The effect of static magnetic field of mid-shaft femoral fractures healing in Rabbits

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Summary

This study was done on eighty rabbits to investigate the effect of static magnetic field on the transverse femoral fracture fix by intramedullary pinning. Experimental animals were divided into two main groups, control group contain twenty rabbits, while the treated group were divided into three subgroups as the following.

Control group induce mid-shaft fracture and fixation by intramedullary pinning.

Treated groups which divided into three subgroups, induces fracture similar as in control group and treated as the following:

1. Treated subgroups one (T1), expose to magnetic field (5 gauss).
2. Treated subgroups one (T2), expose to magnetic field (75 gauss).
3. Treated subgroups one (T3), expose to magnetic field (300 gauss).

The radiographic and Histopathological findings at 7, 14, 21, 28 and 35 days after operation indicated that the magnetic field accelerates the fracture healing, however, there are differences among treated subgroups , which revealed that T1 and T2 group have somewhat similar results, while T4 best results. The intramedullary pins did not show any side effect on the treatment with magnetic field therapy.

Key words: Magnetic field, bone, healing, physiological reactions.

Introduction

Bone is an essential component in the body described as a connective tissue helping to support and bind together various parts of the body, bone acts as a structural building allowing tendons and ligament attachment and facilitating kinematics motion, also supports and provide protection to the soft and vital internal organs [1].All bones serves as storage for inorganic matrix such as calcium and phosphor release these substances when demands. In addition, these substances give the bone strength [2]. Bone consists of two types of cells osteoblast and osteoclast and organic matrix formed by the osteoblast in which characteristic bone mineral is deposited in ordinary arrangement .

Their functions are different from one type to another, the first and second to building the bone, while the third to destroy the fragment bone [3&4].

Fracture of bone is an abnormal structure of bone that means discontinuity of the bone tissue, may be broken into two or more parts. And their etiology is external trauma or pathology, but both types of fractures have same stages, these stages are hematoma stage, inflammatory stage, granulation tissue stage, soft callus stage, hard callus stage, and remodeling stage [5].But the quality and speed of the processing healing depend on some factors [6].

To facilitate and quicker make the fracture healing, we should be inducing modern technique. One of these techniques is called Biomegnetotherapy. Biomegnetotherapy is one of the modern techniques to treat the fracture bone healing and faster healing. Essentially, the researchers depended on nature magnets in the beginning [7].

After that, magnetic coil was used to generate magnetic field and use oscillators to generate frequent magnetic field according to special calculations and equations to calculate exposure magnetic field . Magnetic field therapy is safe, has multiple uses, simplified using, and significant results [8]. The aim of this study was to investigate

the effect of magnetic field on fracture healing and the effect of intra medullary pin (IMP) on the magnetic field reversible.

Material and Methods

Eighty adult healthy rabbits both sexes, age more than 6 months weight range between 1.5-2 kg, were used in this study.The animals were put under similar feeding and environmental conditions . .

The magnetic field apparatus consists of five parts :

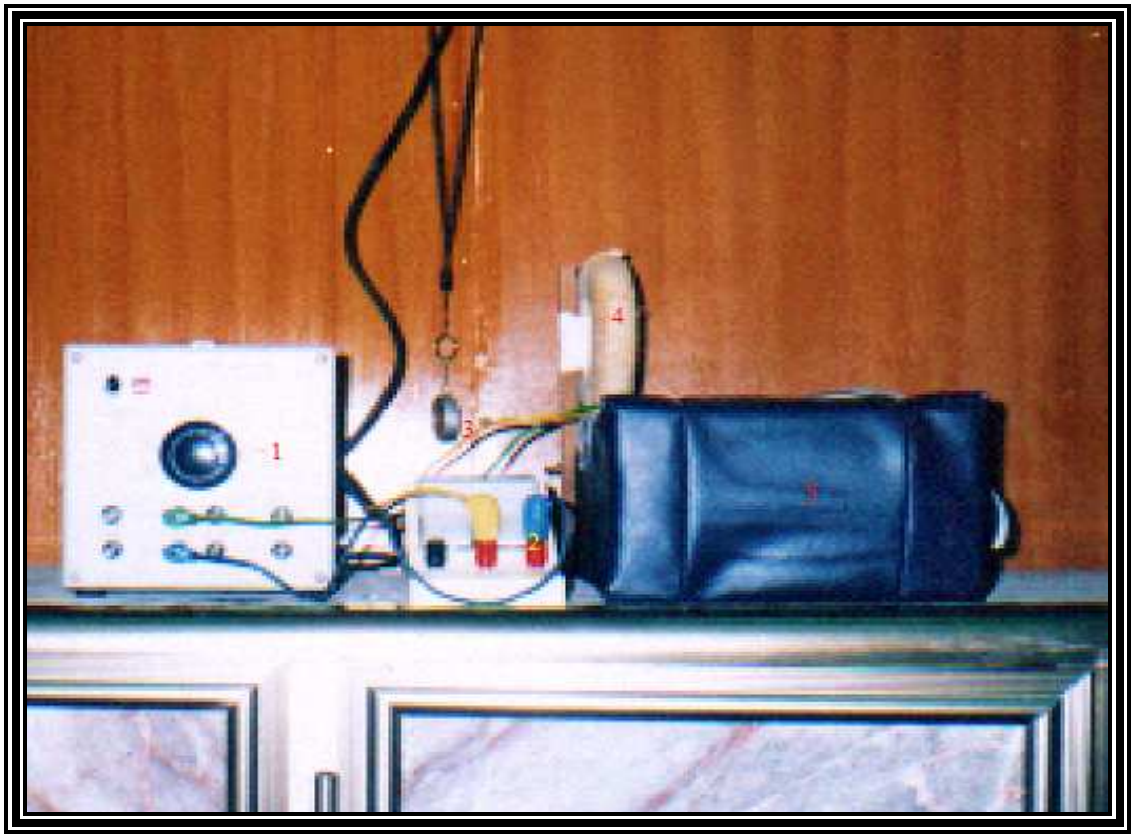
1-Power supply: this apparatus convert alternative current (AC) of electricity to direct current (220V,50 Hz to 30 V,0Hz).

