New trends in fixation of femur fracture in dogs.

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Abstract: The purpose of this study is to evaluate the effects of iliac crest auto bone graft (ICBG), Hydroxyapatite (HA), and the both ICBG & HA together on the healing process of an experimentally induced femoral fractures in dogs fixed with Ender‘s nail (EN). The present work conducted on twenty apparently healthy male dogs divided equally into four groups. Group I left as a control while, groups II& III were treated with ICBG and HA respectively and group IV, treated with ICBG with HA. Clinical, radiographical and histopathological examinations were made for assessment of the healing process. This study concluded that, ICBG with HA provides the most dense callus formation with smooth uniformed contour and the earliest fracture healing with normal attitude and gait rather than the other groups.

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Key words: Dogs – Ender‘s nail (EN) – Femur fracture– Hydroxyapatite (H.A.) – iliac crest autologus boon graft (ICBG).

1. Introduction

Femoral fractures are fixed by different implant systems that included intramedullary nailing, plates and scrwes, external fixator and interlock nails (Cunningham, 2001). It is important to choose an implant system that is capable of adequately neutralizing all the disruptive forces (bending, compressive, or torsional stresses) at the fracture site and allowing bone healing to rapidly progress (Perren, 2002). Intramedullary nailing (IMP) is the most readily used system of internal fixation in small animals (Muir et al., 1993). IMP acts primarily as internal splint of medullary canal of long bone that shares loading with bones maintain axial alignment of the fracture and resists bending forces in all directions applied to the bone (Beale, 2004). IMPs are minimally traumatic as they interfere with endosteal and not periosteal callus formation (Stiffler, 2004). Ender‘s nail (EN) is an flexible unreamed intramedullary nail and relay in three point fixations in the medullary cavity, therefore some amount of micro-motion occurs between the two fragments which in turn stimulate fracture healing (Denny et al., 2000). EN can be inserted in a normograde manner (the pin is started at one end of the bone and driven toward the other end) via a trochantric, intertrochantric and subtrochantric approaches (Egol et al., 2000). EN fixation claim a number of advantages as it requires only a small incision which does not expose the fracture site so that blood loss and the risk of infection are both minimized, also it is available and has low price (Gaeber et al., 2011). A bone graft is defined as the transplantation of living bone from one location to another (Goldberg, 2001). A graft transplanted from one site to another within the same individual is called an autograft (Millis and Martinez, 2003). Osteogenesis is defined as the formation of new bone. This process occurs when viable osteoblasts and/or osteoblast precursors (stem cells) are transplanted with the bone graft (Puricelli et al., 2005). Osteoconduction refers to a bone graft or implant's ability to provide a structural framework on which host cells reconstitute. This scaffold enables the ingrowths of vessels, osteoblasts, and stem cells so that union occurs with the host skeleton (Phieffer and Goulet, 2006). Panagiotis (2005) stated that, iliac crest is the most common source of autograft bone due to its rich source of progenitor cells and growth factors, limited donor site morbidity, fair bone quantity and relative ease of harvest. Calcium phosphates such as hydroxyapatite (HA) have a chemical similarity to the mineral component of bone (Bucholz, 2002). Le Geros, (2002) mentioned that, in order to promote early new bone formation to enhance tissue strength of the grafted area, particulate HA was mixed with iliac bone marrow to rebuild stress bearing areas.

The aim of this study is to focus a light on the healing process of femur fracture with different treatment protocols. Moreover, it tried to compare between them to get the best treatment protocol with least complications.

2. Materials and Methods

This study was carried out on 20 mongrel healthy adult male dogs; at the Department of Veterinary Surgery, Faculty of Veterinary Medicine, Suez Canal University. All dogs were kept under the same managemental and nutritional regimens during the experiment. Food was withheld 6-8 hrs before the operation. Each dog was premedicated with I/M injection of chloropromazine hydrochloride in a dose of 1mg /kg. The site of operation (lateral aspect of the right thigh) was aseptically prepared, and then general anesthesia was conducted by I/V injection of thiopental sodium 2.5% solution until the main reflexes were disappeared. The right femur of all animals was experimentally fractured transversally by using a wire saw in the mid-diaphysis under constant cooling with a 0.9% NaCl solution with antibiotic these is in (Fig. 1).

