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ENHANCING BONE DEFECT HEALING IN RABBITS BY USING AUTOLOGOUS BONE MARROW ASPIRATE AND ELECTRICAL STIMULATION

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ABSTRACT

Electrical stimulation can promote bone formation by stimulating cellular activity, enhancing the proliferation and differentiation of osteoprogenitor cells, increasing local blood flow, and upregulating the expression of bone-related genes and proteins. When combined with BMA, they further contribute to bone healing by promoting osteogenesis, osteo-induction, and modulating the local healing environment. In this experiment, 36 rabbits were used, and they were randomly divided into three groups (CG = Control, BM= Bone Marrow and BM^{E} = Bone Marrow + Electrical stimulation). Under general anesthesia, all animals underwent osteotomy and a small defect up to 2 mm was created in the fibular bone using a fine electrical rotating saw. After osteotomy, group CG was subjected to percutaneous injection of 2 ml of normal saline at the fracture site. Group BM was subjected to percutaneous injection of 2 ml of autologous bone marrow. Group BM^{E} was subject to injection of 2 ml of autologous bone marrow percutaneously and electrical stimulation at the fracture site three days after osteotomy, using a capacitive coupling device electrical stimulator with a power source of 9 volts and an output of 30 KHz, for 30 minutes twice daily for four weeks. This experiment was assessed by clinical, radiographical, postmortem, and histopathological examinations at the 2nd, 4th, 6th, and 8th postoperative weeks. In conclusion, BM^{E} group rabbits demonstrated excellent healing signs, almost starting from the second post-operative week. The defects restored its normal anatomical shape with a high degree of remodeling process compared to groups CG and BM.

Keywords: Osteotomy; Electrical stimulation; Bone growth; Rabbit; Fibula.

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INTRODUCTION

Pets are prone to a variety of unintentional illnesses, including orthopedic conditions. A fracture typically results in soft tissue injury of various degrees, including periosteum damage, torn arteries, and bruised muscles. Pet animals frequently experience it as a result of trauma, such as falling from a height or being struck by a car. The animal's age, anatomical placements, and the kind of fracture, which might be hairline, multiple-piece, or compound, all affect how severe the fracture is (Bahney *et al.*, 2019; Muhamad *et al.*, 2020).

Bone fracture healing: is an intricate and fluent regenerative process that aims at restoring the damaged bone to its pre-injury state and cellular composition. Electrical bone growth stimulators (EBGS) are supplemental forms of therapy to enhance the orthopedic healing process. It is divided into two options, the invasive and the non-invasive electrical stimulator. The invasive method of the electrical stimulator has more complication rates, compared with the noninvasive electrical stimulator device. The invasive method requires surgical implantation of the electrodes within the fragments of fractured bone and implantation of the battery in an intramuscular or subcutaneous space (Conta *et al.*, 2021). The non-invasive electrical stimulator device is achieved through a process called capacitive coupling. In this technique, two electrodes are placed on the skin on either side of the broken bone defects. A current from 9 volts battery passed between the electrodes and the patient that cannot feel the current. The most noticeable effects are that this type of EBGS can cause bone cells to proliferate and accelerate fracture healing (Leppik *et al.*, 2018). The effect of electrical stimulation has been studied in several animal models to repair bone defects, fresh fractures osteotomies, and non-union. Electrical stimulation exhibited enhanced cell proliferation, calcification, and induced mechanical strength in fractured bone (Nicksic *et al.*, 2022).

Stem cells in the bone marrow have therapeutic potential in orthopedic surgery, because they can undergo asymmetric division to produce cells that become more specialized, such as osteoblasts or chondroblasts, which can enhance fracture healing. Stem cells are primitive unspecialized cells, they keep their ability to self-renewal, but they can differentiate into limited cells and tissue, usually to particular tissue or physiological system of origin (Poliwoda *et al.*, 2022).
The recent study plans to identify and evaluate the rate of bone healing in surgically induced fracture defect in fibular bone in rabbits by applying a combination of two therapeutic modalities; first is percutaneous autologous bone marrow injection at the site of fracture; second is capacitive coupling electrical stimulation at the site of osteotomized bones.

MATERIALS AND METHODS

In this study, thirty-six healthy local breed rabbits, 5-7 months old, weighing approximately 2 kilograms were used. They were divided randomly into three groups, each group included 12 rabbits. All rabbits were kept in cages during the whole experimental period under hygienic conditions and fed with water and a standard ration, and the litters of cages were changed daily. The experiment was carried out in the Teaching Veterinary Hospital, College of Veterinary Medicine, University of Sulaimani (CVM/UOS).

Ethical approval

All the procedures and approaches of this study were conducted and approved according to the principles of the ethics by the College of the Veterinary Medicine Research Committee, University of Sulaimani, Kurdistan Regional Government, Kurdistan/ Iraq.

Anesthetic technique

Anesthesia was induced by intramuscular injection of Xylazine hydrochloride and ketamine at a dose of 0.5 mg/kg and 10 mg/kg, respectively (Soundara, 2005).

Surgical procedure

Animals were placed on lateral recumbency on the surgical table, so that the leg to be operated upon was on the top. The area of the lateral aspect of the tibia and fibula was prepared surgically by clipping, shaving, and disinfecting with povidone-iodine. The operated leg was carefully draped, leaving enough space to allow surgical operation. An incision approximately 2 cm long was made in the skin over the fibula, then the subcutaneous tissue and fascia were dissected, fibular muscles were also separated, and the fibula was then exposed. Complete transverse diaphyseal osteotomy and bone defect of up to 2 mm in size of the fibula was created using a fine electrical saw with a patterned blade 10 mm wide and 0.5 mm thick (Figure 1. A to F). No fixation was used, because the tibia and fibula are adhered together by the interosseus membrane, therefore adequate stability was achieved.