2-Magnetic Coil: modified magnetic silk has these characteristic by(type grad P1/2L, H2, diameter 21mm). a coil has 1000 turns isolated by special plastic materials.

3-Analogue or digital Ammeter: to measure the output current from power supply.

4-Compass: used to indicate the magnetic field (MF) direction

5-Modified Beds: these beds were locally manufactured to restrict the rabbits during the treatment. The picture of apparatus is shown in Fig (1)



(Figure,1): the apparatus of magnetic field generation and bed

1-power supply
2-ammeter
3-compass
4-magnetic coil
5-bed

Animals Preparation and surgical operation:

Food was withheld for 6-12 hrs and water for 5 hrs prior to operation . the area of thigh region was prepared under aseptic surgical technique. . Anesthesia was induce with mixture of Ketamine, Xylazine at a dose 15mg-5mg / kg B.w intramuscularly respectively [8]. a longitudinal skin incision is made along the line from the greater trochanter to lateral surface of patella. The fascia along the cranial border of biceps femors is incised, by reflecting the belly of the biceps femors caudally and vastus laterally cranially, most femoral shaft can be exposed. Induce transverse fracture manually by surgical hand saw. Pin will be inserted by retrograde

approach [9]. Intramuscular injection of procaine penicillin 800.000 IU/kg. B.w once daily for 4-5 days after surgical operation, silk suture was removed after 7 days and removal of intramedullary pinning after 35 days of operation.

The experimental animals were divided into two main groups, control group(20 rabbits)and treated group was divided into three subgroups were exposed to magnetic field (5G,75G and 300G)respectively ,which applied by direct contact of source of magnetic field (center of magnetic coil) opposite the lateral surface at the site of fracture at 15 minutes /daily for 15 days as (Fig-2).

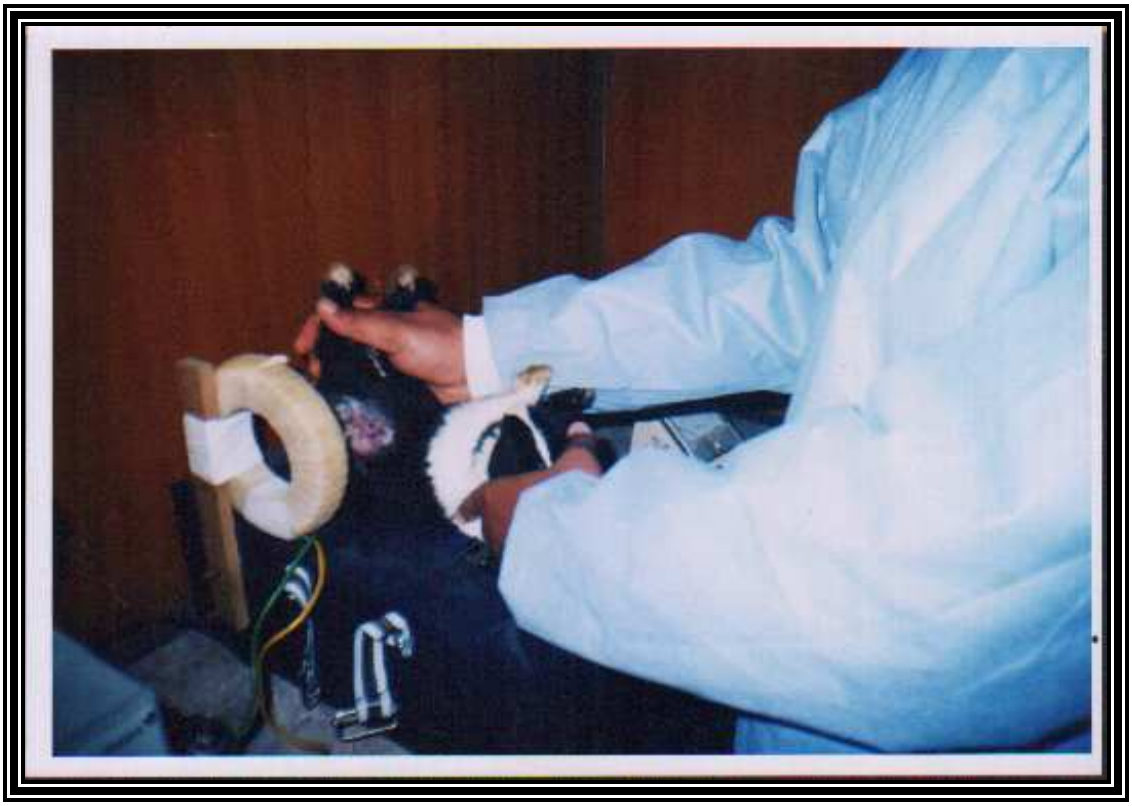


Fig (2) Magnetic field therapy (magnetotherapy)

Magnetic Field Generation and Its Measurement

Magnetic field was generated when a DC current influx to the coil. We can measure the current by analogue or digital ammeter.Practically, we can mesared the magnetic field by gauss-meter or magnetometer, but this apparatus is unavailable.

