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Effect of Low Energy Laser on the Healing of Tooth Extraction Wound: (Histological Study in Rat)

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Abstract: Aims: This study was done to investigate the effect of low energy laser therapy on bone healing at the extraction site**. Materials and methods**:(24) male albino rats were exposed to the extraction procedure of the maxillary first molar on the first day of a seven day experiment and these animals were divided into two main groups; the control group and the laser group. The laser experiment involved using (Ga-As infrared diode laser) from optodent by directing the probe over the extraction site. The control group consisted of 4 rats, and the laser group was subdivided into 5 subgroups of 4 rats each. The laser dose was as follows: B1: a single dose of 5 minutes immediately after extraction., B2: a single dose of 35 minutes immediately after extraction., B3: 7 doses for 7 days as 5 minutes /day from day 1-7., B4: 3 doses for 3 days as 10 minutes /day at days 1, 2, 3., B5: 3 doses for 3 days as 10 minutes/day at days 4, 5, 6., The specimens were prepared for histological study, then examined under light microscopy, **Results:** They revealed a difference in the rate of bone healing between the control and laser groups and multiple doses of low energy laser were effective in enhancing bone formation than the single dose, showing that retardation in the healing may be due to the anti-inflammatory effect of the laser or due to upset of inflammation by mast cells. The healing of the bone in (B5) group was much prominent than in other groups. **Conclusions:** low energy laser may be beneficial in the enhancement of healing which will minimize the infection, and reduce patient suffering and the incidence of post-extraction complications.

Keywords: alveolar bone, Ga-As, healing

1.Introduction

Wound healing is a fundamental biological phenomenon. It includes all aspects of cell proliferation, cell differentiation synthesis and secretion of proteins, proteoglycans and other extracellular substances [Ross, 1971].

Tooth extraction is a compound wound healed by a series of complex osseous phenomena involving not only the socket itself, but other adjacent areas as well [Astrand and Carlssen, 1968] The initial response to the tooth extraction is not limited to the alveolar socket and the gingiva at the edge of the wound, but the alveolar bone as the jaw also plays an important role in the repair of a tooth extraction by the action of blood supply of hematopoetics system [Todo, 1968].

Regeneration of cancellous bone in the healed alveolar socket results in not only a reconstructed bony architecture, but a fully developed marrow complete with a hematopoietic system [Amler, 1977].

There are a number of general factors which may influence the rate of healing of oral wounds (including tooth extraction wounds) such as the existence of the intercurrent disease, the degree of trauma, vascularity of the wound, local temperature, the presence or absence of infection, the age of the host and nutritional or hormonal conditions [Cook et al,1972]

It has been shown that alveolar bone is more sensitive to nutritional and metabolic influences than are long bones because of the presence of teeth and the nature of the blood supply [Furstman and Rothman,1972]

There are many attempts done to increase the rate of healing of tooth extraction wounds by using many techniques and agents such as suturing [Simpson,1960] reduction of the alveolar bone height [Alling and Kerr,1957] application of different types of medicaments [Hubbel and Austin,1941] and bone inducing agents [Frederic et al,1974], hormones [Lim et al ,1995] as well as thermal energy and radio waves with high frequencies [Cook et al,1972]

Low power laser or called low energy laser, soft laser, mild laser , and cold laser, were used in wound healing to accelerate this process, because of its low energy output, intensity , and biostimulating effect [Kert and Rose,1989].

In this study we try to investigate the effect of L.E.L. on bone regeneration following tooth extraction and correlate the effect of dose and time of irradiation of laser to the amount of bone formation.

2.Materials and methods

2.1 Animals

Twenty-four Spraque Dawley male albino rats aged from (6-8) weeks, weighting (250-300) gram were used throughout the experiments. All the animals were subjected to the same environmental conditions.

The 24 rats were divided into two groups and all these rats were exposed to the extraction of the maxillary first molar tooth.

For the histological examination; the 24 rats were divided into two main groups as follows:

The control group: consisted of 4 rats; and they were not exposed to L.E.L. Irradiation.

The laser group: exposed to L.E.L. And this group consisted of 20 rats, which were subdivided according to the laser treatment protocol into 5 subgroups as follows:

Group B1: 4 rats exposed to a single dose of laser for 5 minutes immediately after extraction total dose (1.5J).