Method of bone marrow aspiration

Immediately after finishing the surgical operation, the area around the hip joint was clipped, shaved and disinfected. The site of trochanteric fossa of the femur was located, a stab incision was made and a spinal needle with stylet was inserted into the trochanteric fossa. A moderate pressure was used to rotate the needle through the periosteum and cortex into the bone marrow cavity, the stylet was then removed, and a 10 ml syringe containing 1 ml EDTA was attached to the needle. A volume of 2 ml of bone marrow was aspirated (Laird *et al.*, 1964).

Experimental design

I. Experiment CG (CG= control group); rabbits (*n*=12) were subjected to a percutaneous injection of 2 ml of normal saline at the fracture site after completion of surgical operation.

II. Experiment BM (BM= bone marrow group); rabbits (*n*=12) were administered percutaneous injection of 2 ml of autologous bone marrow at the site of osteotomy after completion of the surgical operation.

III. Experiment BM^{E} (BM= bone marrow + E=electrical stimulation) rabbits (*n*=12) were administered with the same autologous bone marrow as group BM. In addition, the osteotomized area was subjected to transcutaneous electrical stimulation with 30 KHz for 30 minutes twice daily for one month. Electrical stimulation was carried out through a process called capacitive coupling in this process two electrodes were placed on either side of the broken bone, wrapped by straps around the skin. The two electrodes positive and negative poles were connected to an apparatus operated by a 9-volt battery capable of inducing 30 KHz.

Evaluation Criteria:

Bone fracture healing was monitored and evaluated by clinical examination, radiological interpretation, post-mortem inspection and histologically.

Clinical examination

Clinical assessment of the lameness in rabbits of each group was evaluated by weekly observation for weight bearing and pain by palpation of the fractured limbs. Pain was graded from 0 (no pain), 1 (mild pain), 2 (moderate pain), 3 (sever pain). The degree of pain and soundness was assessed using statistical analysis.

Radiological examination

Radiographs of the osteotomies taken by c-arm x-ray machine with image intensifier at the 2^{nd}, 4^{th}, 6^{th} and 8^{th} postoperative week for rabbits in all groups and the degree of healing was evaluated.

Post mortem examination

By the end of the 2^{nd}, 4^{th}, 6^{th}, and 8^{th} post-operative weeks, three rabbits in all groups were sacrificed. Specimens of tibia and fibula of the operated limps were detached.

The skin is removed and soft tissues, such as muscle, fascia, tendons and ligaments were carefully removed by scalpel blade. Postmortem evaluation of these specimens taken from control and experimental groups was assessed by gross observation and palpation of the osteotomized bones

Histological examination

Three rabbits in all groups were sacrificed at the 2nd, 4th, 6th, 8th post-operative week, and the consolidation of bone healing assessed macroscopically and by palpation. The fibula of each animal was removed, dissected from the surrounding soft tissue, and fixed in 10% formalin. The specimens were decalcified in a mixture of hydrochloric acid and formic acid solution for about 24 hours. After decalcification, the specimens were rinsed in water and then transferred to an ammonia solution for 30 minutes to neutralize acids left in the specimens. The specimens were washed in running tap water thoroughly for up to 24 hours (Suvarna & Bancroft, 2019). Then they were dehydrated overnight and embedded in paraffin wax, then routinely processed for staining with hematoxylin and eosin according to the. The samples were examined and photographed using a light microscope and an AmscopeTM, Japan camera, respectively.