I(Amp)	X(cm)	B(G)
0.1	4.5	5
0.7	2.3	75
1.9	0	300

I(Amp) : current in ampere
X(cm) : distance from the center of the coil
B(G) : calculation magnetic flux

Four rabbits from control group and from each treated subgroups were imaged radiographically at interval (7, 14, 21, 28 and 35th) postoperative days which used70Kvand 7mAS.

Histopathological Study:

From control and each treated subgroups were sacrificed after radiographic picture were taken at interval of (7th,14th,21st,28th,35th) days after operation. Histopathology specimens should be taken at bone fracture site. The specimens were put in 10% neutral buffered formalin for fixation. Decalcifying processes after that was performed by solution of Formic acid-Sodium citrate for 12 days, during this period the solution should be exchanging and turn off the bones parts in solution [10& 11]. After 12 days the bone became softening which examine by insertion of needle then the bone trim into two on which include the fracture site in central of biopsy, after that wash with water for 3 days. The

bone was longitudinal dissection and prepared the slide as a routine manner and stain with Harris hematoxlin and eosin stain.

Results

The clinical observation were exhibited by pain, swelling, movement of the leg and discoloration of the skin operation sites were noted in the first three days after operation and disappear gradually in control group after 7 days, while other treated subgroups disappear after 3-5 days after operation. There are yellow spots at site of operation in some cases of third treated group (300G),which gradually disappear after 14 days post operation. The results of radiographic findings of control group at a period of (7,14,21,28 and 35th days) post operation .

After seven days, were observed no evidence of periosteal reaction and line fracture appears, Fig (3).On the 14th post operative days, was noticed clear appearance of line fracture and evidence callus formation.

On the 21st post operative days, an increase callus formation and still appear of fracture ,Fig (7).On 28th post operative days, shows increase callus formation and disappear gradually fracture line.

On 35th post operative days, decrease callus formation and disappear fracture line. Fig. (11).

While in treated subgroup (T1) ,on the 7th post operative days, notice evidence of periosteal reaction and appears fracture line. Fig (4).

On the 14th post operative days, shows callus formation and disappear gradually fracture line,on the 21st post operative days, shows increase callus formation and partially fracture line appear, Fig(8).on the 28th post operative days, show increase callus formation and pass into distal and proximal ends of line fracture. on the 35th post operative days decrease callus formation. Fig (12).

Treated subgroup (T2),on the 7th post operative days, shows evidence the callus formation (periosteal and endosteal reaction). Fig (5). on the 14th post operative days, shows evidence the callus formation and disappear fracture line, on the 21st postoperative days, increase density of callus and whole disappear of fracture line, Fig (9),on the 28th post operative days, shows decrease callus formation and formation and decrease density of fracture site, on the 35th post operative days, notice the bone semi normal the radiolucent and decrease the callus is early of remodeling stage, Fig (13)

Treated subgroup Three (T3),on the 7th post operative days, increase callus formation evidence of periosteal and endosteal reaction. Fig (6),on the 14th post operative days, appear callus formation and disappear line fracture, on the 21st post operative days, increase radiolucent of bone site fracture and shows decrease callus formation as well as disappear facture line. Fig (10),on the 28th -35th post operative days, show decrease callus and started of remodeling stage, Fig (14).

Fig (3) T1 group on 7th day, show periosteal reaction.

Fig (4) control group on the 7th day, show no evidence of callus formation

Fig (5) T3 group on 7th day, show appear callus formation

Fig (6) T2 group on 7th day, show periosteal and endosteal reaction

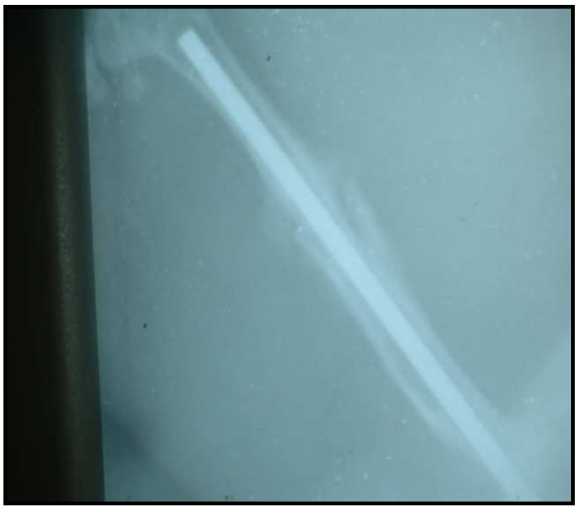


Fig (7) T1 group on the 21st day, show increase callus formation.

