion is low level laser therapy by continuous diode laser at the 810 ± 10 nm can be used for repair of induced chronic defect of Tibia in rabbits.

Keywords: Bone repair; Continuous diode laser; induced chronic defect of Tibia; Low level laser therapy

Introduction

Laser is a short termination for (light amplification by stimulated emission of radiation [1]. Low level laser therapy (LLLT) can be used successfully in repair mandible defect in rabbits, by increase number of cells proliferation and migration of the osteoblst cells, and its activation in bone formation [2]. laser therapy can promote healing of the chronic defect in the tibial bones in rabbits which achieved by radiographic and physical finding [3], other results work of the radiological and histopathological examination of laser exposure on the fractures of the distal third radius in dogs revealed a positive effect on promoting fractures healing [4,5]. In others works result Nazht and his group in 2018 mention the stimulatory effect of LLLT at 850 nm for 5 minutes on the xeno-sheep bony implantation in the femoral fractures in rabbits [6].

Laser applications play an important roles in orthopedic surgery by accelerating fractures healing and promoting repair bone defect, because of its positive effect on osteogensis, fibroblast cells activation, collagen synthesis, osteoblast cells proliferation and activation with osteoid depositions and mineralization with hard bone formation [2,7,8]. LLLT has a positive effect on the inflammatory phase by reducing the inflammatory signs, pain and its analgesic effect, also its positive roles in orthopedics surgery, on bone metabolism during fractures healing [9-16]. The pathphysiological action of laser as reported by [17] that the photonic energy of laser absorbed by cells mitochondria of the irradiated target which converted to chemical kinetic energy and finally leads to more production of ATP, which is the source of energy in the cell and necessary for cell activations and synthesis of DNA, RNA, and proteins that are important in cellular proliferation.

The aim of the present project is to evaluate the efficacy of the continuous diode laser therapy at 810 ± 10 nm on the repair of induced Tibia chronic defect in rabbits and evaluate this goal by clinical observation, radiological examination and histopathological finding at the end of the experimental period 2 and half months p.o. (One month from laser exposure).

Materials and Methods

Eight adults local breed rabbits were used to induce 3 mm diaphyseal chronic defect in Tibia. the medial aspect of tibia was prepared by clipping shaving the hair and wash with soap and tap water then disinfected the area with 70% ethyl alcohol, the defect created by electrical drill with dropping isotonic sterile normal saline to prevent thermal necrosis, the operation done under general anesthesia by intramuscular injection of 2% xylazin hydrochloride and 10% ketamine hydrochloride respectively, the defect implanted with stainless steel screw for one and half month p.o. (Figure 1), then the stainless steel screw removed to create bone chronic defect in Tibia (Figures 2A and 2B), the animals daily observed for any abnormalities or complication with intramuscular administration single daily dosage of penicillin streptomycin as broad spectrum antibiotics for 3 days p.o., the experimental animals were divided to two equal group each contained four rabbits, the control group followed for normal healing processing without laser irradiation, while the treatment group

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at the medial aspect of tibia (Figure 3), exposed for single daily dosage of continuous diode laser at 810 ± 10 nm, 500 Ma for 10 minutes at 72 hours interval for 14th days(Figures 4 and 5). This work done in the surgery department of Veterinary Medicine College/university of Baghdad/Iraq under observation and ethical committee.

The parameters which were used for the evaluation as follows.

1. Daily clinical observation, the physiological behaviors and animal's gaite, or any complications or abnormalities which may occur at the site of operation.
2. Radiographic finding at the end 1st and 2nd weeks post laser irradiation.
3. Histopathological examination for both macroscopic and microscopic finding at the end of the experimental periods two weeks post laser irradiation (two and half month p.o.).

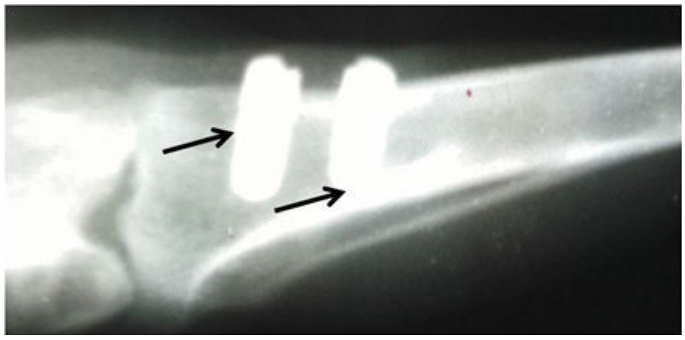

Figure 1: Radiographic image shows the stainless steel screw long black arrow, implanted in the Tibia to create the chronic defect.

