

The effect of low level laser therapy on bone healing in male rats

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Abstract

The study aimed to evaluate the beneficial effect of LLLT on bone healing, and to compare between the effect of using two doses (4 and 8 J/cm²) of laser. Sixty adult male Wistar rats were used and randomly divided into three equal groups, control and two laser treatment groups 4J / cm² and 8J / cm². Bone injury (2 mm in diameter osteotomy) (limited size bone stoma) was created by drilling in the right tibia bone of each animal after making 2cm length longitudinal skin incision on the antero-lateral aspect of the leg by use scalpel, and displaced the anterior tibialis muscle laterally. After that the muscle was replaced and the incisions was sutured with 3/0 silk suture materials. The two laser groups (4J/cm² and 8J/cm²) are received a close-contact pulsed Gallium-Aluminum Arsenide Laser (GaAlAs) on the operation site (with energy density of 4 and 8J/cm² in a wave length of 660nm, power output 50mw, and pulsing rate 146 Hz.) immediately post operation, and then repeated the dose daily for seven successive days. Control group left untreated. Bone healing processes were followed-up by taking histopathological specimens after euthanasia of animals at 7, 14, 21 and 28 days after the surgical operation. Results displayed that the laser therapy used in both doses were an evenly continuous enhance the healing process of the bone, by acceleration process of healing and reduce inflammation period in compared with the control group. Histologically the bone defect in 4J/cm² group was filled early with a dense mass of granulation tissue interposed with polydactyl projections of spike like bony trabeculae giving the shape of new developing bone. The osteoblasts were seen large in size lining the bone trabeculae. The trabecula containing numerous osteocytes. Intra membranous and endochondral ossification were developed early. The cartilage tissue was disappearing at 28 days and the calluses seem like more developed. In 8J/cm² group early visualization of both intramembranous and endochondral ossification surrounded by new periosteal tissue. Thick and dense branching interconnected trabeculae were appearing fill the bone defect. The endochondral ossification early invade the cartilage tissue from the periphery, also the intra membranous and the endochondral ossification were interconnected. No signs of inflammation, and early disappear of granulation tissue were seen. Finally thick highly cellularized woven bone was seen characterized by presence of wide multi-branched interconnected trabeculae connecting each other. In conclusion the both doses (4 and 8J/cm²) were used seen effective in stimulating and enhance the bone healing, and the 8J/cm² was given better stimulation effect than the 4J/cm².

Key words: LLLT, laser, bone, fracture, bone healing, rat.

تأثير العلاج بالليزر واطئ الطاقة على التئام العظم في ذكور الجرذان

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الخلاصة

هدفت الدراسة الى تقييم الآثار المفيدة للعلاج بالليزر واطئ الطاقة في شفاء العظم ومقارنة تأثير استخدام جرعتين 4) جول و 8 جول\اسم²) من الليزر. استخدمت في الدراسة 60 جرد (ويستر) ذكر بالغ قسمت عشوائيا الى ثلاثة مجاميع متساوية (مجموعة سيطرة ومجموعتين ليزر). احدث ثقب بالثاقب (القطر 2 ملم) (فتحت العظم محدودة الحجم) بعظم الساق اليمنى لكل حيوان بعد اجراء جرح طولي في الجلد بالمشروط بطول 2سم على الجانب الامامي الوحشي للساق وازاحة العضلة القصبية الامامية جانبيا ، بعدها أعيدت العضلة الى موقعها وجرى خياطة الجرح بخيط الحرير 0/3.

مجموعتي الليزر أعطيت جرعتي الليزر 8 جول و4 جول اسم² (الليزر النبضي (GaAlAs) الطول الموجي 660 نانومتر، الطاقة 50 ملي واط، معدل النبضة 146 هيرتز) على موقع الجرح مباشرة بعد اجراء العملية (لمرة واحدة في اليوم) ولمدة سبعة ايام متتالية. ومجموعة السيطرة تركت بدون معالجة. جرى تعقب عملية شفاء العظم من خلال الفحص النسيجي المرضي بعد اخذ عينات العظم بعد قتل الحيوان في الايام 7، و 14، و 21، و 28 يوم بعد العملية الجراحية. أظهرت النتائج ان العلاج بالليزر المستخدم في كلا الجرعتين عزز وبشكل مستمر عملية شفاء العظم بواسطة تعجيل عملية الالتئام واختزال فترة الالتهاب مقارنة بمجموعة السيطرة. نسيجيا في المجموعة 4 جول مليء العيب العظمي مبكرا بكثافة كثيفة من النسيج الحبيبي يتخلله بروزات اصبعية متعددة من الحويصلات العظمية الشبيهة بالشوكة معطية شكل عظم حديث التكوين. الخلايا البانية للعظم شوهدت كبيرة بالحجم تبطن الحويصلات العظمية التي تحتوي العديد من الخلايا العظمية. التعظم الغضروفي والتعظم داخل الغشائي تطورا بوقت مبكر. النسيج الغضروفي اختفى في 28 يوم ولوحظ الثفن اكثر تطورا. في مجموعة 8 جول لوحظ ظهور مبكر للتعظم الغضروفي والتعظم داخل الغشاء محاطا بسحق خارجي جديد. العيب العظمي لوحظ ممتلأ بحويصلات بينية ثخينة وسميكة. التعظم الغضروفي غزا مبكرا النسيج الغضروفي من الجوانب والتعظم كانا مترابطين. لم نلاحظ علامات التهابية كما حصل اختفاء مبكر للنسيج الحبيبي. وفي النهاية لوحظ عظم جديد سميك كثير الخلايا يتصف بوجود حويصلات متعددة التفرعات مترابطة مع بعضها. نستنتج ان الجرعتين المستخدمة كانت مؤثرة في تحفيز وتعزيز شفاء العظم وكانت الجرعة 8 جول افضل من 4 جول.

الكلمات المفتاحية: الليزر واطى الطاقة، العظم، كسر العظم، شفاء العظم، الجرذان.

Introduction

Bone, as a part of the skeletal system, is responsible for mechanical support for the soft tissues and muscles, body shape and movement (1). It also contains of calcium, a mineral that is essential for proper cell function. Blood cells and platelets are produced in the marrow, the central cavity of bone. Bone fracture is a medical condition in which there is a break in the continuity of the bone. It is among the most frequent injuries of the musculoskeletal system in man and animals, which cause many economic losses, disabilities, culling of animals, and slaughter of meat production animals (2). Bone fracture healing is a complex physiological process involves complex processes of cell and tissue proliferation and differentiation (3). Many players are involved, including growth factors, inflammatory cytokines, antioxidants, bone breakdown (osteoclast) and bone-building (osteoblast) cells, hormones, amino acids, and uncounted nutrients. It initiates a series of cellular and molecular events leading to structural reconstitution and tissue regeneration, commonly described by a four-phase model consisting of an inflammatory phase, two repair phases with soft callus formation followed by hard callus formation, and a remodeling phase, or more recently by an anabolic/catabolic model (4). During the fracture, cortical bone, periosteal tissue, and surrounding soft tissues are ruptured, destroying the blood vessels and consequently causing tissue bleeding. Thus,

cells of the blood and bone marrow, such as immune cells, erythrocytes, and stem cells come to the place and are disrupted from the oxygen and nutrient supply at the injury site (5). This process leads to local tissue hypoxia and an inflammatory response, which is a result of migration of inflammatory cells, leukocytes, and macrophages into the fracture gap, triggering the formation of granulation tissue. The local inflammation initiates bone regeneration by stimulating the migration of mesenchymal stem cells (6). The principle of using low level laser therapy (LLLT) is to supply direct biostimulative light energy to body cells. Absorbed laser energy causes stimulation of molecules and atoms of cells. Using low-intensity laser radiation on the tissues does not cause rapid and significant increase in tissue temperature on the other hand; it has biochemical stimulation effects on cells, which creates multiple biological changes (7). These types of radiation affect the photoreceptor of cells and by stimulating the electron transport chain, modulate the cellular action. On bone healing the effects related to LLLT include increased vascularity, increased osteoblast activity, organization of collagen fibers, and changes in mitochondrial and intracellular levels of adenosine triphosphate. It is a noninvasive method to stimulate ontogenesis, and accelerate the healing of bone defects (8). Kawasaki and Shimizu (9) used a Ga-Al-As laser with an output of 35.3 W/cm² to stimulate bone formation in tooth extraction

sockets. They found that the amount of bone formation in the treated side of the laser irradiation group was significantly accelerated (1.75-fold) compared with that of the non-irradiation group ($p < 0.01$). Silva *et al.*, (10) investigated the laser's dose-dependent effects on bone formation and reported increases in bone mineralization after three sessions of 4.8-J/cm^2 irradiation on femoral bone defects in rats. From these studies, we can see that it is likely that the use of laser energy can accelerate the healing process of bone. The aim of this study is to evaluate the beneficial effect of low level laser therapy of the wave length and the doses used in this experiment on bone healing and to compare between the effect of the two doses (4 and 8J/cm^2) on bone healing.

