

HISTOPATHOLOGICAL STUDY OF THE DISTAL THIRD FRACTURES OF RADIUS IN DOGS, TREATED WITH LLLT,

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ABSTRACT

The aim of study was a histopathological evaluation of the effects of low-level laser therapy (LLLТ) such as diode laser on healing fracture of distal third of the radius in dogs, at the end of 1st, 3rd and 6th week post operation. 18 male adult street dogs were used to obtain transverse fracture at the distal third of the radius under highly aseptic technique, the anesthesia was done by using the combination of (15 mg/Kg.B.W) ketamine hydrochloride, and (5 mg/Kg.B.W) xylazine, the fractured limb fixed with (Gypson) as an external fixation device, and window is obtained at the anterior surface of the radius at the fracture sites for direct irradiation of the laser. The experimental animals were divided into two equal groups each contain 9 dogs, the control group left without any irradiation, while the treatment groups received daily single dose of laser (850 nm, for 5 minutes at 72 hours interval for 14th days). Histopathological finding at the end of the 1st week revealed early formation of irregular trabecular bone (immature woven bone), compare with the control group at the same period, then these trabecular bone became later more regular, dense and width at the end of the 3rd week in the treatment group, then quickly changed to lamellar bone in the end of the 6th wk P.O. in the treatment group compare with the control group. It concluded that The LLLT promote and accelerate fracture healing, in the distal third of the radius in dogs

INTRODUCTION

Bone fracture is a medical condition in which there is a break in the continuity of the bone, and it is one of the most popular problems of small animals

especially dogs, resulted from high force impact, stress, trauma or as a result of certain medical conditions that weaken the bones (1). Fourteen percent of long bone fractures involved the distal 1/3 of the radius and ulna. This represents the most common site of radial fracture in the dog and it accounted for 85% of the radial fractures (2). These fractures are associated with a higher incidence of delayed or nonunion and the cause may be in some breed is due to the precarious blood supply of this area (3 and 4).

Low level laser therapy (LLLT) was used to enhance and promote fracture healing as a Physical stimulation therapies (5). Improvement of bone healing is by various circumstances, such as by dental extraction, or bone fractures (6), it is efficient in decreasing post injury inflammation, speeding up soft tissue treatment and exciting the growth of new blood vessels (7). LLLT is a biophysical way of intervention in the fracture repair procedure, which, through several mechanisms, accelerates the treatment of bone injuries and enhances callus growth in the initial phase of the procedure of recovery, also laser can speed up bone cells growth by improving osteoblastic action; (8). Several in vivo and in vitro research have investigated the use of laser device treatment in the biomodulation of bone cells repair through its photochemical and photobiologic qualities. Also, it may activate osseointegration and can enhance bone cells ingrowths and functional recovery (9, 10 and 11). Due to the good outcomes on bone cells metabolic rate and on bone fracture consolidation the use of LLLT has been encouraged in clinical practice, the laser device speed up the procedure of bone fracture fixation and cause an enhance on the callus volume and bone cells mineral solidity (12). (13) Revealed a significant enhance in the growth of osteoblasts and enhance the amount of mineralized bone cells after laser therapy

The study was designed to investigate the histopathological effect of LLLT on the fracture healing at the distal third of radius in adult dogs at the end 1st, 3rd and 6th wk post operation (P.O).

MATERIAL AND METHODS

Eighteen adult male street dogs, apparently health weighing 17-20 kg body weight were used. They kept in special dogs cages which belong to the surgery and obstetric department /college of veterinary medicine/university of Baghdad, receiving the same condition for one week prior to operation. All the animals take a single dose of antihelmintc drugs (ivermectine). The animals were fasted 24 hrs prior the operations and water 6 hrs, Anesthesia was achieved by intramuscular injection of a mixture of 15mg/kg B.w ketamine hydrochloride, and 5mg/kg 2% xylazine. .10 cm in length of the skin Incision was done in the distal third at the anterior surface of the radius, the subcutaneous tissue dissected. Elevate and retract the extensor tendons laterally as needed for fracture visualization(14), induced Transverse fracture by using electrical saw and used normal saline to avoid thermal reaction .a positioning the subcutaneous tissue using simple continues pattern using no. 2/0 chromic catgut and closed the skin by interrupted suture pattern using no.0 silk, then fixed the fractured bone with external fixation (Gypsona) and induced window direct to the site of fracture for single daily irradiated dose of laser in the treatment group. The animals were divided to two groups, the control group of 9 dogs which were lifted without irradiation, the second group is a treatment group which were irradiated daily with single dose of laser (Diod laser. Continuous Frequency, Energy density: 148.4 J/cm², 850nm) for 5 minutes at interval 72th hours for 14th days post operation,daily Injection of single dose of antibiotics for 3-5days,

The animals were a scarified at the period of the end of the 1st,3rd ,and 6th weeks, and spacemen were taken and prepared for histopathological processing (15).