Study design

The dogs in this study were divided into 4 equal groups. The fractured femur in dogs of group I (control) was fixed with Ender nail (EN) using a slow speed drilling (Fig. 2), while in group II it was fixe with EN and the iliac crest autologus bone graft (ICBG)(Fig. 3 A& B). The fracture in animals of group III was fixed with EN in addition to Bioosteo; Hydroxyapatite (HA) granules, manufactured by biomecanica Ind. productos orthopedicos LTDA, (Fig. 4), while it was fixed with EN with both ICBG and HA granules in animals of group IV.

Clinical examination

Dogs were clinically observed daily during the study period for recording any postoperative complications. For assessing functional disorders of the affected limb during walking, a scale with five levels 0-4 was used according to Fox et al. (1995) (Table 1).

Radiographical examination

Radiographs were taken at 3, 6, 9, 12 and 15 weeks postoperatively.

Histopathological examination

The specimens were taken after euthanasia of the dogs, using overdose of thiopental sodium at the same time of the radiographic examination. The specimens were fixed in 10% neutral buffered formalin solution and prepared for light microscopic examination by processing though graded alchols and embedding in paraffin blocks. 5µm thick sections were cut and stained by H&E.

Statistical Analysis

Preliminary data for all experiments were statistically analyzed using the appropriate analysis of variance using Tukey test to compare the degrees o f lameness in all groups. A Computer program software SPSS version 19.0 was used to analysis the data of these experiments. Least significant different (L.S.D) at 5% level was used separately to evaluate the response of this trait.

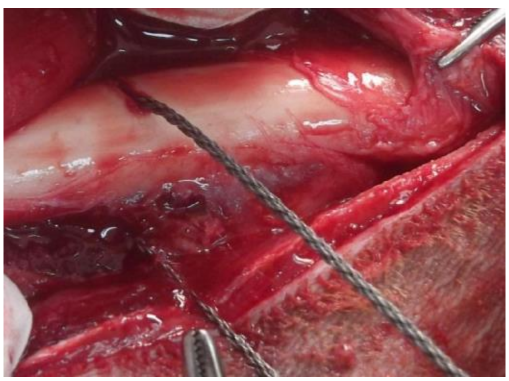

Fig. (1): Showing induction of a mid-diaphyseal femoral fracture using a wire saw.

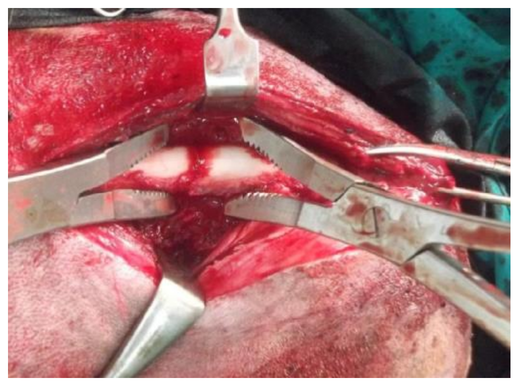

Fig. (2): Showing complete reduction of the fractured bone after insertion of the nail.