Materials and methods

Experimental animals:

Sixty adult male Wistar rats weighing (230-280gm), (10-14) weeks old were divided into three equal groups (control and two laser treatment groups' 4J/cm^2 and 8J/cm^2). All animals exposed to osteotomy induced by drilling of bone. Control group left without treatment, and laser treatment groups were exposed once to the laser beam (4J/cm^2 and 8J/cm^2) for 7 successive days. Five animals per group were euthanized by anesthetic overdose at 7, 14, 21 and 28 postoperative days. Specimens from the osteotomized bone were taken for histopathological examination to evaluate the progress of bone healing process.

Surgical procedure:

Surgical operations were made under general anesthesia by a mixture of xylazine ketamine given by IM injection (50mg/kg. B.W. ketamine, and 10mg/kg. B.W. xylazine) (11). Osteotomy was induced in the right tibia bone of each animal. The operation site was prepared aseptically. Longitudinal skin incision (2cm) on the antero-lateral aspect of the leg was made by use of scalpel, the anterior tibialis muscle was displaced laterally with forceps to expose the bone. Standard osteotomies 2 mm in diameter (limited size bone stoma) (Fig. 1) were made in the middle of tibia bone by special driller

(Dental marathon-3 champion, saeyang, Korea) (Fig. 2) while irrigation the site of drilling with saline to reduce heating of tissue, after that the muscle was replaced and the incisions was sutured with 3/0 silk suture materials.



Fig. (1): Show the 2mm diameter hole (stoma) in the tibia bone induces by drilling the bone.

Laser treatment:

Gallium Aluminum Arsenide (GaAlAs) diode laser (Omega XP Laser system device, UK) was used (Fig. 3). The 660nm wave length, 50mW power density 146HZ pulsing rate, 0.5cm spot size, single probe was selected for laser treatment. Immediately after osteotomy the laser treatment was started in laser groups (laser treated animals 40 rats). 20 rats exposed to 4J/cm^2 and 20 rats exposed to 8J/cm^2 , repeated daily (once a day) for (7) consecutive days. The irradiation was performed at one point on the area of bone injury, by the punctual contact technique. The control group (20 rats) was not exposed to laser light beam.

Preparations of specimens:

After the animals were euthanized, tibia bones were disarticulating. The muscle and fleshy materials were gently removed, and the bones were fixed in 10% formalin for 3 days. Decalcifications were performing by keeping the bones in 10% nitric acid for 2-3 days. When the bones become soft, washing from acid with tap water, dehydrated with alcohol, calcified by xylol, embedding in paraffin, sectioning (5um thickness) by microtome, and stained with hematoxylin and eosin (H&E) stain for examination with light microscope.

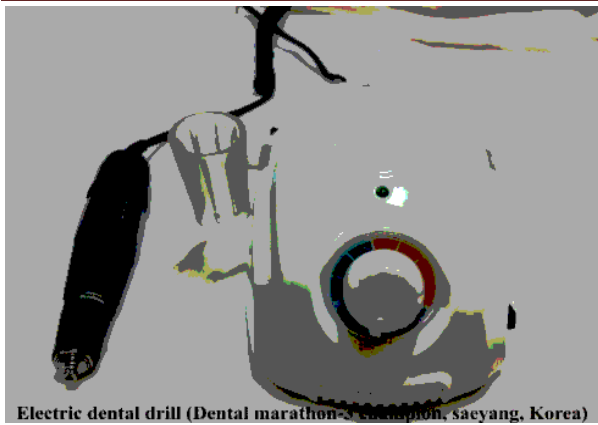


Fig. (2): Electric dental drill (Dental marathon3 - champion, saeyang, Korea) used to make hole in bone.



Fig.(3): Omega diode laser device used for laser treatment.

Results

Control group

After 7 days post operation (PO), more inflammatory cells were seen infiltrated in the bone defect, numerous buds of blood vessels inside the initial medullary contents found at the boundaries of the bone defect. No granulation tissue observed and no interconnected trabeculae or woven bone were seen, except buds of solitary initial formation of trabeculae originated from the border of the bone defect (Fig. 4). At 14 days (PO); The inflammatory signs were subside represented by presence of less inflammatory cells. The bone defect was filled by collagen fibers with minor amounts of granulation tissue. Initial phase of bone repair was seen indicated by interrupted interconnected trabeculae seen diffused in the field (Fig. 5). At 21 days (PO); Large amount of granulation tissue were seen infiltrated with large number of inflammatory cells. The granulation tissues invaded with moderate to thin projections of inter connected trabeculae originated from the borders of the bone defect. Numerous new formed blood vessels inside the granulation tissue were seen. Bone trabeculae has wide lacuna with few osteoblasts and few osteocytes (Fig. 6). At 28 days (PO); No inflammatory cells were seen in this time of observation. There was mild amount of newly formed bone typified by moderate to wide trabeculae with wide lacuna and few osteoblasts. The trabeculae surrounded by granulation tissue. The bone

marrow contains large number of bone cells like mesenchymal cell, osteoblasts and osteoclasts. Osseous tissue was seen also within the bone marrow (Fig. 7).

Laser treatment ($4\text{J}/\text{cm}^2$) group

After 7 days (PO); Dense mass of collagen fiber were seen filled the bone defect. The mass has numerous types of cells like mesenchymal cells, fibroblast, osteoblast, while no inflammatory cells infiltration (Fig. 8). At 14 days (PO); The bone defect was filled with a dense mass of granulation tissue interposed with polydactyl projections of spike like bony trabeculae giving the shape of new developing bone. The osteoblasts were seen large in size lining the bone trabeculae. Some fields no cartilage were seen in it. It seems intra membranous ossification (Fig. 9), while other fields show fibro cartilaginous callus. At 21 days (PO); Thick trabeculae were seen in this time of observation (Fig. 10). The trabecula containing numerous osteocytes on the surface of the trabecula. The cartilage tissue was disappearing and the callus seems like more developed. At 28 days (PO); Thick and wide trabeculae were seen filling the bone defect. The large trabecula shows numerous interconnected spicules connecting each other forming the new callus bone (Fig. 11). Between the trabeculae bone marrow tissue were seen and show ossification of the cartilage were happened where the cartilage tissue replaced by bone.