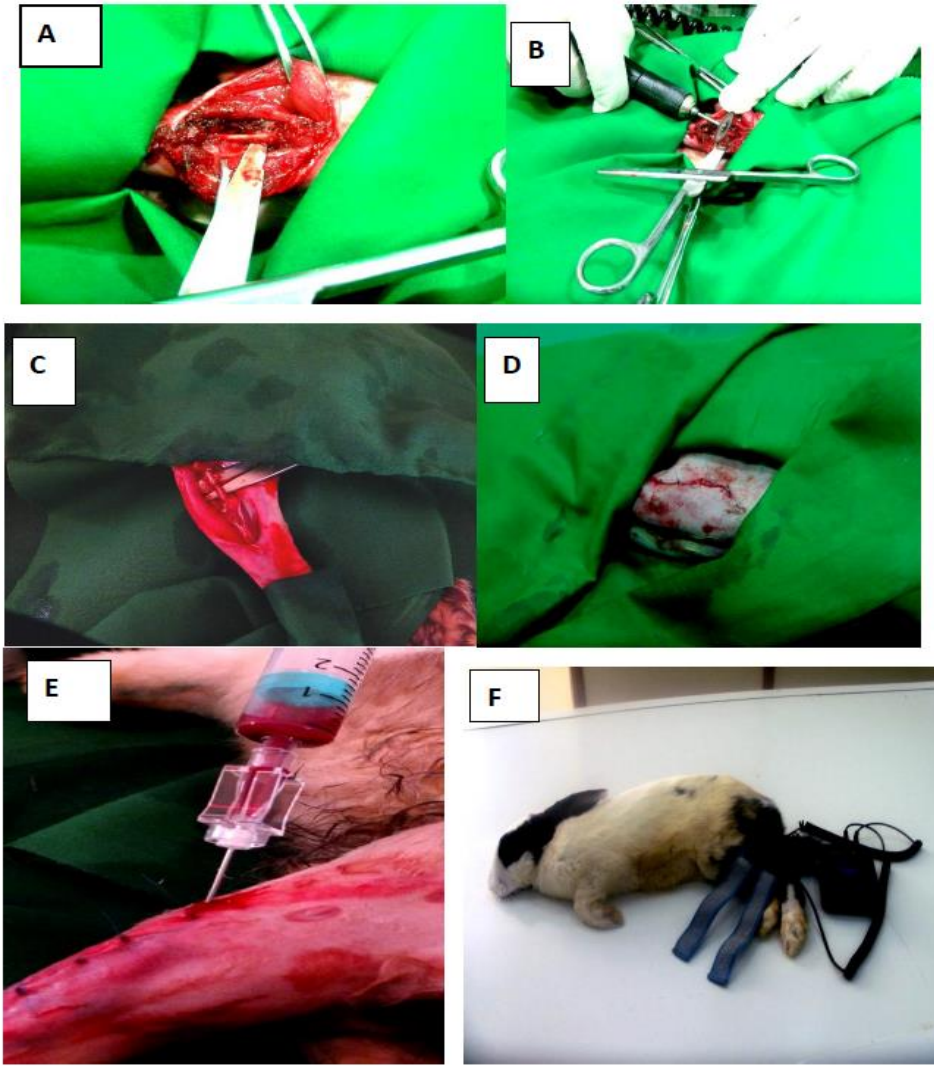

Figure 1: A. An incision about 2 cm long was made in the skin and subcutaneous tissue over the fibula, and the fibula was exposed. B. Procedure of osteotomy of fibula with the electrical rotating saw. C. Transverse fracture defect in the fibular bone. D. Suturing of the incision. E. Percutaneous injection of bone marrow into the site of fracture. F. Electric stimulator device, the two electrodes were placed on the skin of both sides of the fractured fibula.

Statistical analysis

Statistical analysis used SPSS for Windows, version 16.0, employing the non-parametric Mann-Whitney U Test to assess group differences. This method is apt for non-normally distributed or ordinal data, determining distinctions between distributions of independent groups. SPSS enhances methodological rigor, ensuring precision and contributing to study reliability. *P* value $\leq$ 0.05 was regarded as significant, and all obtained data were shown as mean ±SD.]

RESULTS

Clinical examination:

During the first operative week, 6 rabbits out of 12 rabbits in the BME group showed severe pain (score 3), restricted movement, tenderness on palpation and decreased appetite. Meanwhile, the other 6 rabbits showed moderate pain (score 2), and they were able to put some weight on the fractured limb.

In BM group, 8 rabbits out of 12 rabbits showed severe pain (score 3), while the other 4 rabbits showed moderate pain (score 2), and they were able to put some weight on the fractured limb.

In CG group, 11 rabbits out of 12 rabbits showed severe pain (score 3), and only one rabbit showed moderate pain (score 2), and they were able to put some weight on the fractured limb.

At second post-operative week, severe pain (score 3) was still evident in 7 rabbits out of 12 rabbits in the BM group, and the other 5 rabbits showed moderate pain (score 2). In the control group, 10 rabbits out of 12 rabbits showed severe pain (score 3) while 2 rabbits showed moderate pain. In the BME group, 9 rabbits out of 12 rabbits showed moderate pain, and they were able to put some weight on the fractured limb. The other 3 rabbits showed mild pain (score 1).

At the third post-operative week, group CG and BM rabbits were able to put some weight on the broken leg associated with pain in the fractured legs (score 2 and score 1), group BM^E rabbits were able to move around the cage, and no pain (score 0) was elicited when pressure applied around the surgical area.

At the fourth post-operative week, rabbits in group CG were still feeling some mild pain (score 2), and they were not able to put complete weight on the effected limb. These symptoms continued in this group until the sixth post-operative week, thereafter they were able to use the fractured limb normally. Conversely, group BM still had mild pain (score 1) and group BME rabbits were able to carry full weight and walk normally at the fourth post-operative week.

Statistical analysis was used to compare the degree of pain on 1^{st} ,2^{nd}, 3^{rd}, 4^{th}, 5^{th}, and 6^{th} weeks, respectively, for all groups. There was a statistically significant (P<0.05) decrease in the time of clinical healing in the treatment group, compared with the control group (**Table 1)**

Table 1: Statistically scored for each rabbit in all experimental groups according to the degree of lameness in post-surgical days.

Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
BM	2.667 ± 0.14	2.500 ± 0.15^{ab}	1.444 ± 0.17^{a}	0.889 ± 0.11^{a}	0^{a}	0
BM^E	2.500 ± 0.15	1.750 ± 0.13^{a}	1.111 ± 0.11^{a}	0^{a}	0^{a}	0
CG	2.917 ± 0.08	2.833 ± 0.11^{b}	1.889 ± 0.11^{a}	1.778 ± 0.15^{c}	1.167 ± 0.17^{b}	0

The mean± SD for the degree of lameness scoring for the experimental rabbits in all groups. Values are presented as mean ± SEM, ^{a,b} Different superscript letters denote significant difference at p <0.05 ,

Radiological examination:
Radiographs for control and experimental rabbits were obtained at 2nd, 4th, 6th and 8th postoperative weeks. It is worth mentioning here that the type of x-ray machine used in this research was image intensifier x-ray machine in which the image appears on monitor, images were evaluated and assessed for finding variation in the rate of healing. Radiographic images in the CG group, demonstrating that the fractured gaps were still evident at the second postoperative week. While these fractured gaps became smaller in the 4th week. Faint callus formation appeared on the upper and lower fragments, crossing the fracture gap. After 6th weeks, evidence of formation dense callus bridging was seen, while a small gap was still present. Definitely, at 8th week, there was continuity of the cortex and medullary cavity and complete disappear of the fractured gaps (Figure 2).

Radiographic images of the BM group demonstrated early recovery of the fractured bone, where callus formation developed and crossing the fracture site at the 4th week. Interestingly, complete obliteration of the fracture gaps occurred at the 6th week, and restoration of normal bone contour cortical bones shows continuity and reestablishment of the medullary cavity at the 8th week (Figure 3).