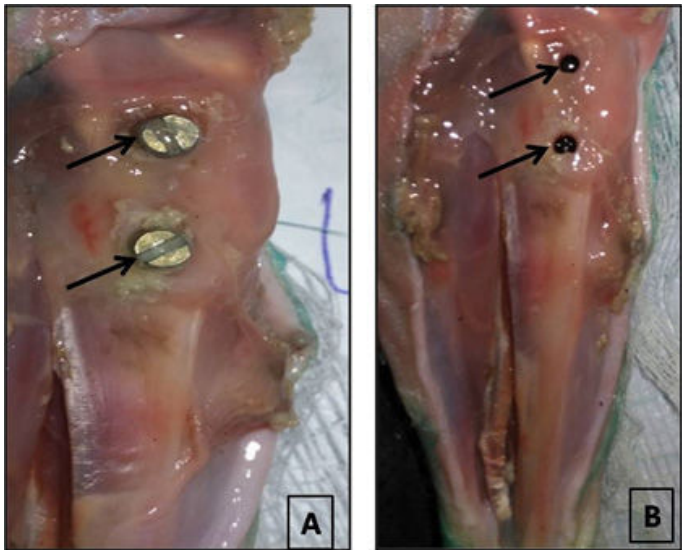

Figure 2: Tibia model shows macrograph 2a) The stainless steel screw well inserted in tibia macrograph 2b) Remove the stainless steel device to create the chronic defect 3 mm in diameter after one and half month.

Figure 3: The treatment group exposed to 810±10nm of LLLT for 10 minutes at the created chronic defect on the medial aspect of Tibia.

Figure 4: Laser apparatus

Portable Laser SN-808A

Specification

Wavelength	810nm±10	Operation Current	<500mA
Output power	300 mw	Operational mode	Continue/pulse(10Hz)
Time set	10-20 minutes	Supply voltage	110V -240V/±50Hz
Laser probe size	Φ37×140 mm	Size	190×135×60(mm)
Weight	0.85kg	Laser class	ⅢB

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Figure 5: Laser model and properities

Results

Clinical observation

The clinical observation were the same in both group, which revealed loss appetite and difficult to move with body depression immediately p.o., then within 24 hours p.o. then animals begin to move and eat with normal physiological activity (urination and defecation) gradually the next day's p.o., the skin incisions healed satisfactory within 5-7 days p.o.

with no complications, the rabbits normally used the limb in bearing the body weight and walking and running from the end of the 1st week until the end of experimental period.

Radiological finding

End of 1st week post laser irradiation: Sclerotic area around the induced chronic defect which is more dense and wide in the treatment group compare with the control group, the defect filled with new bone formation in both group (Figure 6).

End of 2nd week post laser irradiation: The radiographic image show increase in density and widening of the sclerotic area with decrease the defect diameter in the treatment group compare with the control group, the defect filled with new bone formation in both group (Figure 7).

Histopathological examination

Macroscopic examination at the end of the experimental period: The macroscopic findings were the same in both groups which revealed formation of fibrous connective tissues that fill and cover the chronic defect (Figures 8A and 8B).

Microscopic examination at the end of the experimental period: The control group: a)The transverse section revealed marked congested B.V. With cellular infiltration in fatty marrow, containing various types of inflammatory cells, the host bone contain large size of H.C. With tissues debris with irregular trabecular bone formation with number of osteocytes at the surface (Figure 9). B) The longitudinal section shows fibrous tissues deposition with osteoblast cells and osteoid formation at the margin of the recipient bone and the defect border, the compact bone contain various size of H.C. with lacuna contain osteocytes, with large marrow space filled with necrotic debris bone specula's and number of osteoblast (Figure 10). The treatment group: The transverse section revealed numerous congested large blood vessels in the fatty marrow tissue, with hemorrhage area and presence of eosonophilic, new trabeclar bone formation, marrow cavity filled with PMNCS, macrophages, plasma cells, the compact bone of the recipient femoral bone contain number of the osteocytes in the lacuna with few haversain canal, the edge of the host bone replaced by thick fibrous connective tissues and callus formation, the newly developed osteoid tissues supported by active osteocytes cells. Fibrous callus formation with newly developed osteoid tissues fill the space between the host bone (Figure 11).