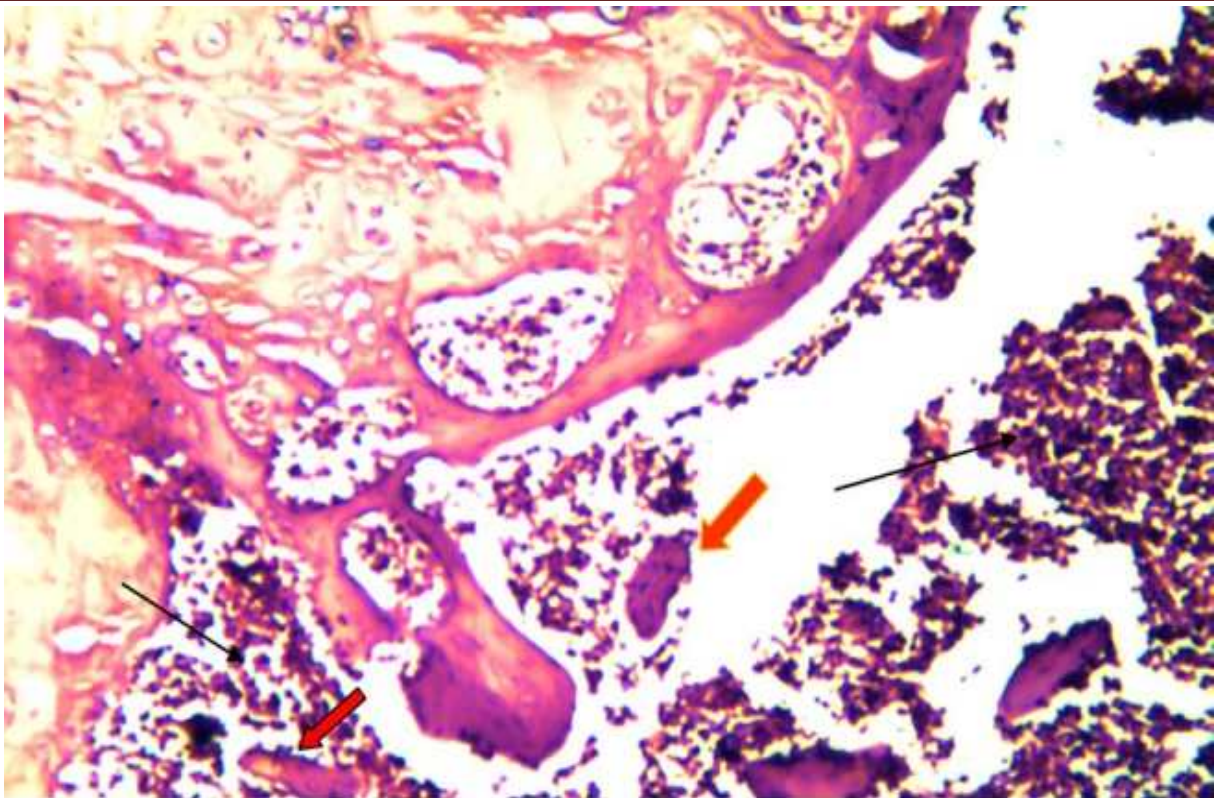


Fig. (4): Control group; 7 days (PO), show presence of more inflammatory cells infiltrated in the bone defect (thin arrows). No granulation tissue, no interconnected trabeculae or woven bone were seen except buds of solitary initial formation of trabiculae originated from the border of the bone defect (red arrow) (H&E Stain X10).

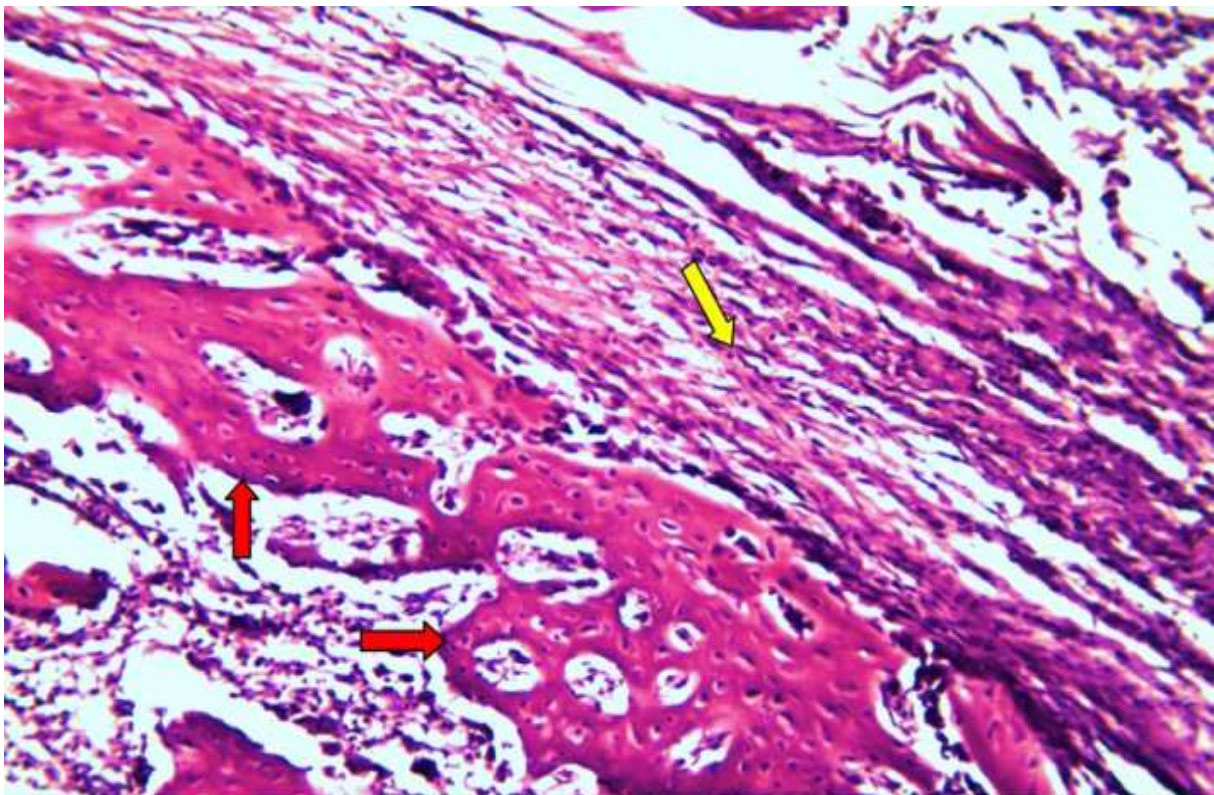


Fig. (5): Control group; 14 days (PO), show presence of less acute inflammatory cells. The bone defect was filled by collagen fibers (yellow arrow) with minor amounts of granulation tissue, and interrupted interconnected trabeculae were seen diffused in the field (red arrows) (H&E Stain X10).

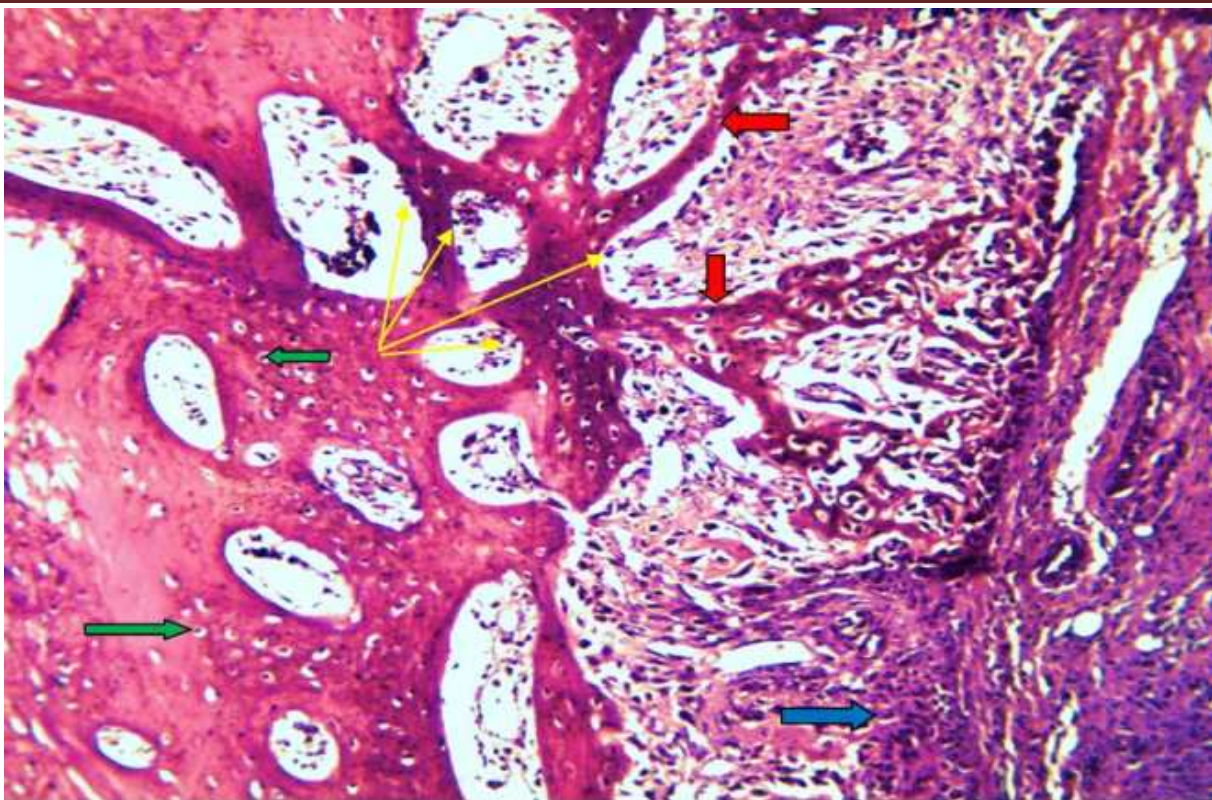


Fig. (6): Control group; 21 days (PO), show large amount of granulation tissue (blue arrow) infiltrated with a large number of inflammatory cells, invaded with a moderate to thin projections of interconnected trabeculae originated from the borders of the bone defect (red arrows). Trabeculae has wide lacuna with few osteoblasts (thin yellow arrows) and few osteocytes (green arrows) (H&E Stain X10).