Group B2: 4 rats exposed to a single dose of laser for 35 minutes immediately after extraction total dose (10.5 J)

Group B3: 4 rats exposed to 7 doses of laser for 7 days as 5 minutes per a day; from day 1-7 of extraction as total dose (10.5 J).

Group B4: 4 rats exposed to 3 doses of laser for 10 minutes each; from day 1- 4 of extraction as total dose (9 J).

Group B5: 4 rats exposed to 3 doses of laser for 10 minutes each; from day 4-7after extraction total dose (9J) .

Every 4 rats of control group and laser subgroups were put in separate cage of big size.

2.1 Laser device (OPTOS)

Optodent is a patent dental unit for infra-red and laser therapy; invented by Mario Scalvini, a researcher of the Italian National Research Council, in 1989 and manufactured by Italia S.N.C. Company [Optod, 1989]

The system is made up of two different emitting sections; the main one consists of a series of infrared emitting, interchangeable and sterilizable handpiece; in such a manner as to treat a wide surface of the dental arch.

The other one makes use of a high-power laser diode cap, carrying the optic fiber beam (interchangeable and sterilizable) for the treatment of specific points of the oral cavity.

Furthermore, the unit contains a germicide compartment with an ultra-violet ray lamp for ensuring constant sterilization of the hand-piece and/or small objects.

The emission may be continuous or modulated at a frequency varying from 1,25Hz-160Hz.

Specification of Optodent:

Supply voltage 220V, power frequency 50Hz, and absorbed power maximum40W, dimensions 340 x120mm, weight 3.5 Kg.

3.Laser section

Type Ga-As infrared diode laser, peak power 20W, average power 8mW, average power (optic fiber) 5mW, wavelength 904nm, impulse

frequency 3000Hz, optical fiber diameter 3 mm. The device was checked by the Institute of Laser /University of Baghdad.

4.Extraction experiment:

Before the extraction procedure was employed, the animal was anesthetized by using Thiopentol sodium chloride (0.2 cc/100gm) as general anesthesia. The animal was held in the left hand by using the special method of grasping rats during injection; which include catching the head, scalp by the thumb and index; while the tail is put between the little and ring finger and the rest of the body rest on the palm. An intra-peritoneal injection technique was used to get general anesthesia and the injection site was the lower left quadrant of the animal according to the pharmacological instruction. When the animal became drowsy, it was put on the fixation stage and the tweezers opened the mouth by putting it between the upper and lower incisors. Then when the assistant reflects the tongue; anenamel hatchet was used to luxate and extract the upper molar tooth from the jaw; the luxation method is done according to the method of [Hansen,1980]. Most of the teeth were intact, although the procedure was not easy and some fracture cases had been occurring, but their results were excluded.

5.Laser experiment

The Laser mode used in this experiment was pulsed in order to get biostimulation action.
The application of laser was by holding the laser probe perpendicular to the extraction site to get deep penetration.
The dose was according to the experimental groups and by using the following equation to calculate the amount of energy in Joules: Energy (Joules) =Mean Power (Watt) x Time (seconds) [Hubbel and Austin,1941]

The irradiation time was used in the experiment, as follow:

1- Single-dose was 300 seconds (5minutes)=1.5 J
2- Single-dose 2100 seconds (35 minutes)=10.5 J
3- Multiple doses (7) of 1.5 J as seconds (35 minutes)=10.5 J
4- multiple doses (3) each for 10 minutes and 3 J as (30 mintutes)= 9J

6.Histology:

The animal was sacrificed with overdoses of ether vapor in a closed jar, on the seventh day of the extraction. The maxilla was separated from the head and then cut at the mid-line by using blade No.11.
After fixation, the specimens were decalcified in 10% formic acid for 10-15 days.Full decalcification of the maxilla was checked by insertion of a pin. After decalcification; the specimens were prepared for Hematoxylin and eosin.

7.Estimation method:

The histopathological findings of post- extraction healing were evaluated semi-quantitatively on an arbitrary scale according to using a light microscope.
By calculating the number of cells concerned in this study (fibroblasts, osteoblasts, and osteoclasts), the amount of blood coagulation was evaluated objectively. Steoid formation was evaluated as bone trabeculae, which were also calculated to assess the healing process of the extraction wound.
The intensity of the healing process being graded as:
(-) absent, (+) slight, (++) mild, and (+++) prominent. The method of assessment was as follows:
Absent< 5, slight < (5-15) cells, mild < (15-20) cells, prominent >20 cells according to(Takeda, 1988) .