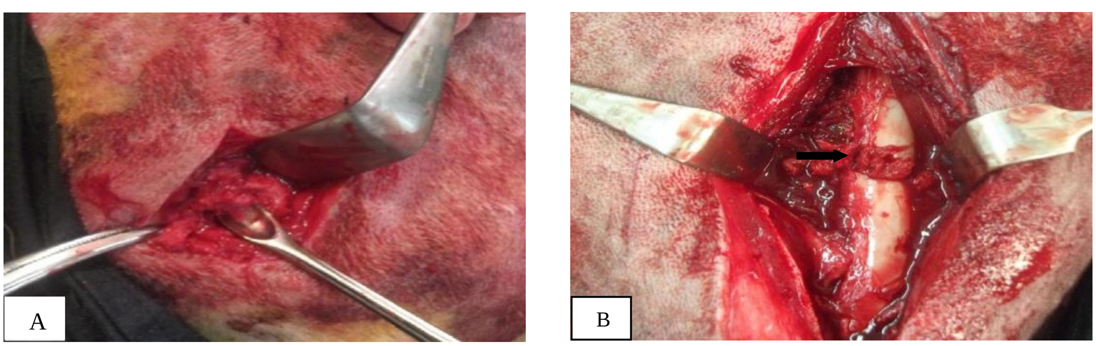

Fig. (3): A. Showing collection of iliac crest autologus bone graft. B. Showing packing of the autologus bone graft (black arrow) into the bone defect.

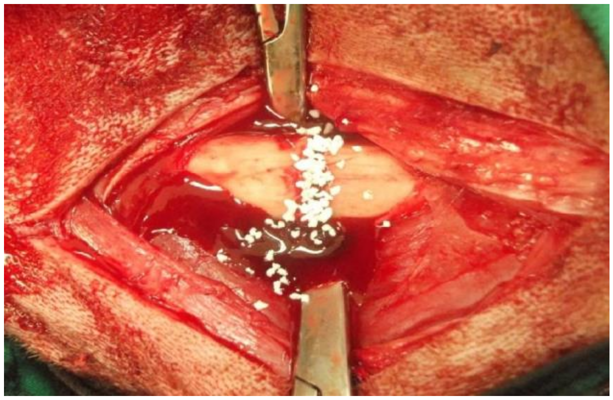

Fig. (4): Showing packing of hydroxyapatite into the bone defect.

Table 1: Classification of walking scales during the convalescent period.

Degree	Description
0	Normal attitude in station and walking without lameness.
1	Difficulties in walking, especially at rapid carriage with fine lameness.
2	Difficulties in walking, intermittent lameness in rapid walking.
3	Evident lameness at every step.
4	The leg pulls out of support in station and in walking with intense pain.

3. Results

Animals treated with autologous bone graft (groups II& IV) developed a sever lameness with a signs of dragging the hind limbs during the early 6 post operative days. Animals of groups II, III& IV showed partial weight bearing on the operated legs during walking and intermittent lameness in rapid walking at the 6th week. While at the 9th week, there were clinically active with normal attitude and no signs of lameness or muscular atrophy. In group (I), the first trials by the dogs to put light weight on their operated legs were during the 12th to the 14th days, while better functional legs usage were seen during the 9-12th weeks. Normal attitude in station and walking were observed at the 15th week post operatively (Fig. 5).

Radiographic interpretation

All animals showed progressively dense periosteal reaction at the 3th week. At the 6th week, the periosteal reactions were getting denser in animals of group I, with presence of periosteal callus bridging between the two fracture ends in animals of group II& IV, while the size of the external callus was decreased in animals of group III (Fig. 6). At the 9th week, the callus was declined in the size in animals of groups II& IV. The animals of group III revealed complete disappearance of the fracture line reforming their normal shape and contours (Fig. 7). The animals of group IV revealed even density and smooth borders of the healed bones at the 12th week (Fig. 8). During the 12th to the 15th weeks, the animals of group I revealed disappearance of the fracture line and the borders of the healed fractured bone were uneven (Fig. 9).