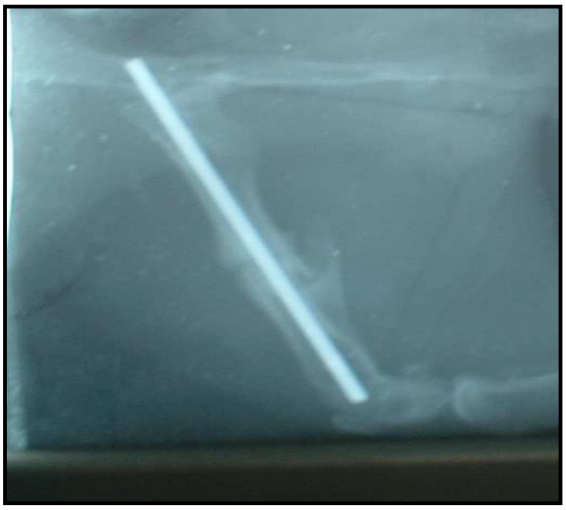
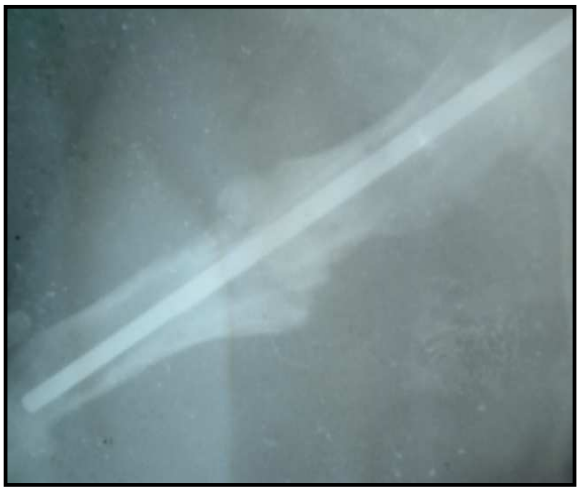


Fig (8) control group on the 21st day, show increase callus formation and appear line fracture.

Fig (9) T3 group on the 21st day show increase disappear fracture line.



Fig(10) T2 group on the 21st day, show increase density of callus.

Fig (11) T1 group on the 35th day, show decrease callus formation .

Fig (12) control group on the 35th day, show pull the IMP and decrease callus formation.

Fig (13) T3 group on the 35th day, show remodeling stage (normal bone)

Fig (14) T2 group on the 35th day, show semi-normal bone, radiolucent and decrease callus formation.

Histopathological findings :-

The histopathology section has been taken as follow (7, 14, 21, 28, and 35) days post operation.
Control group at 7 days of operation , the site of fracture characterized by organizing hematoma, present fibroblast, and inflammatory cells are low and less evidence of bone formation, Fig,(15).
Post operative 14 days, show fibrin deposited at the bottom, present trabecular bone irregular. at 21 days, there was increase irregular trabecular and increase bone formation (osseous tissue) Fig (19). At 28 day post operative, show decrease space between trabecular and increase osteocytes and osteoblast. While at 35 days, post operative, shows osteochondral bone formation and less number of inflammatory cells Fig (23).

Treated subgroup (T1)

At the 7 days post operative, it show increase osteoblast and osteocytes, evidence to osseous tissue or bone formation, Fig(16) on the 14 days post operative, shows increase callus formation (new bone) formation and increase inflammatory cells as well as irregular trabecular , At the 21-28 days post operative shows enlargement of trabeculae and converted woven bone to lamellae bone, noted chondrocytes, and began Haversion canal, central bone and periosteal reaction ,Fig (20).while at the 35 days post operative, shows increase thickness of trabeculae, and show osteoclasts, Fig (24).

Treated subgroup (T2)

At 7 days post operative, show increase inflammatory cells and osteoclasts and osteocytes. Organizing hematoma and irregular trabecular bone was enclose contact with the edge of bone, Fig (17). At 14 days post operative, show increase osteoclasts and osteocytes and irregular trabeculae, increase

matrix bone (fibrin).At the 21-28 days post operative, show increase osteoclasts trabecular bone is irregular and Haversion canal, central bone, osteoclasts appears, Fig (21). In the stage of 35days post operative, show bone central, Haversion canal and increase thickness of trabecular osseous tissue, Fig (25).

Treated subgroup (T3)

at 7 days post operative, show osseous tissue and active bone formation, Fig (18).Osteoclasts and osteocytes increase significantly at 14 days post operative. Irregular trabecular bone, this trabecular bone formation was seen in the floor at the edge. The histological appearance of T3 at 21-28 days interval are regular trabecular bone, Haversion canal, bone central, increase blood vessels evidence of bone marrow formation, Fig 22), at 35 days post operative, show Haversion canal, increase osteoclasts, is repair active phase characterized by hard callus formation and undergoing endochondral ossification within a bridging callus, Fig (26).

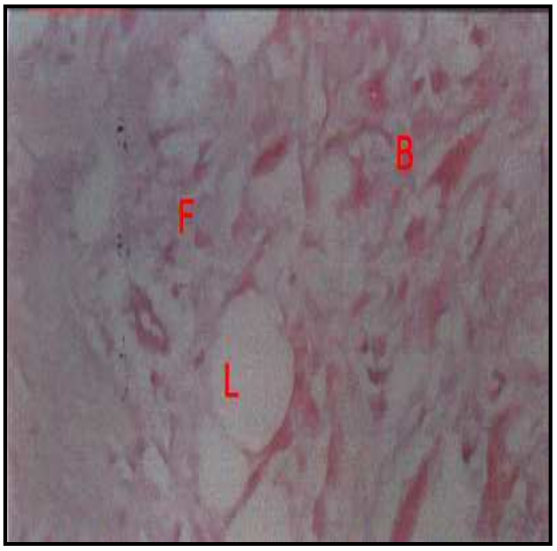


Fig (15) cross-section of T1 group after 7 days, noted congested blood vessels B, fibrosis F, and large lacunae L (H&E, 400x).