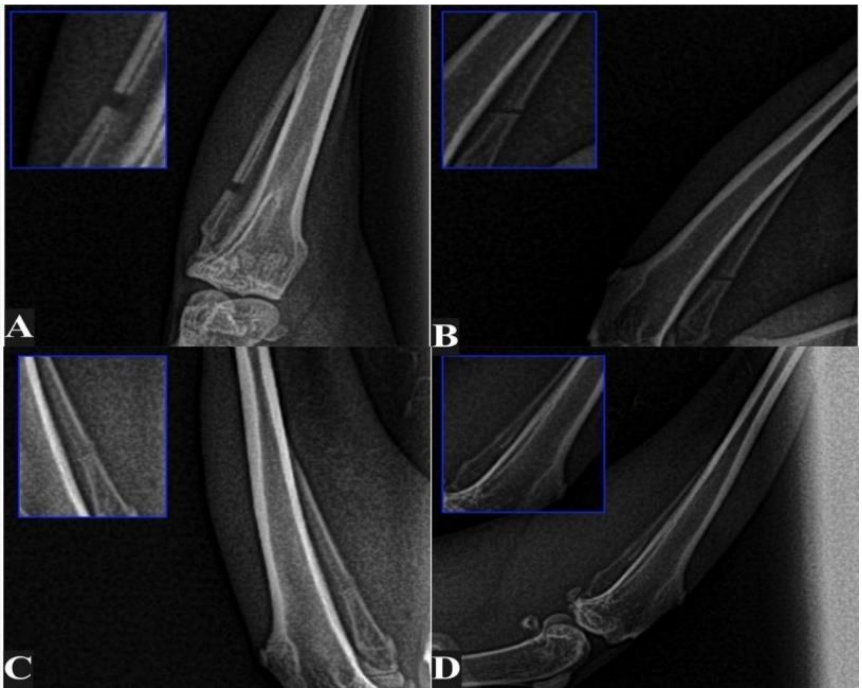

Figure. 2: A. Radiographic image of the CG group in the 2nd week, fractured gap is evident. B. Fracture gap is becoming smaller at the 4th week. Faint callus formation appeared on the upper and lower fragments, crossing the fracture gap. C. dense callus bridging the gap, a small gap is still present in the 6th week. D. There is a continuity of the cortex and medullary cavity at the 8th week.

Radiographic views in BME group showed faster recovery of the fractured segment, in contrast to the control group as well as to the BM group. Callus production was evident and bridged the fracture site at the 2nd week, and complete obliteration of the fracture gap was evident at the 4th week. Then, after the callus became smooth and regular at the fracture site at the 6th week. Finally, remodeling of the callus with normal cortical shadow reestablishment with restoration of the medullary cavity was completely evident at 8th week (Figure 4). Totally, it was found that the rate of fractured healing was enhanced using bone marrow aspirate, and it became faster when the bone marrow aspirate was supplied with specific doses of electrical currency.

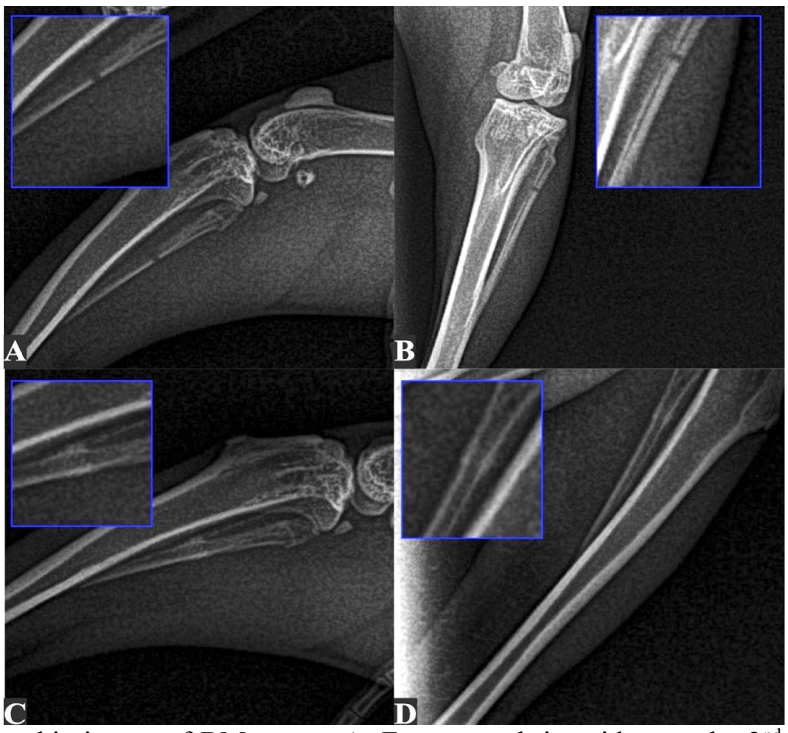

Figure 3: Radiographic image of BM group. A. Fracture gab is evident at the 2nd weeks. B. Callus formation is present crossing the fracture site at the 4th week. C. Complete obliteration of the fracture gap is evident at the 6th week. D. Restoration of normal bone contour cortical bones shows continuity and reestablishment of the medullary cavity at the 8th week.