8.Results:

The histopathological findings of post-extraction healing were evaluated according to the presence of blood coagulation, the number of (fibroblasts, osteoblasts and osteoclasts) ,and osteoid or bone matrix as new bone trabeculae. The results are summarized in (table 1).

9.The control group:

The epithelium completely covered the alveolus with a thin keratin layer and discrete retepegs. The

alveolar sockets were filled with fibrous connective tissue.

In the crestal area, there was active osteoclastic resorption of bone and some spicules were separated from the apical portion of the socket wall and were resorbed.

In apical area of the socket, new bone was developing along the lateral wall and at the base.

Numerous osteoblasts in bead-like configurations were seen to form osteoid, and bone formation appeared as smooth surfaces. (Fig.1).

The B1 group:

The socket was filled with blood clots and no sign of an organization or bony changes (Fig-2).

The B2 group:

The epithelization in this group was not completed and the sockets were filled with fibrous connective tissue, which was more cellular than other groups including the control group. The sockets were filled with numerous blood vessels.

The lateral walls of the socket showed bony changes. New bone formation found in the fund's and lateral walls, which appeared as bone spicules surrounded by osteoblasts, and contain numerous osteocytes. (Fig-3)

The B3 group:

There was no epithelium covering the socket, but the blood clot covered the upper part of the socket. The crestal alveolar bone undergoes resorption, which gave us irregular margins.

The inflammatory cells were seen just below the blood clot at about the middle of the socket.

The apical portion of the socket was filled with congested blood vessels more than other groups.

There was a sign of new bone formation, which appeared as small spicules surrounded by osteoblasts with osteoclast cells at the surface of the old bone, which indicated bone resorption. (Fig-4)

The B4 group:

There was no epithelium covering over the socket. The alveolar socket was filled with fibrous connective tissue which became more abundant with fibers and fewer cells when compared with the control group and other groups.

There was an inflammatory response at the mesial root area especially at the coronal area.

There were numerous bone trabeculae extending from the fundus and lateral walls of the socket and at different levels.

The new bone trabeculae were projecting toward the center of the socket. Bone trabeculae were larger and numerous than the control group with osteocytes and there was an obvious osteoblastic activity around the proliferating bone. (Fig-5)

The B5 group:

The upper portion of the socket was covered by unorganized blood clots. There were numerous bone trabeculae found in the apical and middle third of the socket.

Bone spicules arranged in different configurations. These spicules also surrounded by osteoblasts. There were abundant blood vessels all around the healing area (Fig-6).

Table (1): Healing of post-extraction socket in the control and lased groups at the seventh day of the extraction

Group	Blood Coagulation	Fibroblasts	Osteoblasts	Osteoclasts	Osteoid
Control group	-	++	++	+	+
B1	+++	-	-	-	-
B2	-	+++	+	+	+
B3	++	+++	+	++	+
B4	-	+++	+++	+	++
B5	+	+	+++	-	+++

* Graded of the intensity: (-) absent, (+) Slight, (++) Mild, (+++) Prominent

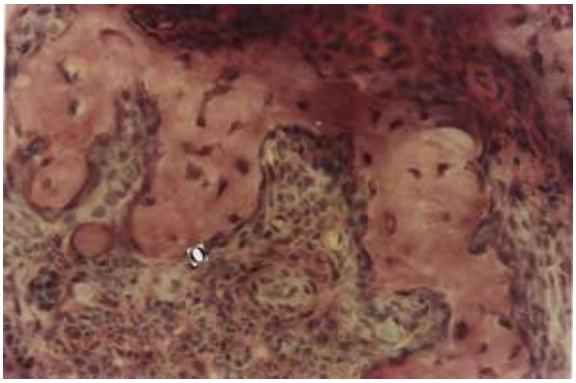

Figure (1): Osteoblasts in bead like configuration surrounded new bone trabeculae the base of the socket (O), Control group, (H/E x 40)

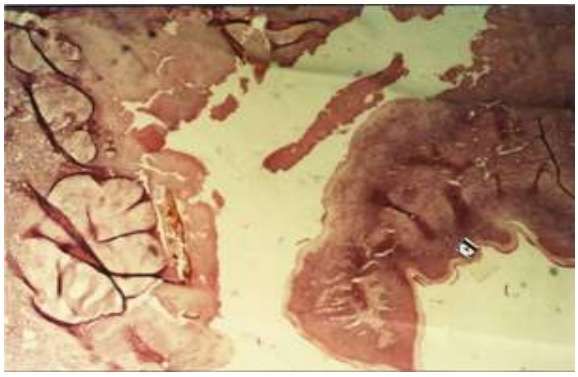

Figure (2): The gingival epithelium (E); covering the socket ,group B1 (H/E x4)