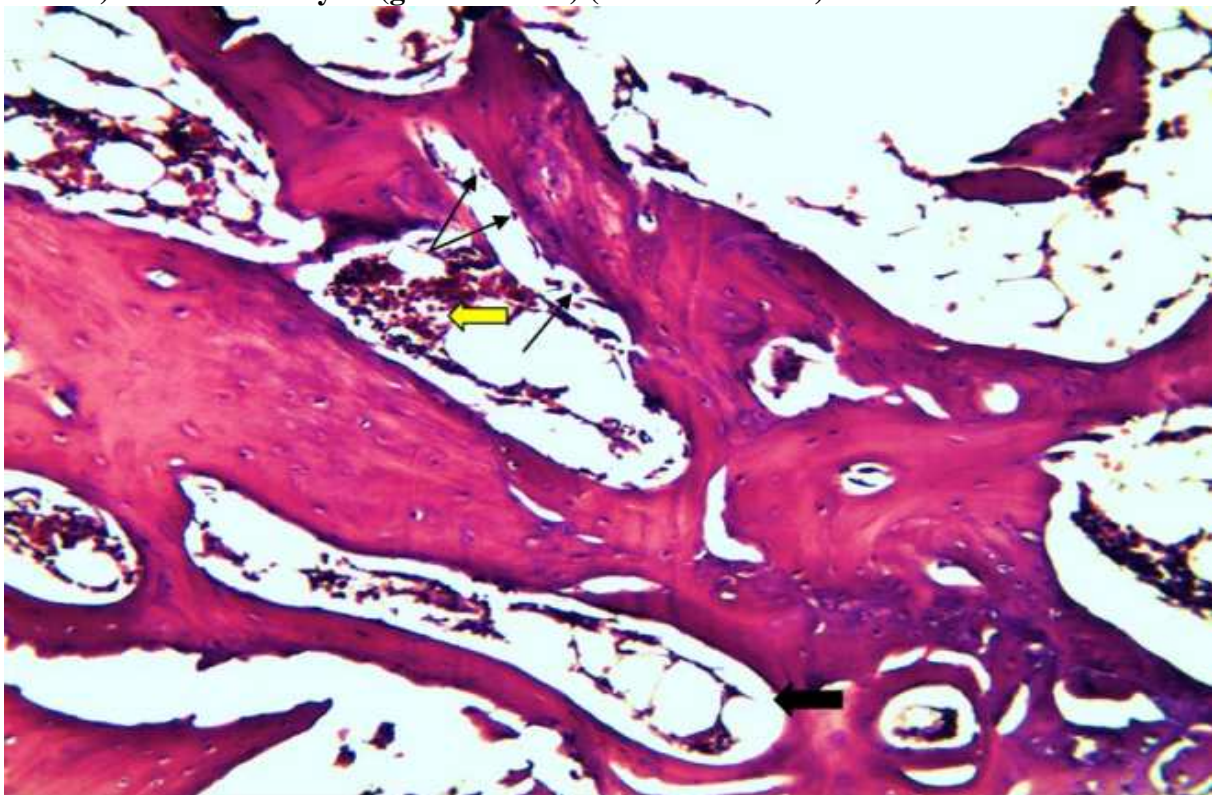


Fig. (7): Control group; 28 days (PO) show mild amount of newly formed bone typified by moderate to wide trabeculae with wide lacuna (black arrows) and few osteoblasts (thin arrows). The bone marrow (yellow arrow) contains large number of bone cells like mesenchymal cells, osteoblasts and osteoclasts.(H&E Stain X20).

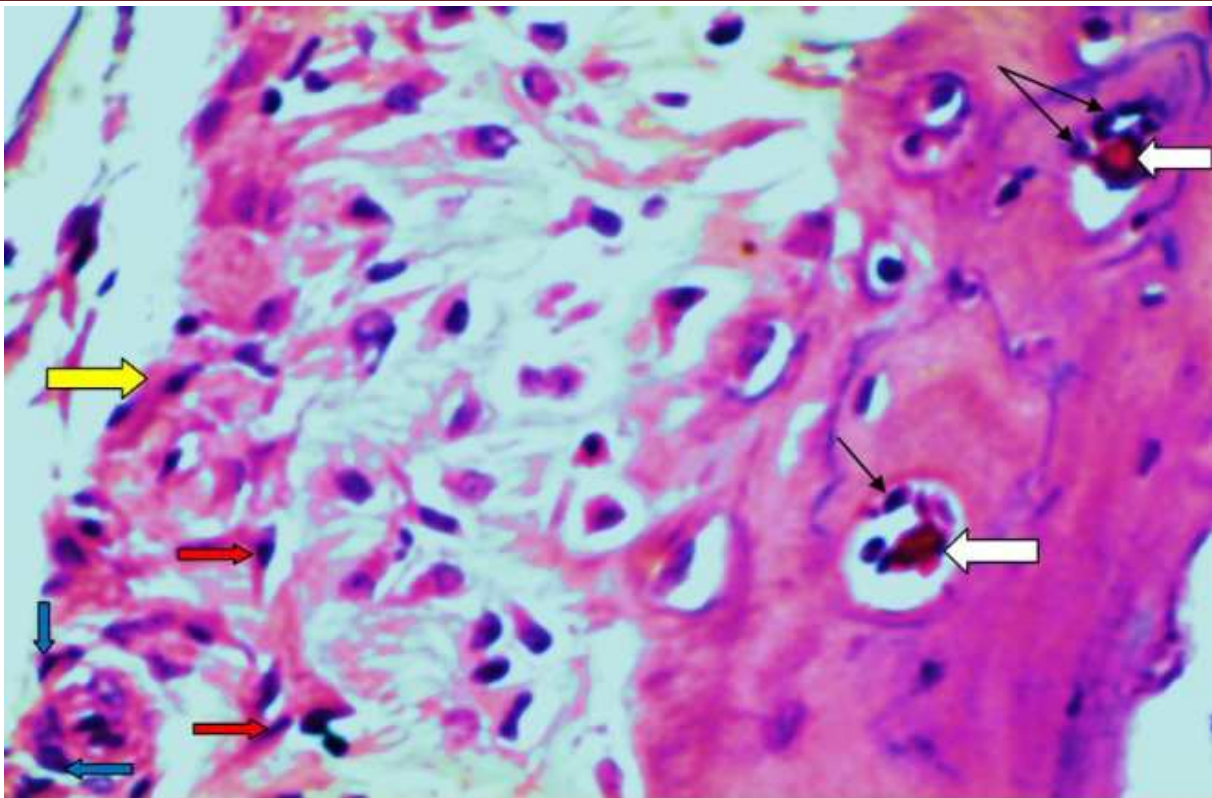


Fig. (8): Laser treated group ($4\text{J}/\text{cm}^2$); 7days (PO), show dense mass of collagen fiber (yellow arrow), numerous type of cells like mesenchymal cells (blue arrows), fibroblast (red arrows), osteoblast (thin arrows), while no inflammatory cells infiltration. The lacunas of the adjacent bone were filled with blood cells (white arrows). (H&E Stain X40).