Histopathological findings

Three weeks post-operative, the external and the internal callus of the control group consisted of a loosely haphazard arranged fibrous connective tissue that was admixed with many newly formed blood capillaries (Fig. 10). A moderate number of inflammatory cells were seen, mainly macrophages, lymphocytes and some plasma cells. The fracture gap of bone graft group was impacted with very thin bony trabeculae of cancellous bone, and it was covered with externally and internally with granulation tissue calluses (Fig. 11). Animals of group III showed a moderately dense external callus that was composed of interwoven bundles of fibrous connective tissue intermingled with newly formed capillaries, irregular areas of cartilage, and small areas of newly formed bone tissue (Fig. 12). Animals of groups II& IV showed similar changes of bone graft in addition to a moderately dense external callus with small areas of cartilage. Six weeks post-operative, control group had a moderately thick layer of granulation tissue covering the external and internal surfaces of the fracture with focal cartilaginous areas. The cancellous bone filling the fracture gap, in animals of groups II & IV, started to show areas of fusion with the pre-existing bone at the level of the cortex and medulla. The calluses of the group III were mostly replaced and composed of cartilaginous matrix which bridging the two fracture ends (Fig. 13). On the ninth week, the control group's callus became thicker containing dense fibrous connective tissue admixed with multifocal to coalescing areas of cartilage in addition to ossified areas (Fig. 14). The fracture gap of the group II contained anastomosing thin bony trabeculae fusing the fractured parts. However, animals of group III showed disappearance of calluses, the cortex at the both sides of the fracture had mature compact bone, and moderately regenerated bone marrow (Fig. 15). The medullary cavity of the Group IV showed a moderately thick trabecular bone conjugating the fractured ends, and slightly regenerated bone marrow. On the twelfth and fifteenth weeks, the two fracture ends in control group were joined cancellous bone. Groups II and IV showed normal thickened bone cortex that joined with a lamellar bone, and moderately regenerated bone marrow (Fig. 16). In animals of group III, the bone retained its normal architecture and contour, with normal bone marrow (Fig. 17).

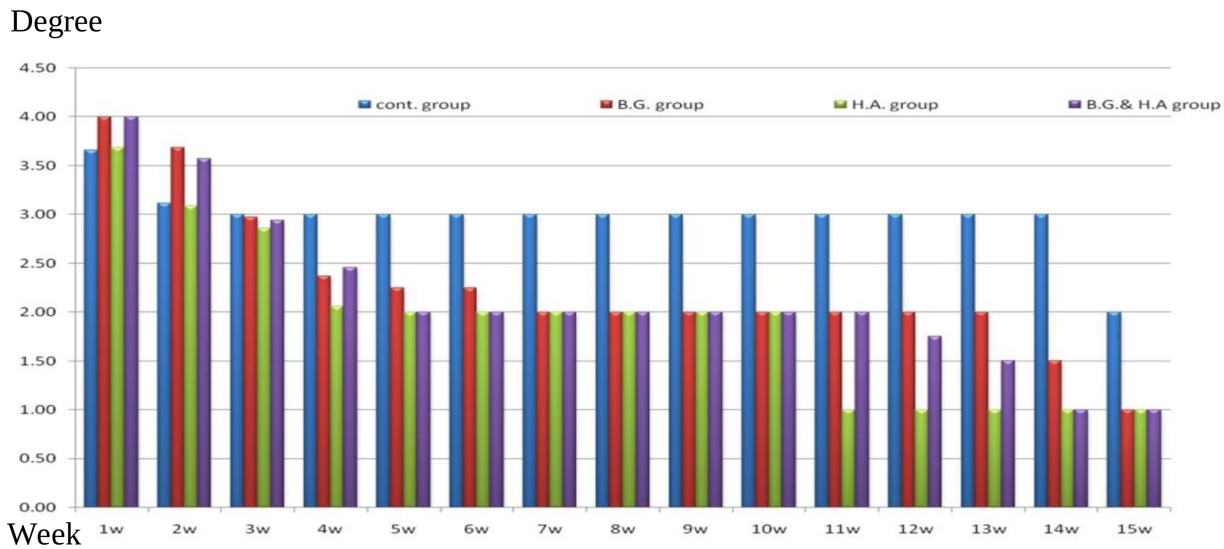

Fig. (5): Degrees of lameness (mean ranks).

Fig. (8): Anterio-posterior radiograph of a dog of group (IV) twelve weeks post-operatively showing even density and smooth borders of the healed bone.