RESULTS

1. End of 1stwk. Post Operation.

A. Control group: hemorrhage, newly formed blood vessels in bone marrow with, few numbers of young fibroblast, in other section, fibrin networks deposition, and many inflammatory cells like mononuclear cells infiltration consist mainly of macrophage and plasma cells around congested blood vessels. (fig.1).

B. Treatment group: hemorrhage, sever dilatation and congestion of capillaries, trabecular bone formation that appear as irregular thin delicate lined with osteoblast that appears flat and fibrin networks, with many inflammatory cells infiltration in bone marrow as well as necrotic bone fragment, in other section quantity of newly

formed thin walled blood vessels appear in bone marrow the fatty marrow will fill the space between trabecular. Numerous osteoblasts at the out surface and vascular connective tissue. Scattered proliferation of mature fibroblast near the irregular trabecular (fig.2).

2. End of 3rd wk Post Operation.

A.control group: active osteoblasts cells around the newly formed trabucular bone and the osteocysts cells in their lacuna, in other section apparent of peripheral, moderate mononuclear cell infiltration in bone marrow consist of macrophages plasma cells, mainly seen around the blood vessels (fig.3).

B.Treatment group: increase thickness in the newly formed immature woven bone, with evidence of newly formation of havresin canal in the remnant of vascular mesyncahymal cells around tissue around large congested space, mononuclear cells with fibrin networks infiltration in the preostium and fibrous connective tissue in (fig.4).

3. End of 6th wk Post Operation.

A.Control group: Well developed trabecular bone, lining with osteoblast, the mature trabecular bone arranged around large vascular space, infiltration of mononuclear cells, the space gap filled with irregular mesyanchymal tissue with mature granulation tissue together. (fig.5).

B. Treatment group: thickening of the trabecular bone formation around haversian canal, compact lamellar bone formation, with appearance of havreasin system, lining by active osteoblasts , the mature bone contain osteocyt in their lacuna, with number of osteoblasts with esinophilic osteoid tissue and counting cement line (fig.6).

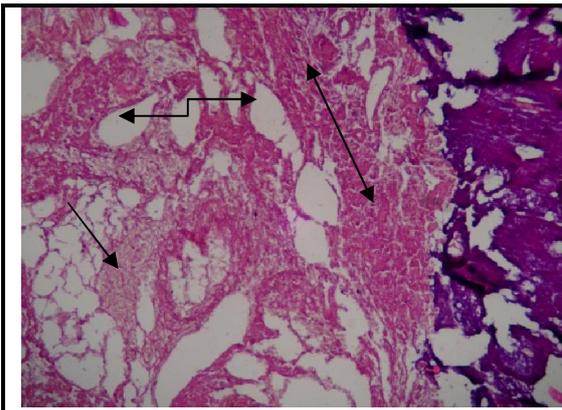


Fig.1.Histopathologic section of control group at the end of 1stwk P.O. shows newly formed blood vessels ←↗, hemorrhage ←↔, fibrin networks deposition mixed with inflammatory cells in bone marrow → (H&E 10X).

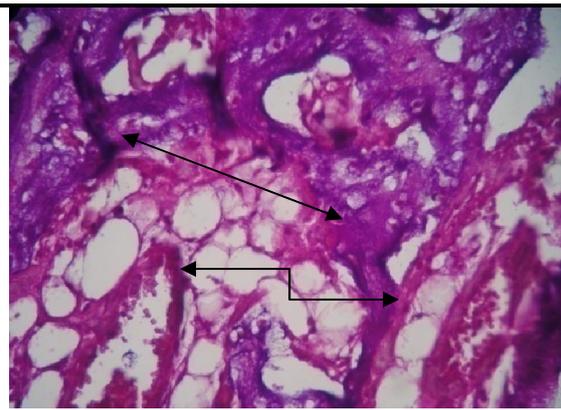


Fig.2.Histopathologic section of treatment group at the end of 1stwk, shows well developed blood vessels appear in bone marrow ←↗ with newly formation of trabecular bone (H&E 40X). ←↔