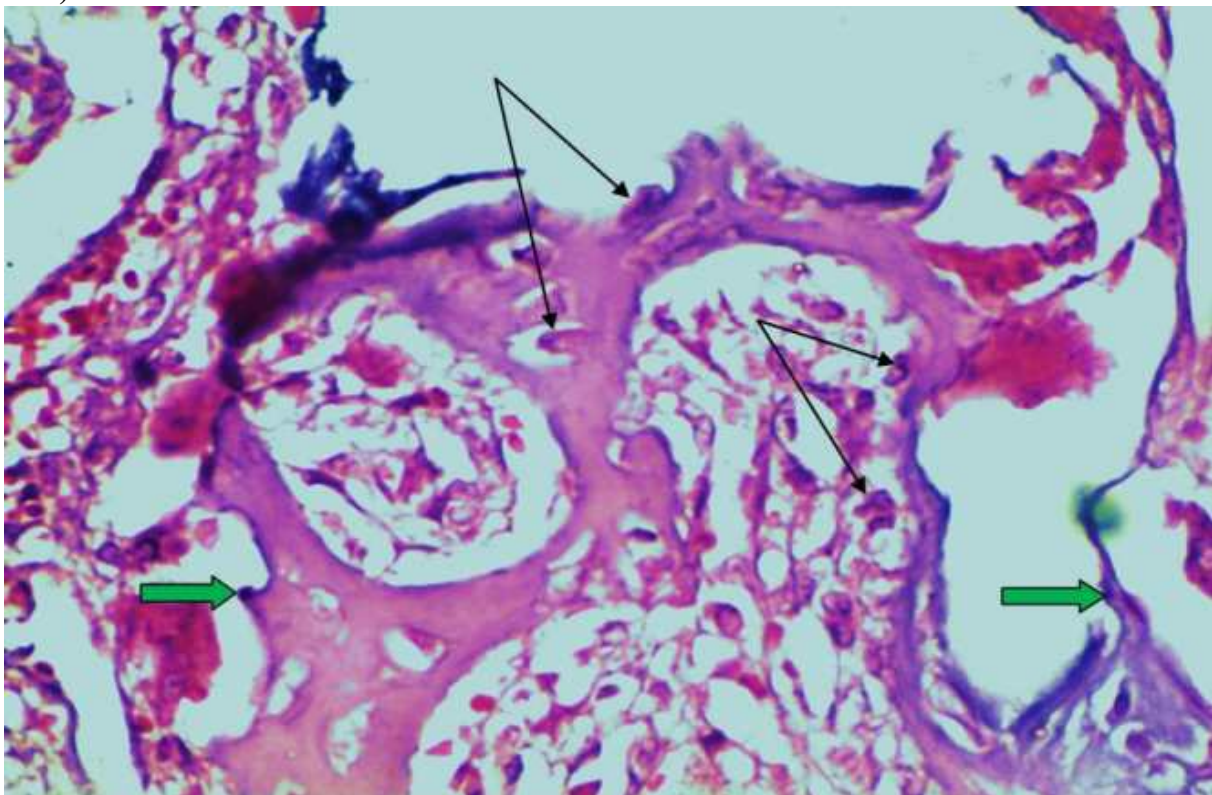


Fig. (9): Laser treated group ($4\text{J}/\text{cm}^2$); 14 days (PO), show spike like bony trabeculae confirming the shape of new developing bone (green arrows). The osteoblasts were seen large in size lining the bone trabeculae (thin arrows). No cartilage was seen in this field. It seems intra membranous ossification (H&E Stain X40).

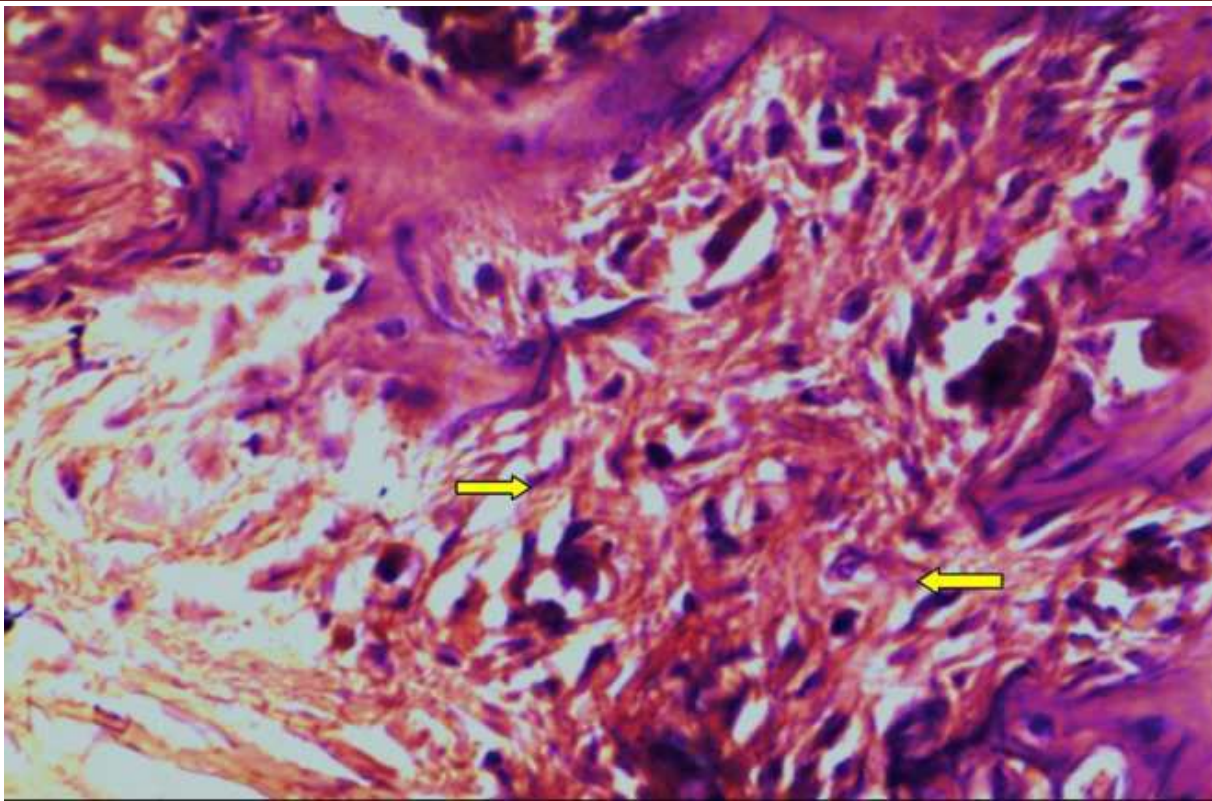


Fig. (10): Laser treated group ($4\text{J}/\text{cm}^2$); 21days (PO) show thick condensed collagen fibers between the trabeculae (yellow arrows). (H&E Stain X40).

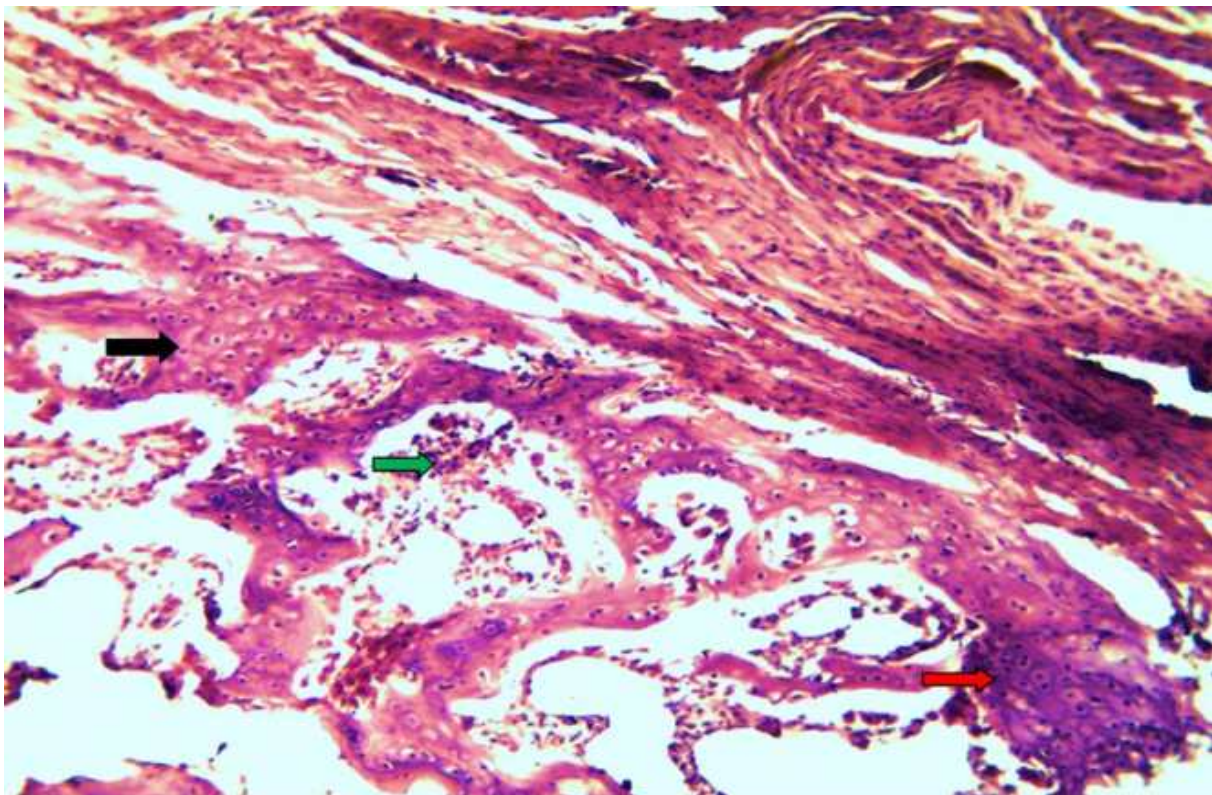


Fig. (11): Laser treated group ($4\text{J}/\text{cm}^2$); 28 days (PO), show thick and wide trabeculae (black arrow), ossification of cartilage tissue (red arrow), and bone marrow tissue surrounding by the trabeculae (green arrow). (H&E Stain X10).