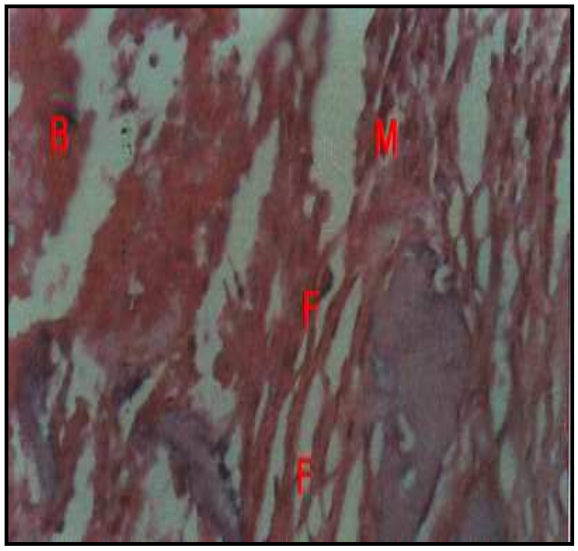


Fig (16) cross-section of control group after 7 days, noted infiltrated blood B, fibers F, infiltrated inflammatory cells M (H&E, 400 x).

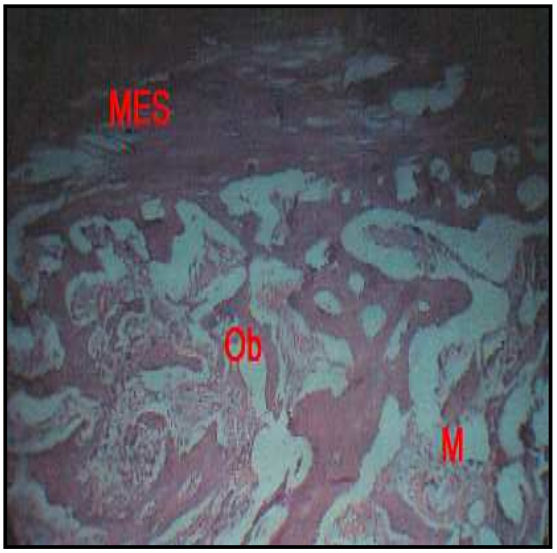


Fig (17) cross-section of T3-group after 7 days, noted mesenchymal cells MES, inflammatory cells M, osteoblastes and osteocytes Ob (H&E,400x).

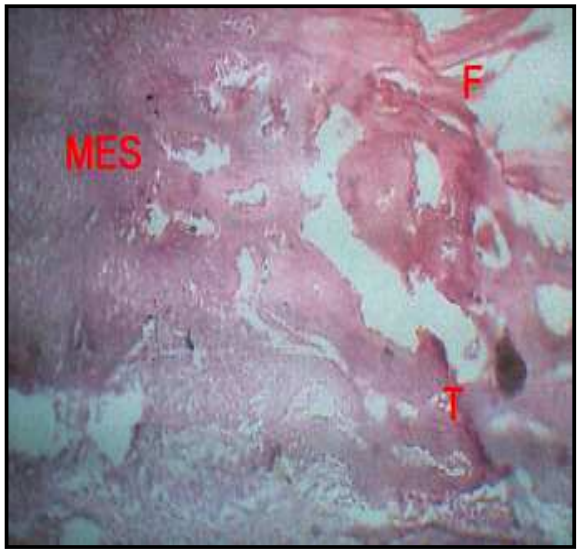


Fig (18) cross-section T2-group after 7 days, noted fibroblasts F, little trabecular bone T, undifferentiated mesenchymal cells MES (H&E, 400x)

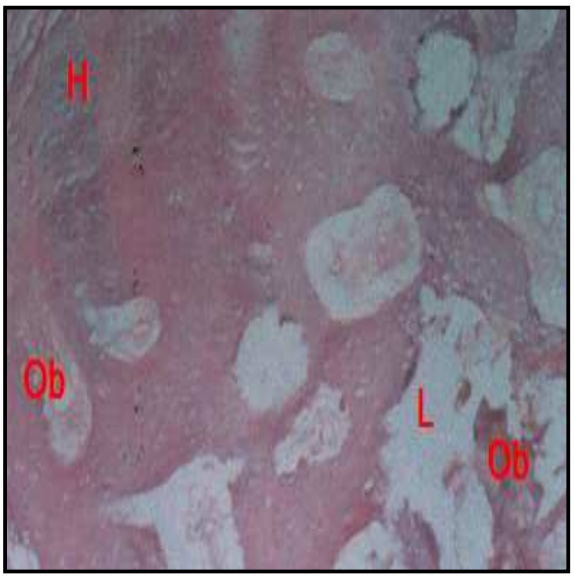


Fig (19) cross-section of T1 group after 21 days, noted osteocytes, osteoblastes Ob and Haversion H canal lacunae is presentation L (H&E,400x).

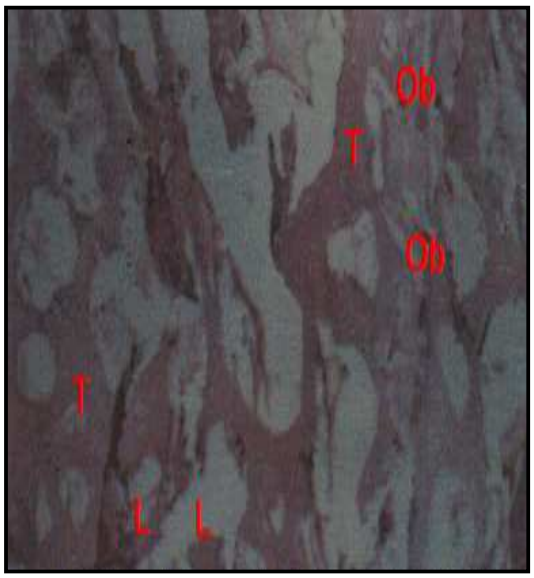


Fig (20) cross-section of control group after 21 days, noted increase trabecular bone T, and osseous tissue formation T, osteoblastes and lacunae (H&E, 400 x).