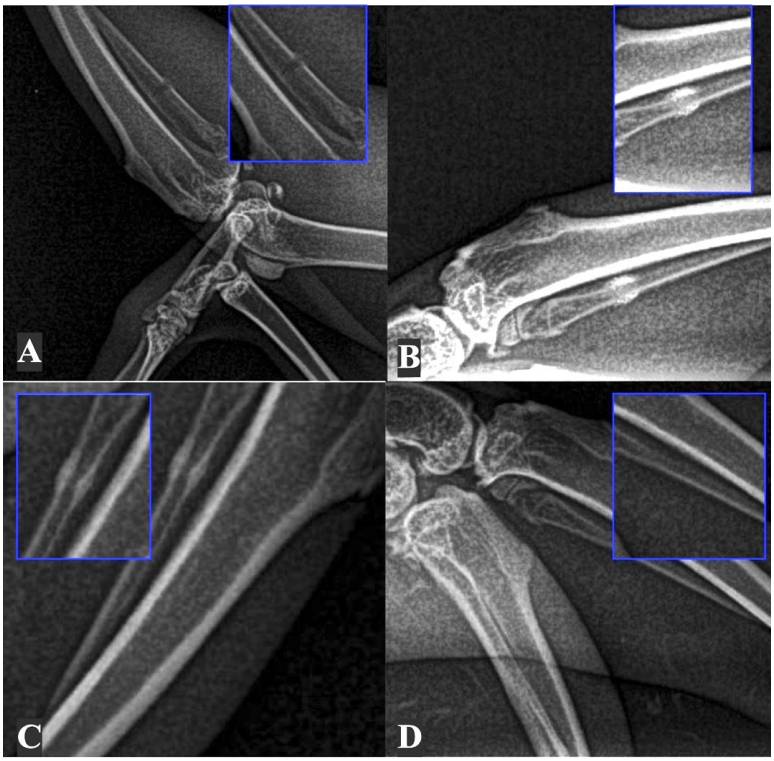

Figure 4: Radiographic image of the BM^E group. A. Callus production is evident and bridges the fracture site at the 2nd week. B. Complete obliteration of the fracture gap is evident at the 4th week. C. Callus became smooth and regular at the fracture site at the 6th week. D. Showing the remodeling of the callus with normal cortical shadow reestablishment with restoration of the medullary cavity at the 8th

Post mortem examination

Results of post-mortem examination of fracture sites were illustrated in **Figure 5 to 7**.

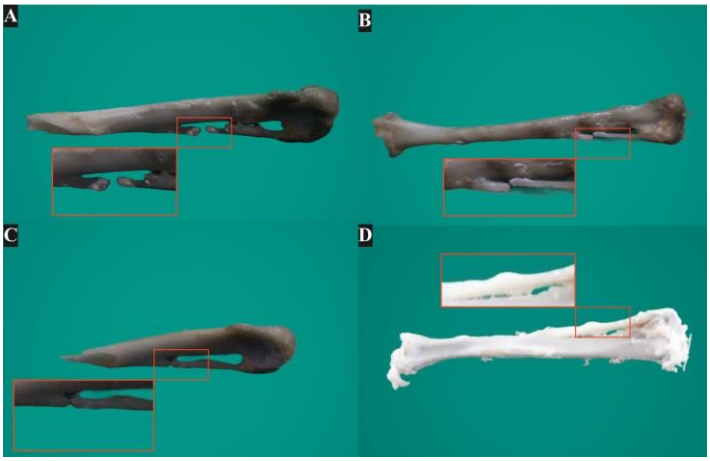

Figure 5: Postmortem image of CG group. A fracture gap is evident at 2nd week. B. Fracture gap became smaller at the 4th week. C, The small gap is still present at the 6th week. D, showing continuous cortex and disappearance of fracture gap. at the 8th week.

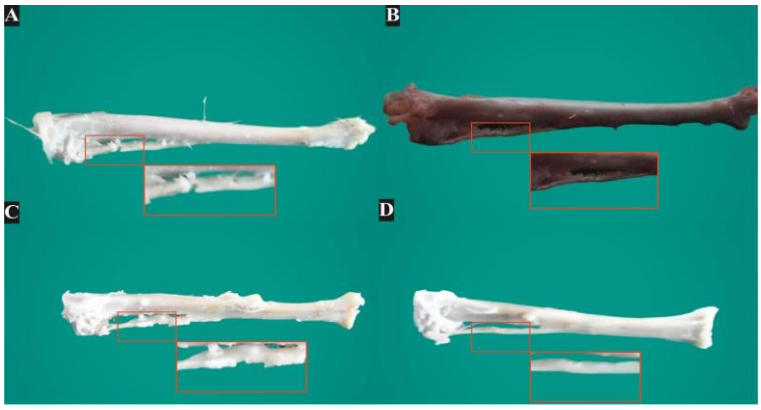

Figure 6: Postmortem image of the BM group. A, fracture gap is evident at the 2nd week. B, Callus formation is present crossing the fracture site at the 4th week. C, showing complete obliteration of the fracture gab at the 6th week. D, showing restoration of normal bone contour with slight irregularity at the 8th week.

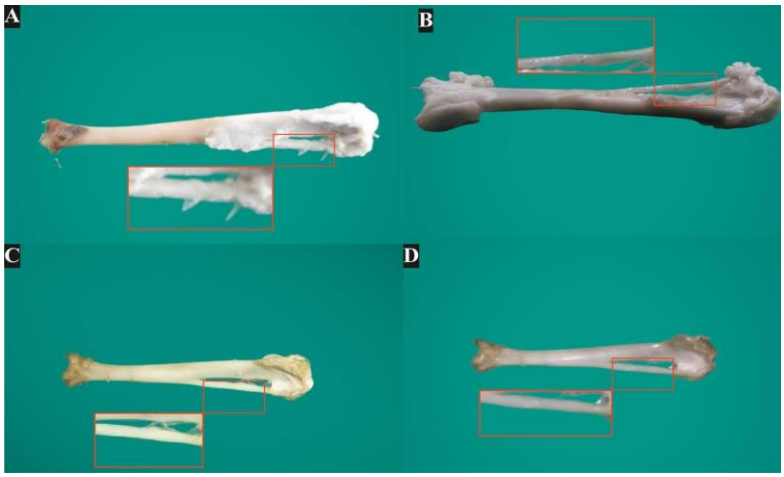

Figure 7: Postmortem image of BM^E group. A, Callus production is evident and bridge the fracture site. at the 2nd week. B, showing complete obliteration of the fracture gap and restoration of the normal bone contour. at the 4th week. C, showing a high degree of healing and remodeling of fibular bone at the 6th week D. showing normal bone appearance at the 8th week.