Fig. (9): Anterio-posterior radiograph of a dog group (I) fifteen weeks post-operatively showing the fractured bone regained its normal contour with disappearance of the external callus. Note: the density and the border of the healed fractured bone was uneven.

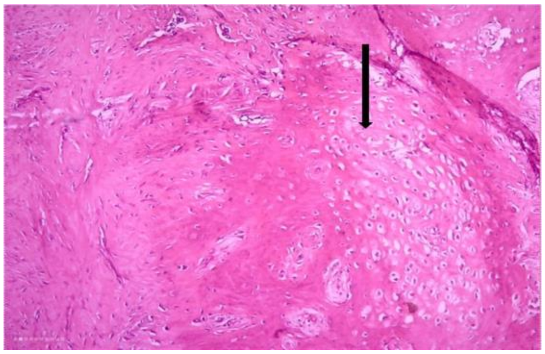

Fig. (12): A cartilaginous area was noted within the external callus (arrow), 3 weeks post-operative, Group III. H&E 100X.

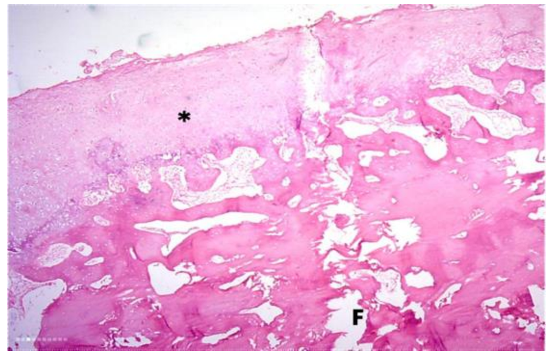

Fig. (13): The external callus is completely replaced by cartilage (asterisk), note the fracture gap (F), 6 weeks post-operative, Group III. H&E, 40X.

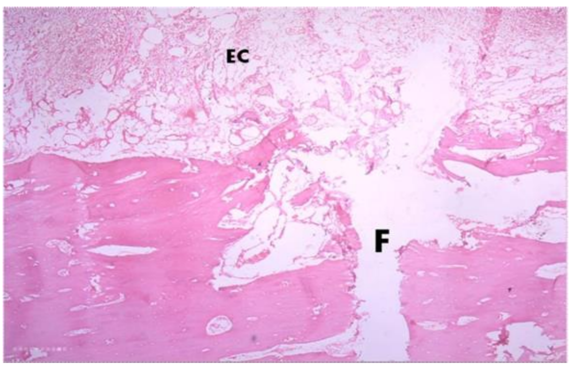

Fig. (10): The fracture line (F) is covered with a moderately cellular external callus (EC), 3 weeks post-operative, Group I. H&E, 40X.

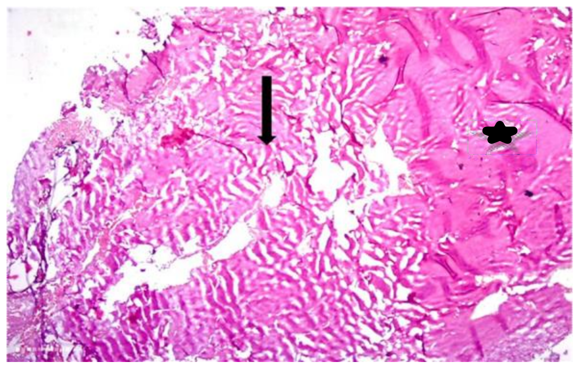

Fig. (11): The fracture gap is filled with cancellous bone (arrow) note the bone cortex (star), 3 weeks post-operative, Group II. H&E 40X.