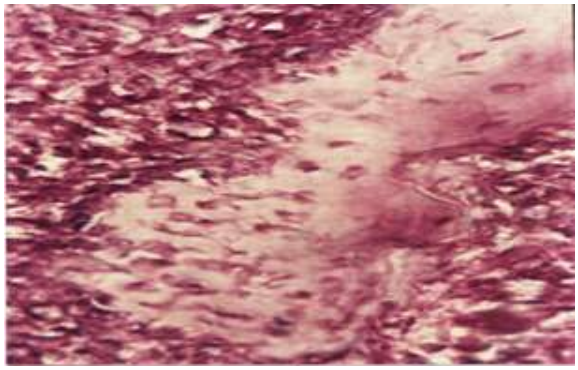

Figure (3): Formation of osteoid tissue in cellular granulation tissue (H/E x40).

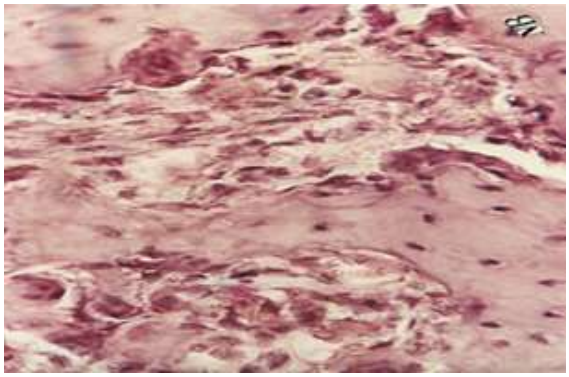

Figure (4): Osteoblastic activity at the surface of new bone trabeculae (NB),*Group B3 (H/E x40)*

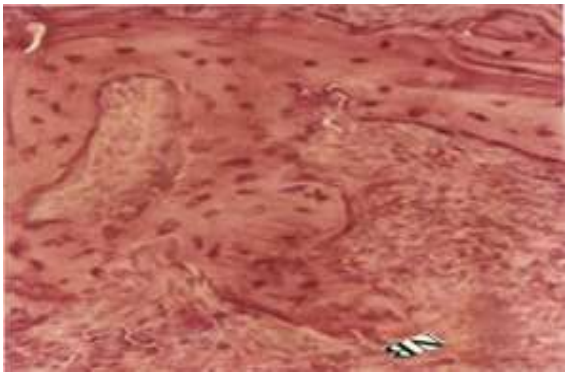

Figure (5): osteoblastic activity at the surface of new bone trabeculae (NB) group B4 (H/E x40)

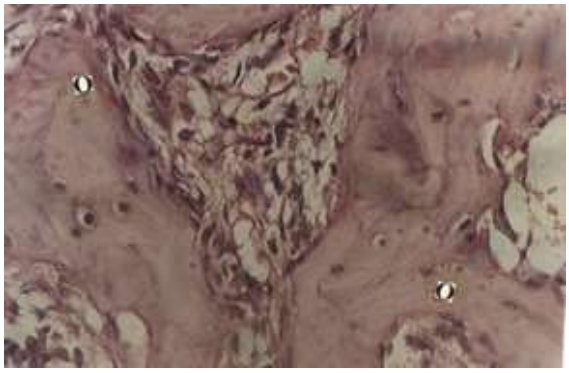

Figure (6): Osteoblasts (O) rimming the newly formed bone Trabeculae group B5 (H/E x40).

10. Discussion:

The laser employed ,employing low level radiant energy; , have has been used to produce a positive effect on the biological and biochemical process of wound reconstitution. It has been reported that L.E.L. accelerated wound healing, reduced pain and enhanced neural regeneration (Green et al ,1969). The present study demonstrates the effect of Ga-As L.E.L. on the healing of bone after tooth extraction in relation to the dose and timing of irradiation.

All the control rats manifested a normal histological appearance, which characterized by complete epithelization with rete-pegs appearance and the socket was filled with fibrous connective tissue.