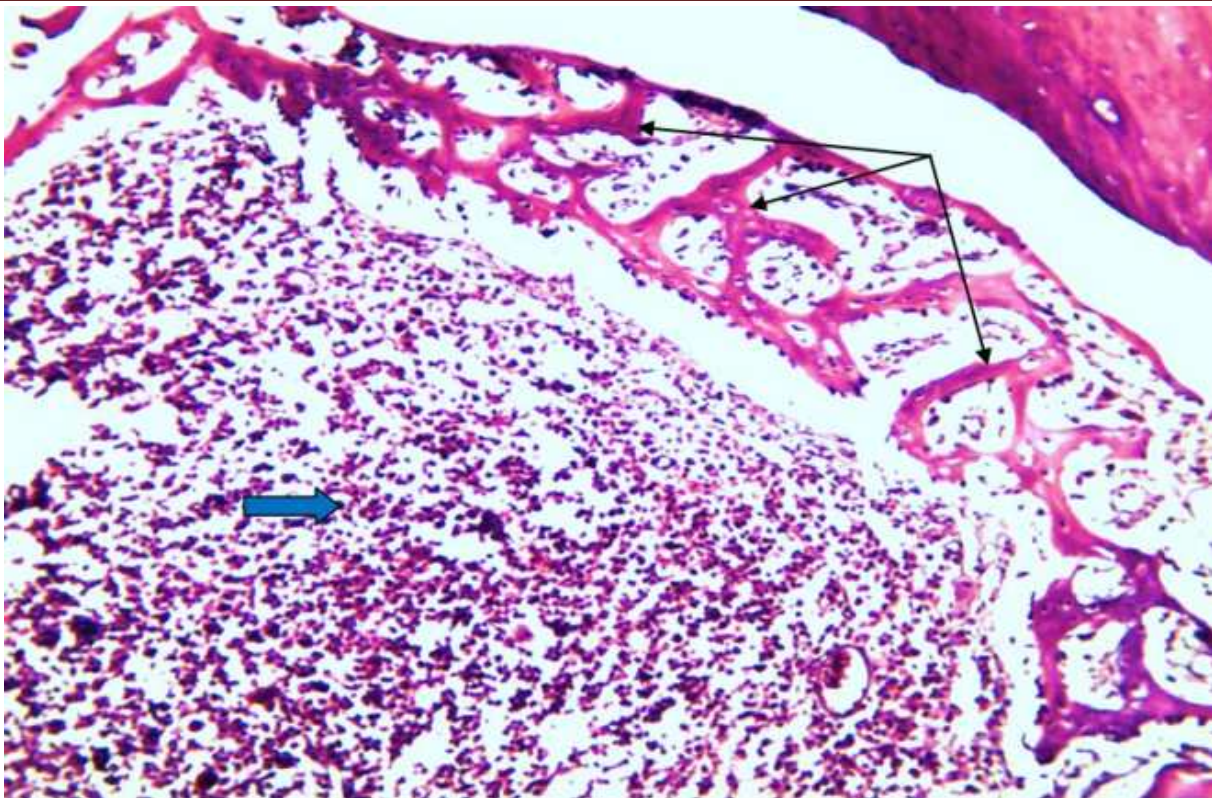


Fig. (12): Laser treated group ($8\text{J}/\text{cm}^2$); 7days (PO), show thin numerous net of new formed trabecular bones (thin arrows). encircling massive infiltration of acute inflammatory cells (blue arrow), with no collagen fiber. The trabeculae were found near the old bone (H&E Stain X10).

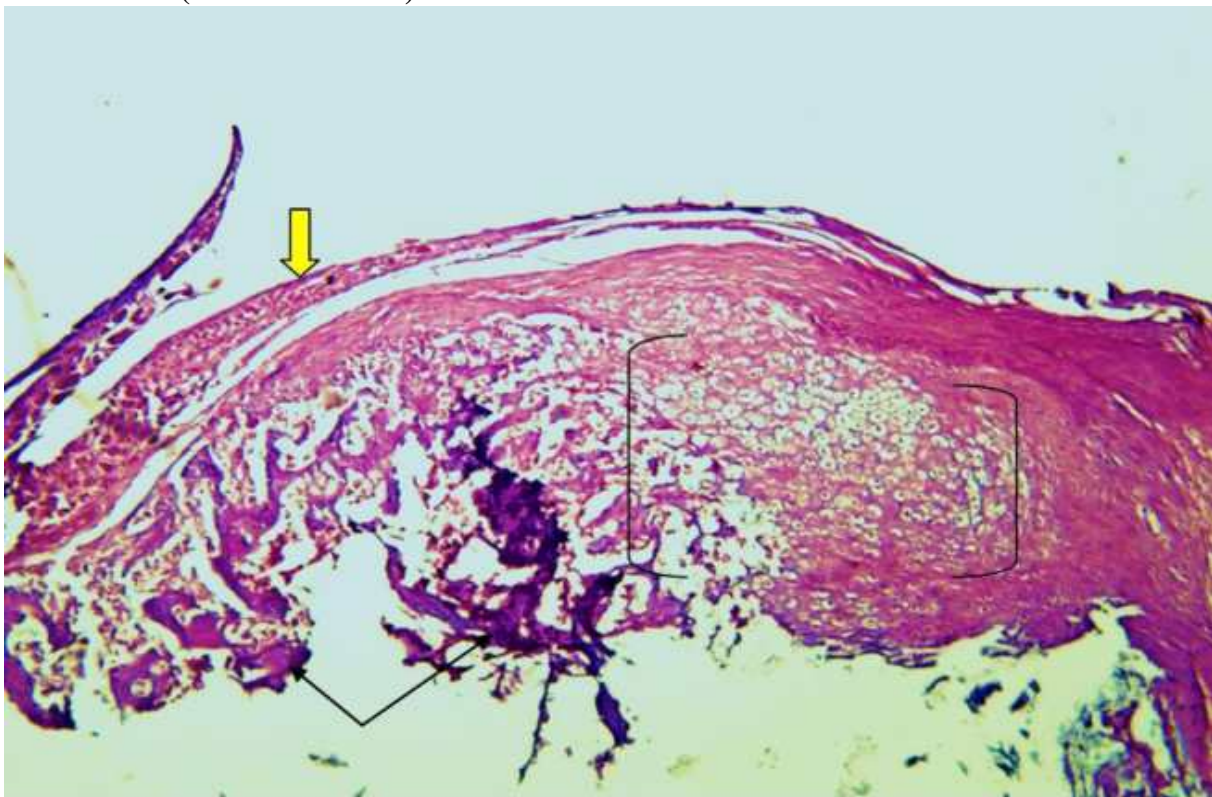


Fig. (13): Laser treated group ($8\text{J}/\text{cm}^2$); 14 days (PO), show early visualization of both intramembranous and endochondral ossification (restricted area). The trabeculae were dense and thick (thin arrows). No signs of inflammation, no granulation tissue were seen, and the entire defect was surrounded by new periosteal tissue (yellow arrow). (H&E Stain X10).