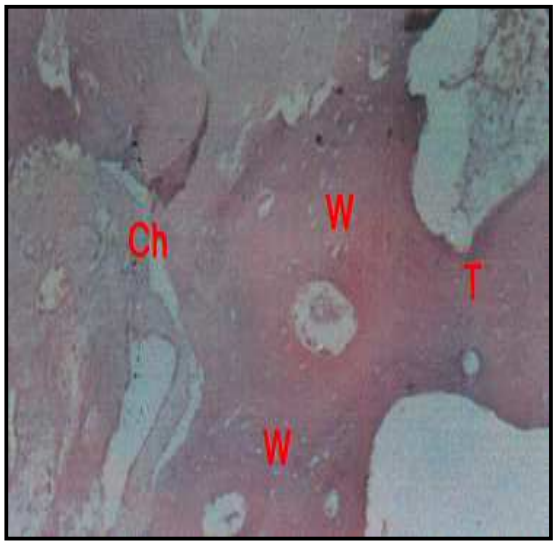


Fig (21) cross-section of T3-group after 21 days, noted trabecular bone T, woven bone W, lamellae chondrocytes Ch (H&E, 400x).

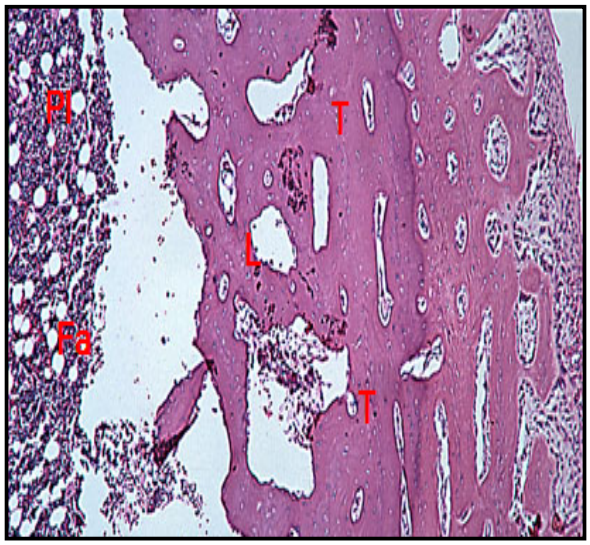


Fig (22) cross-section of T2-group after 21 days, noted thick trabecular bone T, phagocytes Fa, and plasma cells Pl is presenting (H&E,400x).

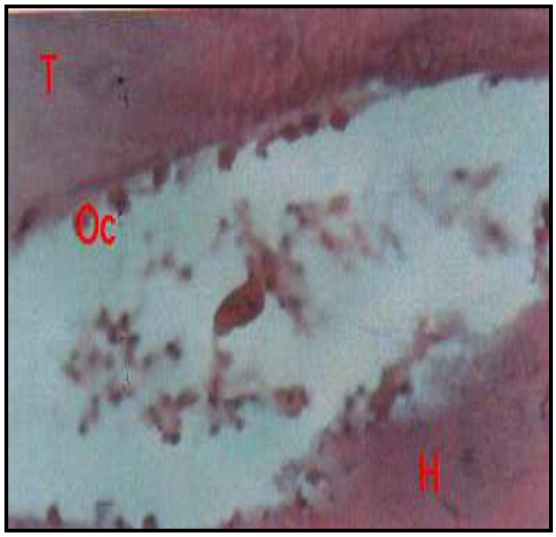


Fig (23) cross-section of T1-group after 35 days, noted Haversion canal H, osteoclasts is presenting Oc (H&E, 100x).

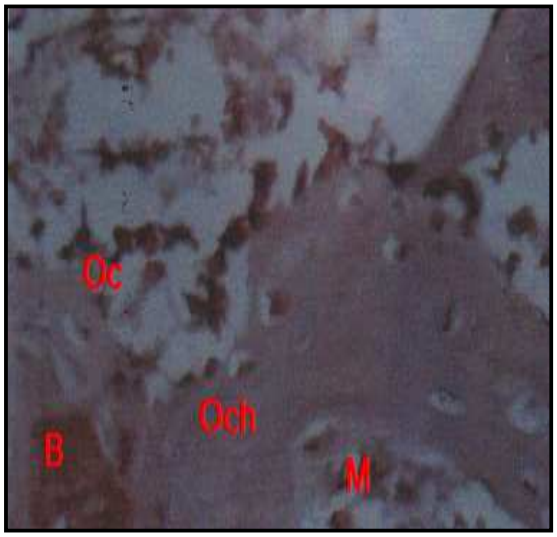


Fig (24) cross-section of control group after 35 days, noted osteochondral bone formation Och and less number of inflammatory cells M, osteoclasts presentation Oc (H&E,400 x).

Fig (25) cross-section of T2-group after 35 days, noted trabecular bone T and Haversion canal H (H&E,400x).