Also, bone resorption was seen in the crestal area; while new bone formation was seen as few trabeculae at the fundus and lateral walls of the socket; and this result of the control group agreed with that of [Tennenbaum and Shklar, 1970] [Johansen,1970] [Smith, 1975][Hansen, 1980] [Menichelli,1986] who found a bone formation on the seventh day of upper molar extraction in rats. The lased groups gave obvious histological responses in their healing process.

The experiments showed a significant difference among the laser groups depending on the dose and its time of application.

This indicated that laser irradiation of (1.5, 3, 10.5) Joules; penetrated the field of experiment and since the depth of the rat molar socket is about 2-3mm; so it was surely affected by laser irradiation during the experiments according to [Hubbel and Austin,1941] who found that one Joule of energy has a depth of effect at one centimeter. Also the application of one Joule of diode laser at right angle, gives an additive penetration of about 30mm [Saperiaet al,1986] Experimental groups receiving multiple doses of laser irradiation presented with obvious differences in bone formation rate and pattern of osteoid deposition.

While On the other hand, the experimental groups, which exposed to a single dose of laser irradiation, exhibited a slight difference in their histological appearance in contrast to the control group or they may show retardation in bone healing.

So in the examination of the effect of a single dose of laser on the first day for 5 minutes after extraction, it had has been found, that there is no effect on bone healing; but in controversy it caused retardation in healing which was characterized by the presence of only blood clot.

This result may be explained that as there is no organization step and clot after its formation and that dose of irradiation may cease the activation of the synthetic proliferative cells, including fibroblast osteoblast cells; which are the principle cells in healing of wounds [Catone and Alling,1997] This agreed with the results of [McMillan,1972] who found that there was no effect of 5 minute dose of L.E.L. on prevention of dry socket occurrence.

This may be due to either the anti-inflammatory effect of L.E.L. on the tissue or due to stimulation to the mast cell activity which was improved to be associated with healing retardation of bone wounds and causing an upset in the initial increase in vascular permeability or retardation in fibroplasia [Satio,and Shimizu,1997].

Also in the examination of the effect of the total dose of irradiation as in group B2, which is at the same to total dosage as in group B3 or in group B4 and B5; , the results showed that there was no effect of L.E.L. on bone regeneration, but maybe in contrast to the control group, there was healing retardation.

These findings suggest that the effect of laser irradiation doesn't depend only on the total laser dosage, but there are other factors based on the timing of using the irradiation and its frequency.

These results of B1 and B2 groups in agreement with these of [AL-Muscati,2000]; who found that the single dose of L.E.L. dose not affect the formation of new mineralized bone and that of [Orikasa,1990] who found a delay in the development of alveolar bone and delay in eruption of teeth in rats.

At the same time these results agreed with those reports that suggested that multiple doses of L.E.L. Are more effective than a single dose for the acceleration of bone formation [Catone, and Alling,1997] [Orikasa et al,1990] [McCarthy,1990] [AL-Muscati,2000] The result of B5 group, gave more bone formation than other laser groups and in the control group. In this group; bone regeneration in the newly formed area was stimulated significantly compared with other groups and filled 2/3 of the socket.

This result disagreed with that of [AL-Muscati, 2000] who found that when laser irradiation was applied during the last 3 days of the one-week experiment, midpalatal suture had no effect on bone regeneration. This might be due to a difference in histological features of the suture area and tooth socket area.

The result of B4 group may be similar to that of control group in appearance and in the configuration of bony trabeculae, but in the laser group; bone trabeculae were more numerous and more cellular.

The result of B3 group had has shown less bone formation than the previous laser groups, and even in contrast with the control group in which the healing process appeared more stable.

This may be due to the continuous application of L.E.L. over the socket; which induced a continuous irradiation and inflammatory response which was obvious by the presence of blood clot and inflammatory reaction at the top of the socket and the presence of numerous congested blood vessels in the middle third of the socket; while the apical part showed bone formation with bone resorption.

Blood is a dynamic living tissue, which shows marked structural alteration in response to injury changes of stress, vascular, endocrine, genetic and nutritional influences the biostimulating action is a characteristic of the L.E.L. on the bone [Saperia et al,1986]

The effect of L.E.L. on the osteogenesis process maybe by its effect on bone morphogenetic proteins and energy required for calcification reaction. The bone morphogenetic proteins offer a potential, accelerating healing and regeneration of the bone and require as initiating stimulus to more undifferentiated cells, including myoblasts, synoviocytes, fibroblasts, chondroblasts and osteoblasts as well as pluripotent mesenchymal cells [Redy ,1998].