Histological Examination

Histopathological examination of bone specimens showed that the fibular bone (Figure 8 A) in the electrical stimulation group at 2nd week showed reparative phase or bony callus formation; where soft callus replaced the blood clot that formed in the inflammatory stage. osteoblasts lined woven bone matrix, which is characterized by a haphazard organization of collagen fibers. Osteoblast formation and osteoclasts in the marrow cavity with necrotic debris. At 1 month (Figure 8 B) showed focal remodeling of compact bone tissue into thin, regularly arranged trabeculae of various shapes. Selected changes were marked by elevated hematoxylin absorption and a different trabecular structure with mostly parallel or multi-directional arrangement of the trabeculae and electrical stimulates at the 2nd month (Figure 8 C) showed remodeling phase: Well formed lamellar or compact bone consisted of (Haversian canal) surrounded by concentic lamellae which contain osteocyte.

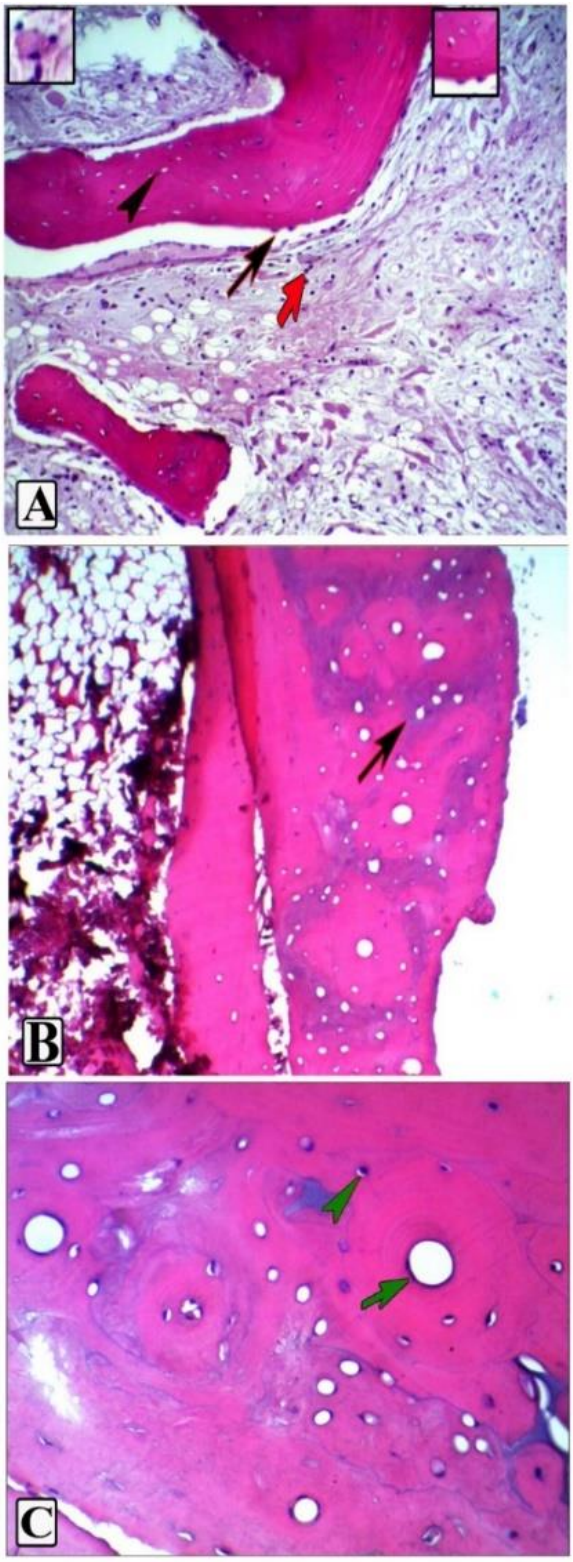

Figure 8: Paraffin sections of bony callus formation in BM^E group stained by Hx & E. A: Reparative phase or bony callus at the 2nd weeks show osteocyte (arrowhead, osteoblast black arrow and inset) osteoclast (red arrow and inset) . B: showing remodeling phase at the 1st month with woven bone formation marked by elevated hematoxylin absorption (arrow). C: remodeling phase at the 2nd month, there is well-forming lamellar or compact bone the Haversian canal (green arrow), osteocyte (arrowhead).

Bone marrow group at the 2nd week showed fibrocartilage, dense collagen fiber formation and new bone formation (Figure 9 A) and at the 1st month showed reparative phase or bony callus formation): Woven bone or immature bone was rimmed by osteoblasts (Figure 9 B) and at the 2nd month (Figure 9 C) showed (Remodeling phase): Compact bone has lighter stain and begins to resemble lamellar pattern structure that replaces the spongy bony callus (Replacing spongy bone by compact bone). Control group at the 2nd week (Figure 9 C) showed Granulation phase: Connective tissue (collagen fibers) proliferation (fibroplasia) with the presence of phagocytes (macrophages which phagocytes debris), single osteoblasts.