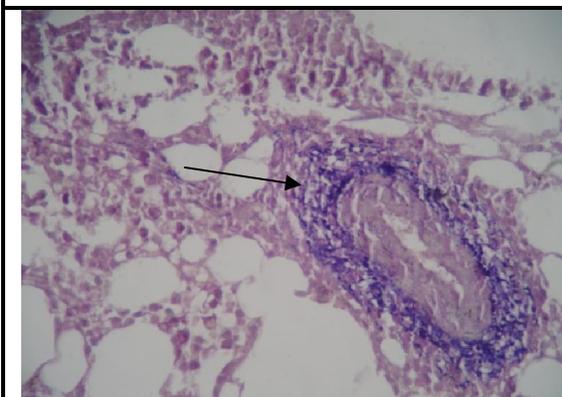


Fig.3. Histopathologic section of the control group at the end of the 3rdwk shows moderate per vascular mononuclear cells infiltration in bone marrow tissue → (H&E 40X).

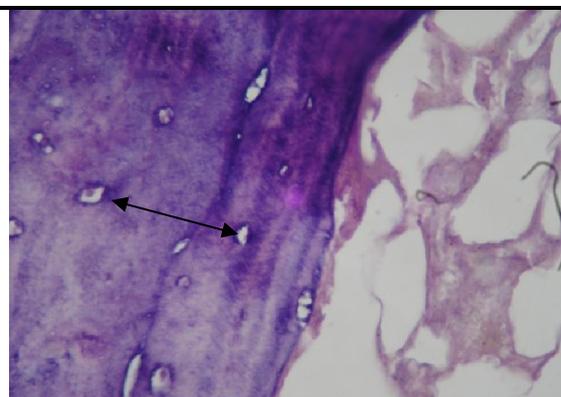


Fig.4. Histopathologic section of treatment group at the end of 3rd wk. P.O. shows evidence of newly formation of havrasin system in the remnant of vascular tissue. ←↔ (H&E 40X).

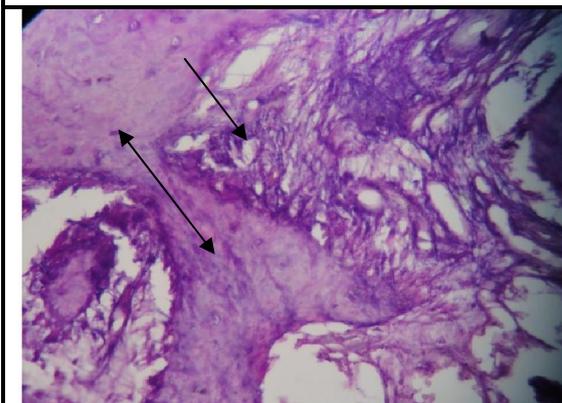


Fig.5. Histopathologic section of the control group at the end of the 6thwk P.O. shows well developed widely trabecular bone ←↔ with irregular mesenchymal → granulation tissue associated with mild mononuclear cells infiltration fill the gap space (H&E stain 40X).

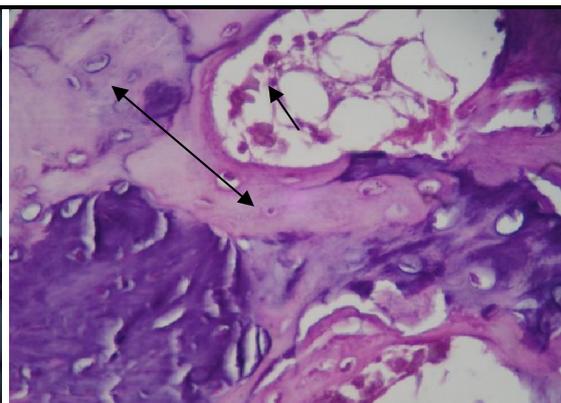


Fig.6. Histopathologic section of the treatment group at the end of 6thwk P.O. shows lamellar bone formation with cement line ←↔ and remnant fatty bone marrow → (H&E stain 40X).