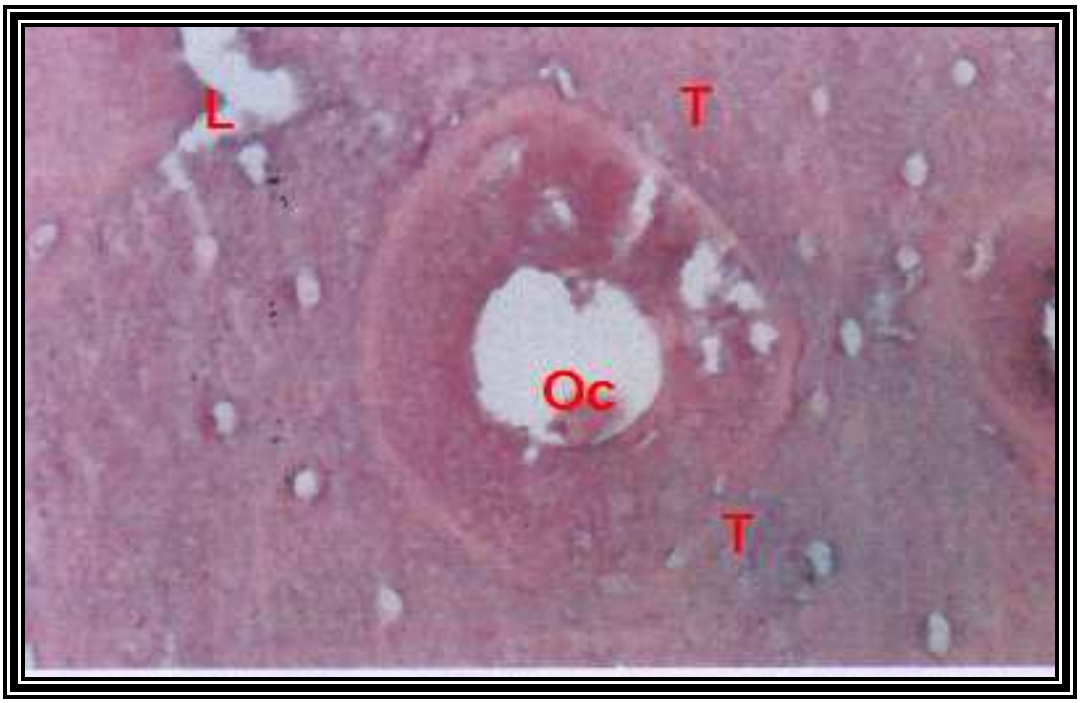


Fig (26) cross-section of T3-group after 35 days, noted thickness trabecular bone T and osteoclasts proliferation Oc (H&E,400x)

Discussion

Five factors play main role in magneto therapy, animal species, body organs, magnetic field tension, frequency, and exposure period. The present study includes all the above parameters except frequency.

Animals species differ from type to other. In the present study used rabbits with three levels of magnetic field tensions 5 gauss, 75 gauss, and 300 gauss. The best results showed with 300 gauss, and also showed in 75 gauss good results, all applications are with period at 15 minutes per day /daily for 15 days, the healing showed at 21days post operation, other study used 40 gauss per 1hr per day/twice daily, the healing showed after 23 days post operation in mid tibial osteotomy in rabbits [8&12]. Therefore, some animals like dog responses to megnetotherapy faster than other animals like rabbits. The causes that effect to faster healing related to bone itself directly and other organs that effect on bone fracture healing indirectly. The function of organ or organ vitality effect the recovery or healing of defect organ. The bone non vitality rather organ compared with other organ like glands, heart, and skin. In present study the bone healing in minimum period 21-23 days post operation and maximum period 33-35 days post operation. This agree with [13&14]. The suggestion of organ vitality of bone is agreed with other organ like tendon, when application of magnetic field on traumatic Achilles'

tendon with (450 gauss) per 1hr /daily, the recovery showed after 39-52 days after application this agree with [15]. The present study indicated that the magnetic field play a role in bone healing this agrees with [16], whom used 3 level of magnetic field (50µT, 100 µT, and 200µT) show different results among groups. Finally the period of exposure effect of positively or negatively depending on the type of tissue and its position according to previous factors.

In present study don't used frequency with magnetic field in treatment of fractured bone, because of undesirable results that reported by researchers such as [17, 18, 19& 20].in agreement with [8,19,21&22], the results disagreed with [27] who confirmed the negative effect of intramedullary pins on fracture healing, this suggestion wasn't confirmed in present study.

The treated subgroups show a rapid recovery faster than control group, this may be due to some factors ,the magnetic field effect directly on microorganisms this suggestion agree with [23], and also effect indirectly by increasing immune function through the oxygenation of white blood corpuscles, the magnetic field affect on wound healing by