The longitudinal section revealed. The marrow tissues showed extension hemorrhage and B.V congestion with infiltration various type of inflammatory cells, new bone formation. Profuse fibrous connective tissues fill the induced gab with active and numerous blood vessels and collagen deposition infiltrated with inflammatory cells mostly poly morphonucleic cells, the new bone formation around the chronic defect characterized by weak elongated trabeclu bone formation containing vascular canal with flat osteoblast cells lining its surface, the blood vessels inside the haversian canal with the osteocytes cells in the lacuna in centrifugal appearances, with many of the empty lacuna (Figure 12).

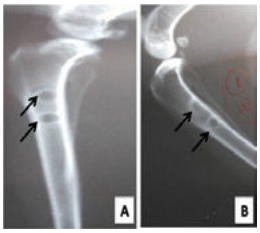

Figure 6: One week post laser irradiation. 6a) control group, 6b) treatment group, represents increase radiographic density, sclerotic area around the induced chronic bone defect which appears wide and clear with decrease the defect diameter in the treatment group compare to the control group , turbidity area inside the pore in the both group represent the newly fibrous connective tissue which may be converted to new bone formation.

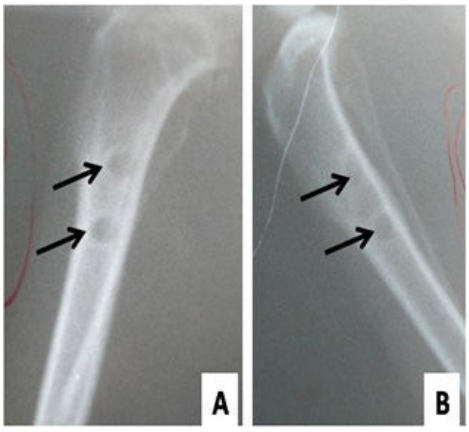

Figure 7: Two week post laser irradiation, 7a) control group, 7b) treatment group no significant changes in the control group, while the treatment group seemed to show more dense area around the induced chronic defect, with decrease the pore diameter.

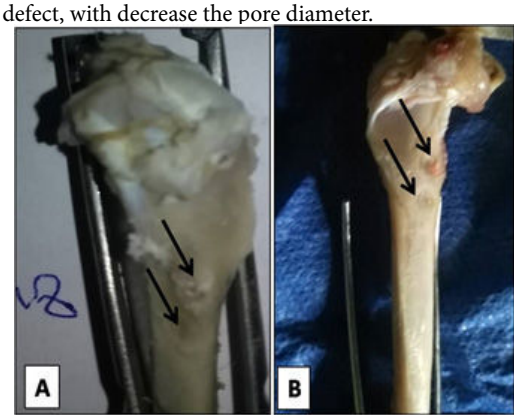

Figure 8: Grossly examination of the tibia defect, 8a) control group, 8b) treatment group, illustrated close the defect with fibrous connective tissue in both group.

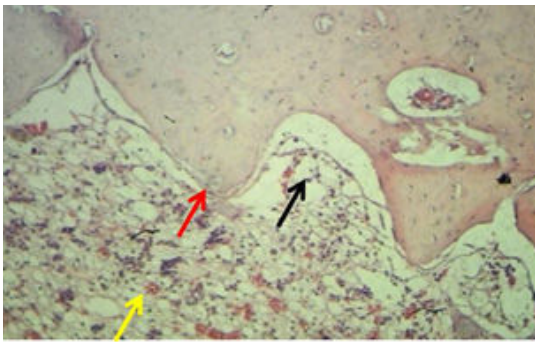

Figure 9: Figure 9. Control group, transverse Histopathological section, Hemopoetic cells(↗) in the bone marrow cells, with increase of blood circulation(↗) and new bone formation (↗)from the endosteum (H and E× 10).

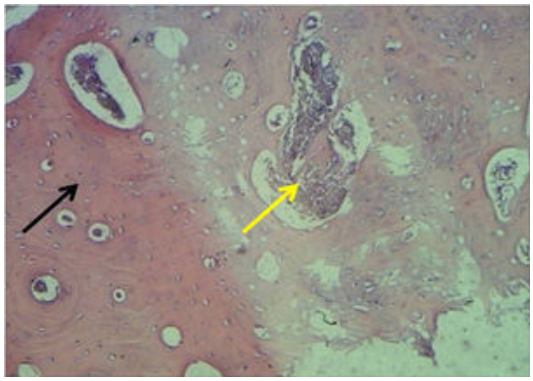

Figure10: Control group longitudinal histopathological section which revealed new bone formation that converted to lamellar bone formation (↗) with some cavity which not converted to mature bone formation these cavity filled with vascular connective tissues (↗), with new bone formation, profuse osteoblast cells lining the new bone formation (H & E× 10).