DISCUSSION

The histopathological section at the end of the 1st weeks post operation which shows new and irregular trabecular woven bone in the treatment group, when compared with a control group which was not clear enough, due to the positive effect to laser exposure on the cell growth and osteoblast action (8). The early effect of LLLT on the fracture healing is due to osteoblastic activity (significant increase in osteoblastic proliferation), which later in the other week may improved osteoid formation, as shown in the histopathological finding in the treatment group in this study at the end of 3rd week post operation compare with control group at the same period (16). The real effect of laser within week with regardless of the wave length is due to the bio modulator impact and its ability for promote proliferation of endothelial cells and formation of a newly highly vascular net work which is essential for repair process (17). In other hand the second action of the laser on the irradiated area is to stimulate fibroblast cells for collagen synthesis and bone growth (18). Many authors referred that laser stimulate osteoprogenitor, osteoblasts and osteoclasts cells which contributing to bone remodeling (19). The increase in the bone matrix deposition and quickly in new bone formation with the clear signs of increase the thickness of the newly formed trabecular bone in the treatment group at the end of 3rd week ,compare with the control group at the same period, These results may attribute to the stimulation effects of LLLT on the new angiogenesis at the site of fracture and increase collagen fibers deposition and promote bone cells proliferation which finally lead to the accelerating callus formation and hesitant fracture healing (20), which evidences that the effects of LLLT through the proliferative stages (12 and 13). the effect of the LLLT on the newly woven bone formation which later became more mature and widths with the presents of the active osteoblast in and around the trabecular bone formation ,and this statement agreed with what mentioned from (21) , that the laser device is able to contributed to the speeding of the bone tissue recovery procedure and discovered good outcomes after the administration of laser in bone tissue defects and after 14 days they discovered an improve of the area of the woven bone tissue in the treatment group when compare with control group.

The lamellar bone which formed quickly in the end of the 6th week in the treatment group compare with the control group at the same period is due to the positive effect of laser. The beneficial effect of LLLT is on the bone tissues recovery

procedure and on the osteoblast cells, and this was agree with the results of others researchers whom shown that laser device therapy is able to stimulate osteoblastic growth and angiogenesis at the bone fracture site of the irradiated tibia, and had considerably more woven bone tissue as opposed to the control group that received no laser device therapy in rat subjects, therefore, it will increase the bone healing process , (21,22 and23). The low dose of the laser which employed in this study gives best results at the end of the first week by the newly bone formation which was fixed during the results of the histopathological finding in the treatment group compare with the control group in which the new trabecular bone formation was not clearly found (23). Low amount of LLLT tends to work better than the same wave length delivered at high levels. (12and13) they found that an important improvement in the growth of osteoblasts after laser irradiation and improve the quantity of mineralized bone cells and speed up the procedure of bone fracture fix and cause a rise in the callus quantity and bone cells nutrient solidity.LLLT which was used as a Physical stimulation therapies enhance and promote fracture healing (5 and 13).

دراسة مرضية نسجية لكسور الثلث القاصي من عظم الكعبرة في الكلاب المعالجة بالليزر واطئ الطاقة

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الخلاصة

الهدف من هذا البحث هو دراسة التأثير المرضي النسجي لليزر واطئ الطاقة نوع الدايدود ليزر على التئام الكسور في الثلث القاصي من عظم الكعبرة في الكلاب نهاية الاسبوع الاول والثالث والسادس بعد العملية. استخدم ثمانية عشر كلب ذكر ناضج من الكلاب السائبة تم استحداث كسرا مستعرض في الثلث القاصي من عظم الكعبرة باستخدام شروط التطهير والتعقيم وتحت التخدير العام باستخدام مزيج الكيتامن هايدروكلويد وبجرعة (١٥ ملغم/كيلوغرام من وزن الجسم) والزيلازين بجرعة (٥ ملغم/كيلو غرام من وزن الجسم) استخدمت (الجبسونه) كجبيرة تثبيت خارجية لتثبيت الكسور. استحدثت فتحة من خلال الجبيرة في الجزء الامامي من عظم الكعبرة فوق منطقة الكسر لتعريض الليزر. قسمت حيوانات التجربة الى مجموعتين وكل مجموعة تحوي تسعة كلاب. مجموعة السيطرة تركت بدون تعريض الى الليزر ومجموعة العلاج تم تعريض منطقة الكسر الى جرعة واحدة يوميا من الليزر بمقدار (٨٥٠ طول موجي لمدة خمس دقائق لكل ٧٢ ساعة لمدة ١٤ يوما). اوضحت النتائج المرضية النسجية في نهاية الاسبوع الاول سرعة تكوين الحويجزات العظمية غير المنتظمة مقارنة مع السيطرة للفترة ذاتها، ثم تحول هذه الحويجزات العظمية غير المنتظمة الى حويجزات

عظمية منتظمة كثيفة واسعة نهاية الاسبوع الثالث وتحولها الى عظم صفائحي مصمت في مجموعة العلاج نهاية الاسبوع السادس مقارنة مع مجموعة السيطرة لنفس الفترة. نستنتج ان الليزر واطى الطاقة يحسن ويسرع من التئام الكسور في التلت الفاصي من عظم الكعبرة في الكلاب.

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