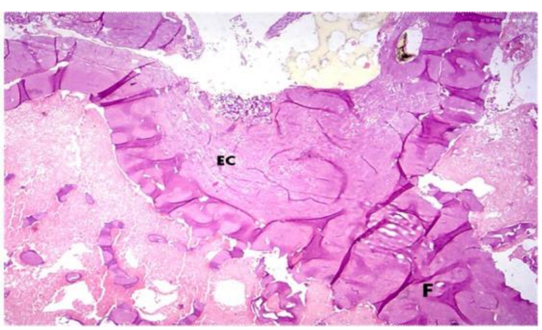

Fig. (14): The external callus is formed from dense connective tissue with newly bone formation and cartilage (EC) that extends to the fracture gap (F), 9 weeks post-operative, Group I. H&E, 40X.

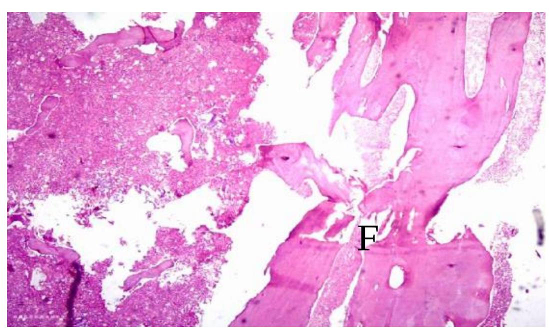

Fig. (15): The bone cortex is almost healed with compact bone, note the fracture line (F), and a moderately regenerated bone marrow, 9 weeks post-operative, Group III. H&E, 40X.

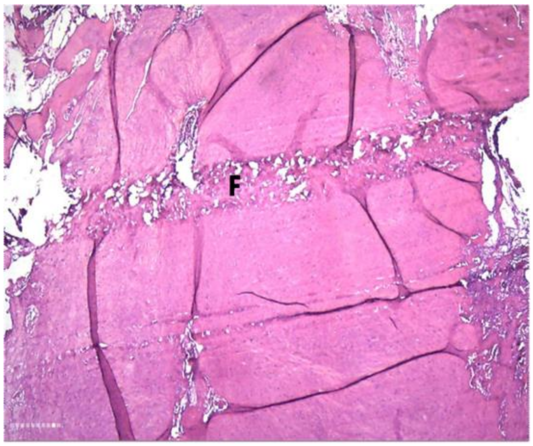

Fig. (16): Newly bone formation is filling the fracture gap (F) and tightly connected the sides of the fractured cortex, 12 weeks post-operative, Group II. H&E, 40X.

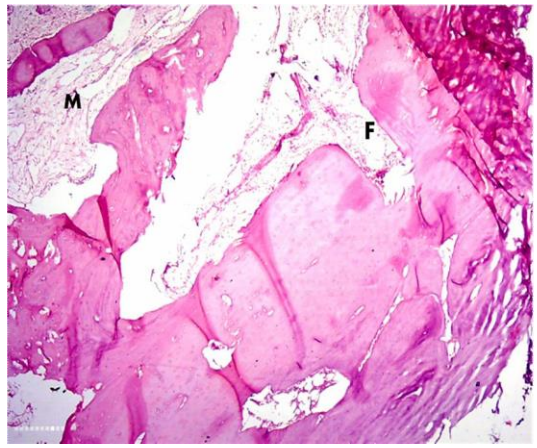

Fig. (17): The bone cortex retained its normal contour and thickness , note the a small remaining fracture line (F) and fully regenerated bone marrow (M), 12 weeks post-operative, Group III. H&E, 40X.