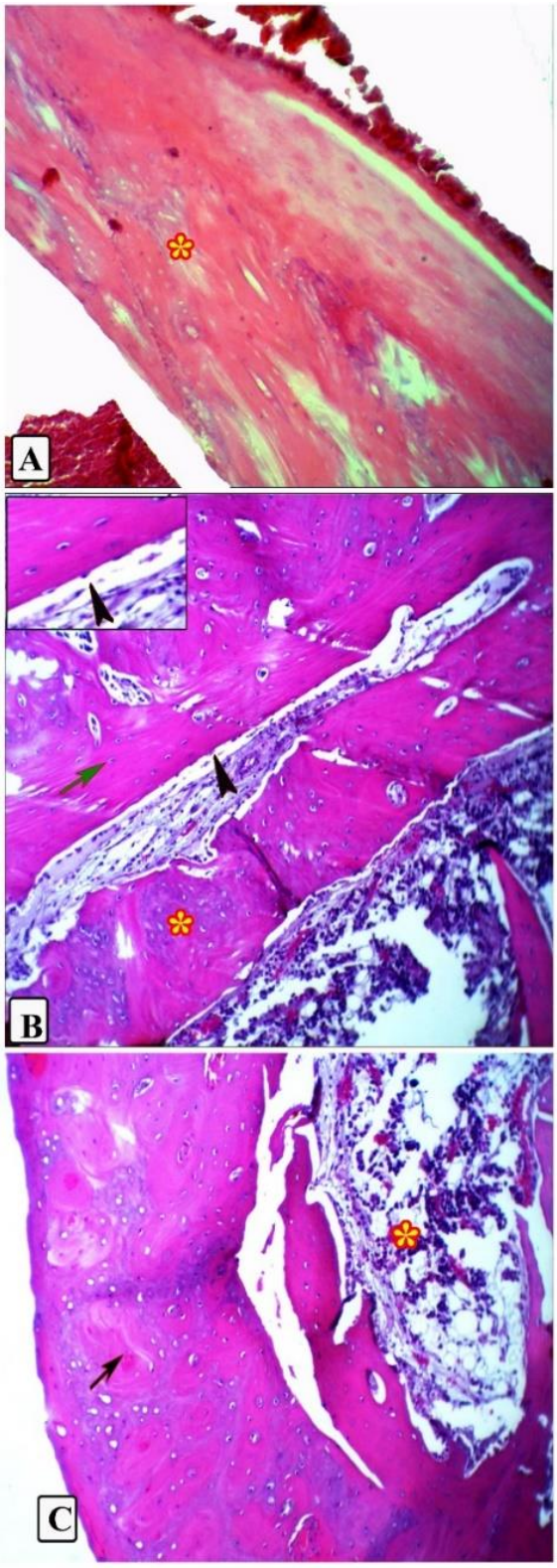

Figure 9: Paraffin sections of bony callus formation of BM group stained by Hx & E. A: Cartilaginous (soft callus formation) of BM group at the 2nd week (star). B: Bony callus formation showing woven bone or immature bone (star) at the 1st month, and osteocyte (arrow) osteoblast (arrow head) and inset. C: Remodeling phase at the 2nd month, compact bone resembles lamellar pattern structure that replaces the spongy bony callus (arrow) bone marrow (star).

In the control group, at 1st month (figure 10 B); showed mature fibrocartilage and dense collagen fiber formation with a new bone formation, while at the 2nd month (figure 10 C) showed new formation of bone from cartilage without connective tissue deposition, indicating reparative phase or bony callus formation. Bone sections showing focal remodeling of compact bone tissue into thin regularly arranged trabeculae of various shapes lined by osteoblasts and contained osteocytes and surrounded by large quantities of bone marrow.

DISCUSSION

In the present study, we assessed the efficacy of bone marrow aspirate alone and in combination with electrical stimulation on bone growth and/or healing. Based on clinical, radiological, postmortem, and histopathological examination, it was found that group BME showed a high degree of remodeling signs, compared with the CG group and BM. To our knowledge, such findings were not reported by investigators who applied autologous bone marrow graft or electrical stimulation alone in augmentation of fracture healing. Electrical stimulation exhibited enhanced cell proliferation, calcification, and induced mechanical strength in fractured bone (Long et al., 2021). According to the experiments carried out by researchers using autologous bone marrow and platelets, rich fibrin enhanced the healing of long bones fractures like femur, distal radial, metacarpal and metatarsal fractures (Ali & Ali, 2019; Long et al., 2021). According to the experiments carried out by Aron et al (2004) it was suggested that potentials of 1-10 volts at frequencies 20-200 kilohertz (KHz) were applied to the electrodes, which resulted in the development of electrical fields of 1-100 mv/cm at the fracture site. These stimulators were non-invasive, and the electrodes were placed on the skin at the opposite side of the fracture site (Long et al., 2021). On the other hand, any other published study

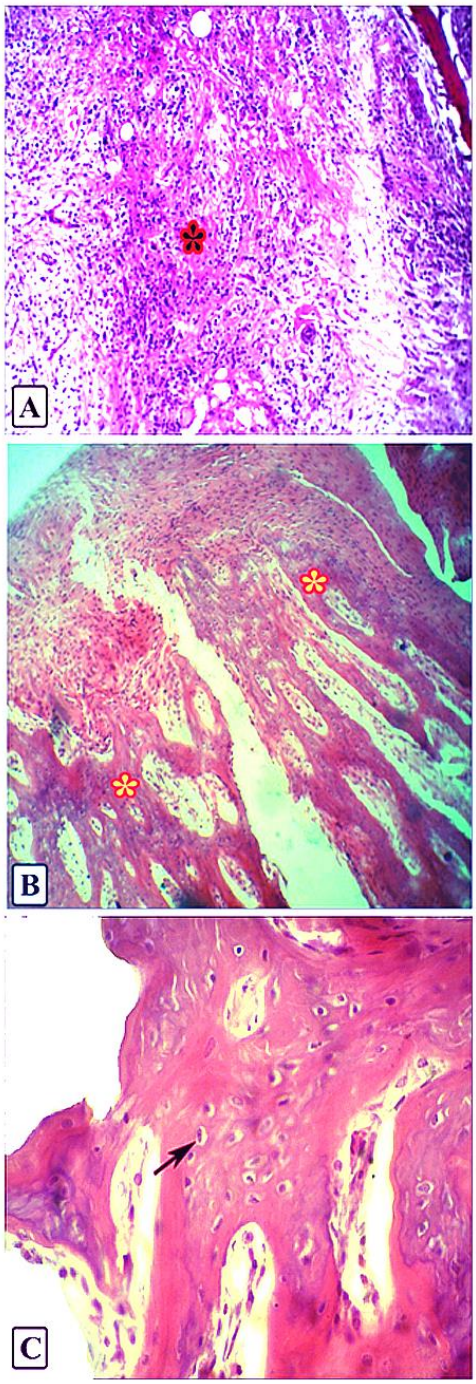

Figure 10: Paraffin sections of bony callus formation in the CG group stained by Hx & E. A: showing granulation phase at the 2nd weeks show connective tissue (collagen fibers) and proliferation of fibroblast (star). B: bony callus formation at the 1st month; with new bone formation spongy bone (star). C: callus formation at the 2nd month, showing new formation of bone with osteocytes (arrow).

revealed the application of various capacitive coupled electrical stimulation on fracture healing. They concluded that a dose-response for capacitive coupling use is an external 9-volt battery source for power generation through two electrodes attached to the skin at 60 kilohertz, and it produces 5-10 mA at the skin, and 15-20 micro-amperes at the target site, which required between 12-20 weeks of use for 24 hours a day to achieve healing (Ongaro *et al.*, 2014; Massari *et al.*, 2019).