The mechanism in which laser irradiation promotes bone formation after tooth extraction may be explained by the L.E.L. effect on either fibroblast or on osteoblas or both of them. The proliferating fibroblasts in the clot seemed to be cells of p.d.l. and vessels along with the alveolar wall [Smith, 1975] [Todo,1968]

As the The fibroblast origin of cells in wound healing was explained by two theories which state that they are either derived hematogeneously or from cells in the connective tissue adjacent to the wound [Ross,1971]

The effect of L.E.L. on fibroblast proliferation seems to be through its effect on increasing venous, arterioles and capillaries circulation and development of newly formed vessels [Ross, 1971]

So the effect of L.E.L. at first 3 days of healing of extraction wound seems to be on the fibroblasts proliferation as well as new blood vessels formation.

The effect of L.E.L. on fibroblasts, will lead to increase in fibers production [AL-Hussenie,1992] [Gugliemohi,and Carbini, 1985] [Green, et al,1969] [Neiburger,2000] [Thawer and Houghton,1999] [Kurita et al, 1985].

Therefor when collagen fibers increased, the bone formation may be increased since the bone formation depends on the deposition of hydroxy apatite crystals along the collagen fiber in a matrix of proteoglycans called osteoid formed by osteoblasts [Amler,1977]

The effect of L.E.L., at the last 3 days of the experiment, might be on the osteoprogenitor cells and osteoblasts, which was present after 3 days of extraction and this disagreed with [Amler, 1977] who found that the activity of osteoprogenitor cells of periosteum increased in the extraction site and reach its peak after 4-5 days then decline gradually, while the activity curve of fibroblasts in the healing process reaches its endpoint 3 days after wounding [Calfin, 1963].

Also, L.E.L. affected bone formation when it was applied after the fourth day from extraction because the bone formation is started on the fourth day according to [Gugliemohi,and Carbini,1985]

L.E.L. affects also the calcification and mineralization of regenerated bone, because calcification requires energy, which is thought to be provided by ATP production that stimulated by the application of L.E.L [In-de-Bracke ,1991]

It is important to note that application of L.E.L. from Optodent device must be in a modulated pattern rather than continuous, to get biostimulation effect [Ibraheim,1999] because the continuous emission has an anti-inflammatory effect rather than biostimulation effect according to [Niccoli-Filho and Okamoto, 1995]

11. Conclusions:

Multiple doses of L.E.L. Were are more effective than a single dose by increasing bone trabeculae especially at a late-stage. The application of L.E.L in stage of bone formation is more effective than early stage.

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تأثير اشعاع الليزر الواطئ الطاقة على شفاء جرح قلع الاسنان (دراسة تجريبية في الجرذ)

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المواد والطرق: تم استعمال 24 جرذ خلال الدراسة حيث تم قلع الطاحن العلوي الاول ومن ثم تقسيم الحيوانات الى مجموعتين قياس وليزر ومجاميع الليزر كانت ايضا 5 مجاميع كالاتي: ب1: تعرضت لجرعة واحدة لمدة 5 دقائق مباشرة بعد القلع., ب2: تعرضت لجرعة مفردة لمدة35 دقيقة مباشرة بعد القلع., ب3: تعرضت ل7 جرع مدة 5 دقائق يوميا., ب4: تعرضت ل3 جرع لمدة 10 دقائق من اليوم الاول الى الثالث., ب5:تعرضت ل3 جرع لمدة 10 دائق من اليوم الثالث الى السادس ., **النتائج:** بعد تحضير العينات للفحص النسيجي باستعمال الميكروسكوب اتضح وجود فرق كبير بين مجموعة القياس والليزر وكذلك تباين بين مجاميع الليزر حيث ان الجرع المتعددة افضل من المفردة وان افضل مجموعة هي التي تعرضت لليزر اخر 3 ايام.**الاستنتاج:** ان من الممكن الاستفادة من الليزر الواطئ الطاقة لعلاج جرح قلع الاسنان لتقليل العدوى والالم. **الهدف:** دراسة لغرض بحث تأثير اشعاع الليزر الواطئ الطاقة على شفاء العظم في جرح قلع الاسنان وكذلك المضاعفات.