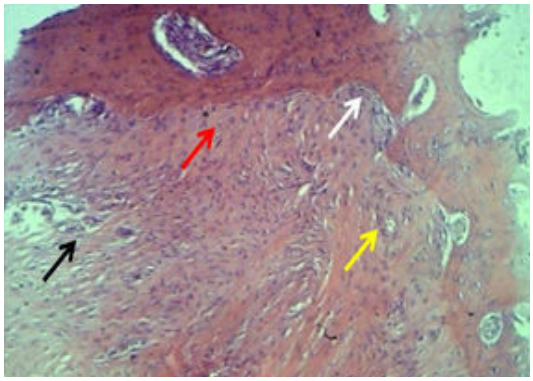

Figure11: Treatment group longitudinal histopathological section which revealed fibroblast cells(↗) proliferation with osteoblast cells (↗) and osteoid depositions (↗)and marked new blood vessels (↗) (H & E× 10).

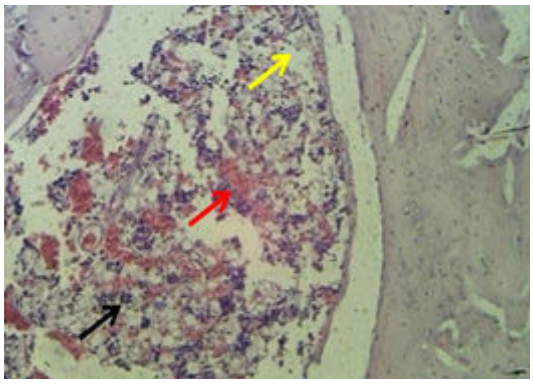

Figure12: Treatment group transverse histopatholoigical section which revealed active hemapotic cells in the bone marrow which filled with fat cells (↗) with highly blood vessels (↗) infiltrated with inflammatory cells (↗).(H & E× 10).

Discussion

The clinical observation were the same in both group and these signs agree with [10,11,14,16] in which they refer to the inflammatory signs that appears immediately after operation with loss of appetite and difficult to move, which then retained to normal after 24 hours p.o.

The radiographic finding of the treatment group which exposed to LLLT in the end of the 1st week post irradiation revealed high sclerotic area around the adage of induced chronic defect that later increase in wide and density at the end of 2nd week post irradiation, with signs reduce in the defect diameter compare with the control group at the same time, these events is due to the stimulatory effect of laser on the osteoblast cells activation and proliferation with osteoid formation and mineralization and these activation may due to increase alkaline phosphates enzymes synthesis and release as mentioned by [3,18].

The macroscopic appearance which shows fibrous connective tissues formation filled the defect in both group, with signs of reduce the diameter of the bone defect in treatment group compare with the control group, that is due to laser stimulation, and this note is agree with [8] That laser application has stimulatory effect on fibroblast cells metabolism and numbers, which may produce profuse of collagen fibers that will later change to mature trabecula bone.

The Histopathlogical findings of the treatment group shown new bone formation in the bone defect which characterized by mature and wide trabecula bone formation with less of the empty space inside, that filled with vascular connective tissues and active osteoblast cells lining the bone surface, in other section the mature new bone formation converted to lamellar bone compare to the control group in which many space of the mature trabecula bone not changed to lamellar bone, these observation is due to osteoblast cells activation and osteon production with calcium and minerals deposition and these agree with [2] that LLLT of 850 nm has a stimulatory effect on the osteoblast cells in bone defect repair in the lower mandible in rabbits while [7] showed that using diod laser of 780 nm faster bone regeneration in mandibles defects of Holtsman rats, than control group.

The positive results of the LLLT on treatment group compare with control group which achieved by histopathological and radiographic finding, is due to the stimulatory effect of LLLT on osteoblast cells activation and proliferation, with production and release ALP enzymes that promote osteoid production and mineralization besides its stimulation on the fibroblast cells and collagen synthesis with osteogensis and these agree with [3,18] in which they refer to the positive effect of LLLT at dosage of 850 nm on the chronic defect in rabbits tibia, which evaluated radiographically and physical analysis.

Conclusion

Laser therapy of continuous diod laser at dosage 810 ± 10 nm can be used in repair induced tibial chronic defect in rabbits.

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