4. Discussion

In the present study ENs were used, which were not rigid and relay in three point fixations in the medullary cavity, these features stated by Denny et al., (2000), whom referred that the formation of the callus required a certain amount of movement at the fracture site. Failure of EN removal was noticed at the end of this study which attributed to that, the nail was flexible bended and the callus at the point of nail insertion was formed. Similar remarks were referred by Durall and Diaz (1996). Bone autografting was utilized with EN fixation in the present study to enhance rapid bone healing as suggested by Puricelli, et al., (2005), whom mentioned that bone autograft is the only graft material that has osteogenic, osteoinductive, and osteoconductive properties. In the first two weeks, animals of groups (II and IV) showed significant increase in the degree of lameness with dragging the hind limbs compared to animals of groups (I and III). Similar findings were reported by Panagiotis (2005), who owed that to the collection of iliac crest bone graft from the same animal. From the 4^{th} to the 15^{th} week, animals of groups (II and IV) showed significant decrease in the degree of lameness compared to animals of group (I). The same result was reported by Phieffer and Goulet (2006), whom attributed this to the osteogenic, osteoinductive, and osteoconductive abilities of autogenous bone graft. HA has shown good osteoconductive ability when used as bone graft substitute in this study. Similar findings were mentioned by Bucholz, (2002), who advocated the use of HA in filling osseous defects, cyst cavities and alveolar clefts. Radiographicaly, a dense periosteal callus was present in the control group at the 6^{th} week which begun to decline in size at the 9^{th} week. A faint fracture line was visible at the 6^{th} week which completely disappeared at the 15^{th} week and the bone started to reform itself. These results were agreed with the results of Sande (1999). A large bridging callus in animals treated with autologous bone graft was seen at the 6^{th} week which gradually declined with complete disappearance of the fracture line at the 12^{th} week. These results were in agreement with Jones (1998). Periosteal callus of even density and uneven borders were found with complete disappearance of the fracture line at the 9^{th} week post operatively, reaching the normal shape and contour of the bone at 12^{th} week in HA group, similar findings were discussed by Thor et al. (2007). The fracture line was completely disappeared at the 9^{th} week in group treated with both iliac crest BG and HA together while, at the 12^{th} week the bone regained its normal contour. These findings were in accordance to Jones (1998). This study revealed that, the periosteal callus appeared radiographically smaller in HA treated animals compared to those treated with autologous bone graft. Similar features was recorded by Thor et al. (2007), whom came to the conclusion that the HA acted as a scaffold into which new bone could grow but were not capable of inducing connective tissues to undergo metaplasia into chondrocytes and osteocytes.

The results of histopathologic examination on the 3rd week revealed a presence of external and internal callus at the fracture line in control group. In Groups treated with ICBG and with both ICBG and HA, the fracture gaps were impacted with very thin bony trabeculae with numerous large empty spaces (bone graft) that resemble the structure of the flat bone. While, in group treated with HA there was a scattered small to medium sized irregular areas of cartilage and small areas of bone tissue throughout the external callus. Similar findings were reported by Thor, et al. (2007). On the 6th week, histopathologic examination revealed that the external surface of the fracture was covered with a moderately thick layer of dense granulation tissue containing focal cartilaginous areas in control group. In groups treated with ICBG and with both ICBG and HA, the fracture gaps were filled with cancillous bone which started to show areas of fusion. While, in group treated with HA the external callus was mostly replaced and composed of cartilaginous matrix bridging the two fracture ends. Similar findings were obtained by Servin et al. (2011). By the 9th week, the external callus became thicker containing dense fibrous connective tissue admixed with of cartilage in addition to ossified areas in control group. In ICBG group, there was anastomosing thin bony trabeculae fusing the fractured parts. HA group showed a good healed fracture that was evident by disappearance of external and internal callus. While, in group treated with both ICBG and HA the medullary cavity showed a moderately thick trabecular bone and slightly regenerated bone marrow. This result was matched with those of Servin, et al. (2011), who demonstrated that the fibrous and cartilaginous tissues remained unmineralized in the control group, whereas the entire callus was calcified in the treated groups. At the15th week, animals of control group revealed the fracture gap was filled also with cancellous bone. On contrary, groups treated with ICBG, HA and with both ICBG and HA revealed complete healing which was evident by normal thickened cortex and the medullary cavity was also retained its normal anastomosing trabiculea. Results of this study were agreed with those of Jochymeka and Gala (2007), whom stated that the last signs of bony union are the redevelopment of the normal trabecular pattern and restoration of the continuity of the medullary cavity and cortex.

It was concluded that, ICBG with HA provides the most dense callus formation and the earliest fracture healing with normal attitude and gait rather than the other groups.

5. References

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