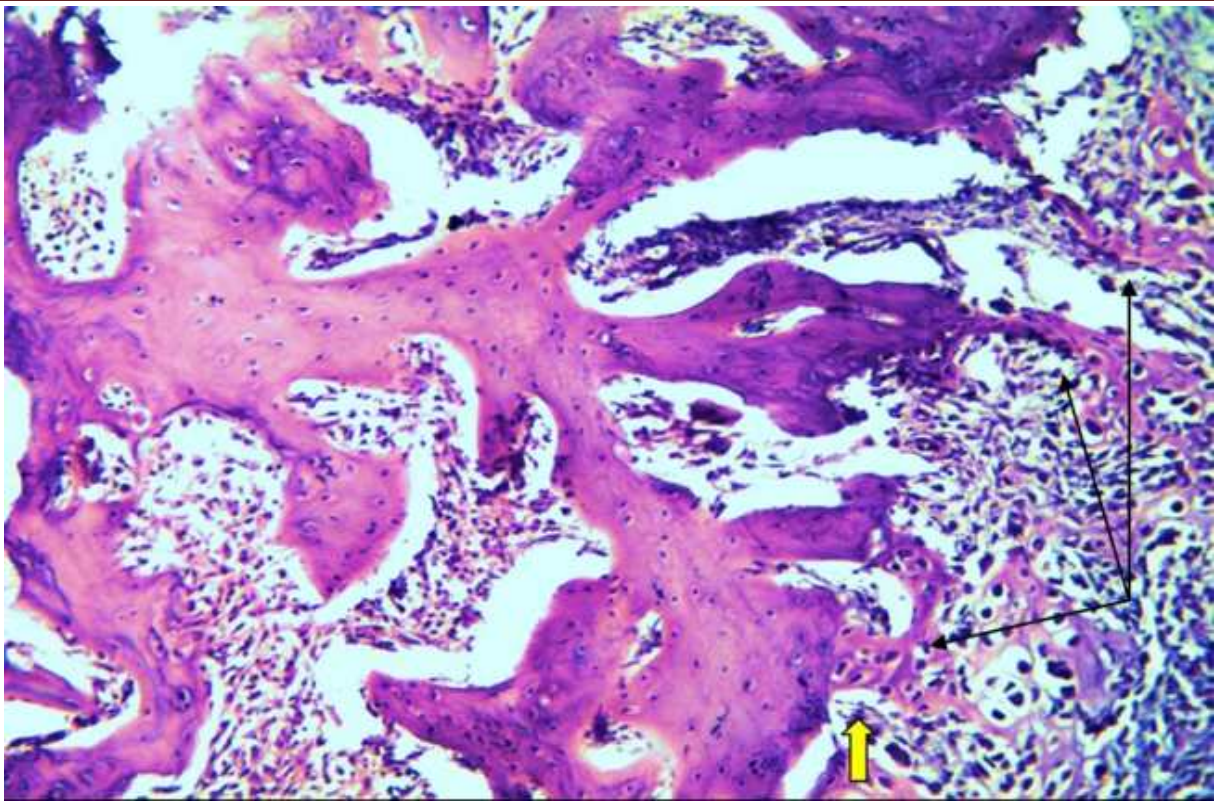


Fig. (14): Laser treated group ($8\text{J}/\text{cm}^2$); 21 days (PO), show thick dense branching highly cellular interconnected trabeculae filling the bone defect (thin arrows). Rare cartilage tissue was seen (yellow arrow). Neither inflammatory cells nor granulation tissue were seen in this time (H&E Stain X10).

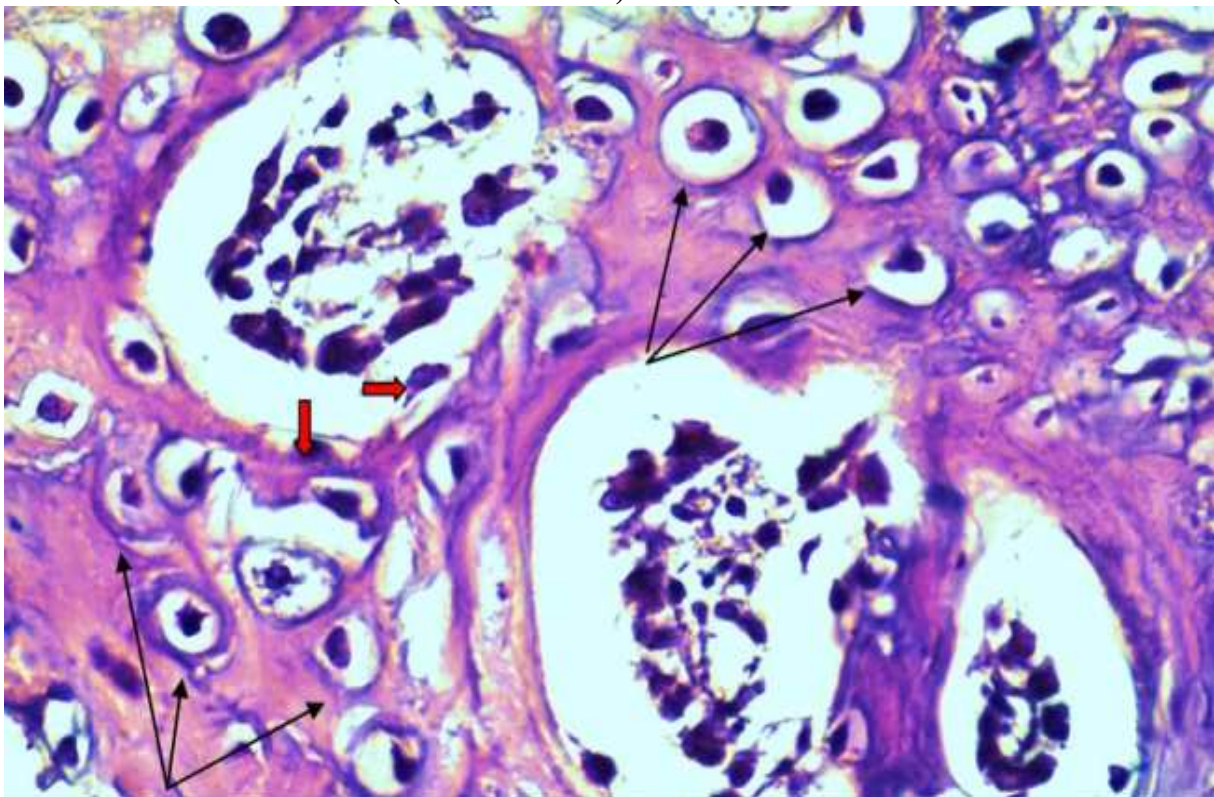


Fig. (15): Laser treated group ($8\text{J}/\text{cm}^2$); 28 days (PO), show numerous active (large hyper-chromatic) osteocytes embedding the trabecular matrix (thin arrows), and many osteoclasts lining the lacunae (red arrows).(H&E Stain X40).

Laser treatment (8J/cm²) group

After 7 days (PO); Thin numerous net of new formed trabeculae bone were seen encircling massive infiltration of inflammatory cells (Fig. 12). At 14 days (PO); Early visualization of both intramembranous and endochondral ossifications (Fig. 13). The trabeculae were dense and thick. The entire defect surrounded by new periosteal tissue. At 21 days (PO); Thick dense branching interconnected trabeculae were seen fill the bone defect. The trabeculae seen highly

cellularized with osteocytes, few cartilage tissue were seen (Fig. 14). At 28 days (PO); Thick highly cellularized woven bone was seen in this time characterized by presence of wide multi-branched interconnected trabeculae connecting each other (Fig. 15), having numerous large active osteocytes embedding the bone trabeculae, a large number of osteoblasts surrounding the trabeculae and numerous active osteoclasts were seen also bordering the lacunae.