increasing vascularization and promote fibrillation to increase the bridges between wound edges [24&25].
Nevertheless, the differences among groups are related to magnetic field tension, this suggestion agreement with [16]. Some cases erupt yellow spots at application of magnetic field, these yellow spots are related to thermal effect of magnetic field, this suggestion is compatible with [26].
The radiographic examination revealed that the callus formation of control group began at second week and fracture line appears clearly. Gradually, callus elevated at fracture line and at edges of fracture at the end of third week. The callus degrade with persistence of fracture line at the end of fifth week. (27).
While in treated the callus mass appeared after 7 days after operation in (T1) and (T2) but saw more elevated in (T3) in fracture line region and edges.
This phenomenon may be due to stimulation inflammatory cells reaction and promote the osteocytes, osteoblasts and osteoclasts to invade the area (mesenchyma) to build the bridges between the two edges, the elevated callus mass also show besides the edges. Calcium, phosphorous, and other bone elements despites because of the factors that are assist the fracture healing by stimulation magnetic field therapy, this results agree with [16]. The persistence to exposure to magnetic field after 7 days stimulate local reaction and general reactions with whole body, especially with organs that indirectly affect fracture healing as parathyroid gland, blood vessels with heamodynamic flow as well as the organs that liberate the energy as liver and pancreas [28&29].The different callus formation and fracture line disappearance after (7-14) days on the processing healing velocity mentioned factors especially depending on indirect factors, but the different factor healing velocities depend on quantity of absorbed magnetic field energy, this confirm with [30&31] During the period of (14-35) days after operation, the mechanism of fracture healing is similar in treated groups, but the velocity of mechanism discuss in histopathology section, these results agree with [12&16]. The biological processing that limit an identity of fractured osseous tissue depends on undifferentiated mesenchyma, differentiated osseous cells (osteocytes, osteoblasts, and osteoclasts) as well as inflammatory cells and fibers.the histopathological section shows differences among control group and treated group, companied with gross pathology development. Undifferentiated mesenchyma, tissue is predominance with few osteoblasts and fibrosis as well as inflammation giant cells at 7 days after fracture in control group.
In sequence, osteocytes, osteoblasts, proliferated and lamellar osseous tissues develops, in beginning it is irregular and thin as well as there are large lacunae appears which is adipose tissue dissolve after tissue preparation.
In sequence, the tissue develops gradually, the osteoblasts in large number, less fibrosis, shrinkage the lacunae, osseous trabecular tissue
developed at 21-35 days after fraction, as well as osteoclasts proliferate gradually at 28- 35 days, this results compatible with[27].
The similarity between T1 subgroup and control group in the first two weeks regards to the weakness effect of magnetic field on the bone fracture and also weakness of the whole body organs that associated with metabolic processes at repair fractured bone. But, (T2) and (T3) magnetic field tension, there are two type of effectsat the fracture site, which increase of blood supply and antimicrobial effect, these show in 7-14 days, there are less number of inflammatory cells and the lamellar osseous tissue appear clearly at 7days in (T3) group. This variety in tissue composition regards to the magnetic effect energy which stimulate the whole organs body to secretion of (PGf2a, PGE2, βTGF, vitamin D3 thironon and calcitonin) besides stimulate macrophages, increase blood flow, fibroblast and after than increase the local reaction. Trabecular osseous tissue early appears after 14 days and predominance at 21 days in (T3), this histopathology section agrees with [16]. The present study is in agreement with other study [22&32]. In conclusions the magnetic field has effect directly on fracture bone healing by stimulation osseous cells and organs that release substance, which increase fracture healing. And then accelerates the process of ossification and bone healing in T3 is excellent than other T1 and T2, and the treated subgroup are better than control group.
This study illustrates the potential benefits of biomagnetic therapy which appear to have greatly influenced the rate of bone healing with good quality callas formation .

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تأثير المجال المغناطيسي الثابت على التئام كسر عمد عظم الفخذ في الأرانب

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الخلاصة

أجريت هذه الدراسة على ثمانين أرنبا من كلا الجنسين لمعرفة تأثير المجال المغناطيسي الثابت على كسور منتصف عمد عظم الفخذ في الأرانب والمثبتة بواسطة مسامير داخل العظم. قيمت التجربة سريريا وشعاعيا ونسجيا ,قسمت حيوانات التجربة إلى مجموعتين رئيسيتين مجموعة السيطرة ومجموعة المعالجة قسمت الى ثلاثة مجاميع فرعية .

*المجموعة الأولى(السيطرة) أحدثت الكسور جراحيا وثبتت باستخدام المسامير وتركت بدون التعرض إلى العلاج بالمجال المغناطيسي .

المعالجة الأولى: أحدثت الكسور جراحيا وثبتت باستخدام المسامير وعرضت المجموعة إلى العلاج المغناطيسي الواطئ ومقداره 5 گاوس.

المعالجة الثانية أحدثت الكسور جراحيا وثبتت باستخدام المسامير وعرضت المجموعة إلى العلاج المغناطيسي المتوسط ومقداره 75 گاوس .

المعالجة الثالثة أحدثت الكسور جراحيا وثبتت باستخدام المسامير وعرضت المجموعة إلى العلاج المغناطيسي العالي ومقداره 300 گاوس.

تمت متابعة التئام الكسر في حيوانات المجاميع المختلفة سريريا للفترات (1, 3, 5, 7و 14) يوما بعد العملية. ونسجيا وشعاعيا للفترات (7, 14, 21, 28و 35)بعد العملية.

أظهرت النتائج(سواء كانت سريريه أو شعاعية أو نسجية) إلى تعجيل عملية التئام الكسور , بالإضافة إلى ذلك هناك اختلافات بين المجاميع المعالجة في سرعة استجابة التئام الكسور, فقد أظهرت مجموعتي المعالجة الثانية والثالثة تشابها نوعا ما في النتيجة , بينما أشارت المجموعة الرابعة المعالجة إلى نتائج معنوية مقارنة مع مجموعتي المعاملة الثانية والثالثة وكذلك مجموعة السيطرة .ولم يظهر السفود داخل العظم تأثير سلبيا على التئام الكسور المعاملة بالمجال المغناطيسي الثابت.