This result was assessed by clinical and radiological examination. Basically, this development depended on the fact that mesenchymal stem cells have therapeutic potential properties in fractures that can reduce the time of healing (Bahney *et al.*, 2019) and the biophysical technique (electrical field) that accelerates the bone formation and healing, particularly in osteotomies and spine fusion (Aaron *et al.*, 2004). The biophysical methods of treatment suggested by the mentioned authors for fracture healing with electric stimulators had some disadvantages, such as skin irritation from electrode disks attached to the skin for a relatively long-term duration of treatment (12-20 weeks for 24 hours a day). Another disadvantage is that the battery is applied 24 hours a day. Therefore, they should be changed or charged every day. When this is compared to our method of treatment, which was easy without complications, no irritation or lesions appeared at the electrode attachments on the skin. We have used one 9-volt battery during the whole experimental duration.

In a previous study, it was reported that capacitive coupling has been shown to have more advantages in patients who have nonunion and complicated fractures, but it has not been shown to be helpful in osteotomies. This investigation revealed that the combination of the two therapeutic regimen applications showed remarkable augmentation to the healing and consolidation of the osteotomized bone in the second post-operative week (Barnes & Greenebaum, 2018).

In comparison to this finding, any fixative splints were not used for fracture fixation of the fibular osteotomies, assuming that the interosseus ligament between the tibia and fibula can give enough support of stability to the fractured fragments. Moreover, this trial showed faster healing of bones being subjected to the two therapeutic regimens without using any type of fixative device (Li *et al.*, 2021).

A histological and histomorphometric study of the tibial fracture in rats was carried out by (Miclau *et al.*, 2007). They have investigated that non-stabilized fractures can develop big calluses with large amounts of cartilage. They also pointed out that stabilization can suppress chondrocyte differentiation and/or proliferation, while continuous instability can induce more chondrocyte differentiation and/or enhance chondrocyte proliferation (Hsia *et al.*, 2017). In the present study, no fixative devices were used to stabilize the fracture fragments, depending on the stabilization ensured by the interosseus ligament between the tibia and fibula. A recent published study believed that movement of the fragments during the process of fracture healing stimulated the periosteum to produce more osteogenic cells to be laid down on the cortex, which produced some thickening on the proximal and distal fragments of the osteotomized fibula (Spittler *et al.*, 2021).

CONCLUSION

On the basis of the results obtained in this study, it was concluded that the combination of the autologous bone marrow graft and electrical stimulation by capacitive coupling method using 9-volt battery and 30 KHz output device has a potential effect to enhance fracture healing and obliteration of the bone defect and subsequent bone remodeling. Thus, animals treated with this combination have restored

almost normal anatomical shape with a high degree of remodeling process compared to the control group.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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تعزيز التئام عيوب العظام لدى الأرانب باستخدام مشفوط نخاع العظم الذاتي والتحفيز الكهربائي

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يمكن للتحفيز الكهربائي تعزيز تكوين العظام من خلال تحفيز النشاط الخلوي، وتعزيز تكاثر وتمايز الخلايا السلفية العظمية، وزيادة تدفق الدم الموضعي، وزيادة التعبير الجيني والبروتينات المرتبطة بالعظام. وعند دمجه مع مشفوط نخاع العظم، فإنه يساهم بشكل أكبر في التئام العظام من خلال تعزيز تكوين العظام، وتحفيز نموها، وتعديل بيئة التئامها الموضعي. تم استخدام ٣٦ أرنبًا في هذه التجربة ، وتم تقسيمهم عشوائيًا إلى ثلاث مجموعات) التحكم CG =، نخاع العظم= BM و نخاع العظم + التحفيز الكهربائي =BME ، خضعت جميع الحيوانات لعملية قطع العظم الشظوي باستخدام منشار كهربائي دوار دقيق تحت تأثير التخدير العام. بعد قطع العظم، تم إخضاع المجموعة CGللحقن عن طريق الجلد بمقدار ٢ ملل من المحلول الملحي الفسيولوجي في موقع الكسر. تم حقن المجموعة BMعن طريق الجلد بمقدار ٢ ملل من نخاع العظم الذاتي. خضعت المجموعة BME لحقن ٢ ملل من نخاع العظم الذاتي عن طريق الجلد و تم إخضاع التحفيز الكهربائي في موقع الكسر بعد ثلاثة أيام من قطع العظم، باستخدام جهاز تحفيز كهربائي متصل بالسعة مع مصدر طاقة ٩ فولت وتردد ٣٠ كيلو هرتز، لمدة ٣٠ دقيقة مرتين يوميًا لمدة أربعة أسابيع. تم تقييم هذه التجربة من خلال المتابعة الاكلينيكية والإشعاعية، والتشريح بعد الذبح، والدراسة النسيجية في الأسابيع الثاني والرابع والسادس والثامن بعد العملية الجراحية. في نهاية التجربة، أظهرت أرانب مجموعة BME علامات شفاء ممتازة بدءًا من الأسبوع الثاني بعد العملية حيث استعادت الشكل التشريحي الطبيعي تقريبًا مع درجة عالية من عملية إعادة التشكيل للعظام مقارنة بالمجموعتين CG و BM . وعليه يمكن توظيف اضافة نخاع العظم مع التحفيز الكهربائي لتعجيل شفاء كسور العظام.