Discussion

The treated bone at 7 days with 4J/cm² shows disappear of inflammation cell. This result supported by (12), who found reduction of inflammatory stages causing faster beginning of other stage of bone healing, in compare to the same period of control group which characterized by more inflammatory cells were seen infiltrated in the bone defect, because of some of the clinical effects of low level laser therapy are reduces inflammation and swelling associated with acute injures in bone due to improved tissue nutrition and oxygenation (13,14). In this study the studied variable the dose but wave length is unchanged (660nm) the short wave length is more absorbed by the cell. The bio stimulatory effect of laser on healing with delayed or normal healing may be associated with high absorption of the 660nm wave length used by cytochrome oxidase, which has been suggested to be one of the primary photoreceptor of the respiratory chain in mitochondria (15). Our result in the 4j/cm² laser treated group demonstrate typified abundant of collagen fibers achieved proliferation of fibroblast, and new blood vessels formation. This resolved in agreement with (16), who found that LLLT at energy density 2J /cm² is couple of enhancing collagen synthesis, and significant increase of both fibroblasts in treated group and endothelium of blood vessels at the maturation of the bone healing process. In this group irradiation was efficient for increasing process of healing, may be due to the enhancement of vascular perfusion (17). Laser photo bio modulation can activate the local blood circulation and

stimulate proliferation of endothelial cells (18, 19, 20). Also in the dose (4J/cm²) but in the 14 days post operation we found presence of granulation tissue which filled the bone defected with poly dactyl projection of spike like bony trabeculae giving the shape of new developing bone , these results are accorded with (21) who found the use of low intensity laser resulted in an accentuated amount of granulation tissue being associated with increased revascularization and hyperemia, this granulation tissue is characterized by intense proliferation of mature fibroblast distributed in a dens connective tissue. The use of GaAlAs laser at lower energy densities had significant effect on the tissue repair through decrease edema, improve granulation tissue and fibroblast proliferation stimulation (20). Our results in 21days post operation in 4J/cm² which found the cartilage tissue has dense chondrocytes are hypertrophic, large in size. Our results of accelerated healing compared to 40 days where the researcher (22) found that the He-Ne laser is capable of inducing cartilage new formation, they induced the bilateral knee cartilage defects in rabbits using spherical bur. Also reported that after 40 days, a well organized fibrous tissue fully filled the lesions of controls group, where as the He Ne laser –treated damage showed that hyaline cartilage filled the lesions completely, Apparently, in the above mentioned studies laser penetration was so deep that it could bio stimulate chondrocytes of articular cartilage (22) and damage cartilage new formation (21). Our study reported that diode laser irradiation increase bone formation from the initial stages of the

healing process, and significantly enhance the newly formed bone tissue by demonstration thick trabeculae are seen in laser treated $4\text{J}/\text{cm}^2$ 21 days post operation and found thick condensed collagen fibers are seen between the trabeculae and the callus seen like more develop. In this time, several studies also support our finding with respect to positive effects of laser on bone formation (23, 24, 25, 26, 27). also this result is consistent with (28) who found; in the early stages, mesenchymal tissue is predominant gradually changing in to cartilaginous tissue, and finally changes to bone after 6 weeks (27). Therefore, the radiographical, histological results failed to demonstrate any stimulatory effect after this time. In our study revealed the trabeculae containing numerous osteocytes on the surface of the trabeculae, thick condensed collagen fibers are seen between the trabeculae, these finding accelerate bone healing in 21 days post laser irradiated ($4\text{J}/\text{m}^2$) tibia in compared with control group, these results agree with finding reported by (29, 30), whom showed that the volume fraction of trabeculae bone decreased more rapidly with time in laser irradiated tibia than the controls. LLLT stimulate the healing by promotion of cell growth and cell proliferation, restoration of homeostasis or increased cell metabolism or equalization of cell function, increase of rate of the healing process by promoting protein production such as collagen and prevention of scarring (30). Our results demonstrated significant increase singes of bone healing in 20 sec. duration exposed to laser irradiation in compare with exposed to 10 sec. in group laser treated with $4\text{J}/\text{cm}^2$. This results contrast with (28) regarding the duration of irradiation in that a longer irradiation did not improve the repair process (area of growth) compared to the short periods. The histological analyses revealed that the laser therapy improved the biological response of bone tissue by stimulating the deposition of newly formed bone at the site of injury. The positive histological finding observed in this study in the treated groups is probably related to the stimulation through the mechanisms aforementioned. The up regulation of these

factors could be related with the attraction of the osteoprogenitor cells and their differentiation into matrix producing osteoblasts, thus increasing the rate of bone formation and bone ingrowths into the defect (31). The healing process is seen more distinguished in laser treated group at the first period post operation. These result seen accorded with (32, 33, 34, 35) whom found the inflammatory process is less intense and more advanced in laser irradiated group than in control group, due to the early onset of the inflammatory response when short wave length are used. More fibroblast immigration and cell proliferation (36, 8) The PPGF, TGF-B and ECM molecules stimulate peri-wound fibroblastic to proliferate and migrate in to the area of injury, once the fibroblast phenotype changes from proliferation to contractile and collagen synthetic (37, 38, 39). (26, 40) showed a significant increase in the proliferation of osteoblasts after 830nm laser irradiation at $20\text{J} / \text{cm}^2$. In addition, the laser seems to accelerate the process of fracture repair and cause an increase on the callus volume and bone mineral density, this results superior our result due the positive effects of LLLT on bone metabolism and on fracture consolidation (25, 39). In bone defect, after 14 days we found an increase of the area of the woven bone tissue in the group treated with laser when compared to the control group. The acceleration of the bone healing process is also reported in other studies, such as the study by (41, 42) that have used the laser Ga-Al-As (830nm, continues 40 mw, $57.6\text{J}/\text{cm}^2$). Phototherapy is characterized by its capability to induce photo biological processes in cell. Its is based on the effects of light energy on cell metabolism of living systems. Biological responses of cell to laser irradiation occur due to chemical and/or physical changes in photo acceptor molecules, components of respiratory chains (24, 43). The results of this study revealed that of group treated with $4\text{j}/\text{cm}^2$ at 21 day post operation, there is collagen fibers; thick trabeculae this observation is coincided with (42) who reported that the photo bio modulator effect of LLLT ($4\text{J}/\text{cm}^2$) result showed histological evidence of increased

deposition of collagen fibers (15-21 days) as well as an increased amount of a well-organized bone trabeculae at the end of the experimental period (30 days). These results are significant compared to non irradiated controls. Since collagen synthesis is normally enhanced in a reparative process, this enhanced suggest, that laser treatment aides in increasing this synthesis. In the laser treated with $8\text{J}/\text{cm}^2$, we found the presence of thin numerous net of new formed trabeculae bone and contain large active osteoblasts are seen large, dark. This result is corresponded with Brazilian group; whom studied activity in bone cells after irradiation with 660nm and found that activity was higher in the irradiation group than in the control group in regards to bone volume (44). The LLLT is increasing the ATP production cellular metabolism and DNA synthesis, these increased the activity of bone cells osteoblasts and osteoblasts around the site of repair with out changing the bone structure. Also this result is consistent with (9), whom investigated the effect of laser therapy on bone remodeling, as evidenced by an increased blast number and new bone formation are more prominent on irradiated animals. The amount of new reparative bone in the hole injury increased between days 10 and 15 post – injury both in control and laser –irradiated rats ($4\text{J}/\text{cm}^2$; $8\text{J}/\text{cm}^2$); However, the filling of the hole injury with new reparative compact bone was more rapid in the laser irradiated tibiae at $8\text{J}/\text{cm}^2$ compared to control and $4\text{J}/\text{cm}^2$. This result coincides with (29) who found laser – irradiation accelerated the healing of compact bone. In the laser group, a significant increase in Alkaline phosphates (ALP) and ATP (28). It is

know that the ALP enzyme is important for calcification of bone and cartilage in normal growth and repair. Results are revealed highly cellularized woven bone seen at 28 days post operation in group treated with $8\text{J}/\text{cm}^2$ and having numerous large active osteocytes embedding the bone trabeculae, a large number of osteoblasts and numerous active osteoclasts. This results agreed with (45) who Raman spectroscopy was used to investigate the effects of laser photobiomodulation (660 nm, $10\text{J}/\text{cm}^2$ on the healing of fracture bone of rats by monitoring the level of (CHA). The reason that the effect of laser photobiomodulation was detectable only at 28-30 days after surgery was probably due to the fact that, during early stages of bone healing, the cellular component is more prominent and more prone to be affected by laser photobiomodulation. Later, bone matrix becomes the main component of the healing tissue. This is why the frequency of application of laser photobiomodulation is important, as the laser irradiation is carried out during the cellular phase of healing, when the number of osteoblasts is increasing. Later, the higher number of cells resulted in a larger deposition of bone matrix, which later incorporated calcium hydroxyapatite (CHA) (46). In conclusion; the LLLT dosage 4 and $8\text{J}/\text{cm}^2$ have beneficial effect on acceleration the healing of surgically induced bone fracture repair in rats. Beyond the common effects of LLLT as improving tissue repair, anti-inflammatory and increasing local micro circulation, compare with control groups. However, energy density of $8\text{J}/\text{cm}^2$ is seen more effective for healing processes than other groups.

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