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Research Article

The Effect of the Administration of Bovine Xenograft on the Accelerated Healing of Femoral Fracture in Domestic Dogs

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Abstract: Bovine xenografting is a method of grafting bovine bones to other species. This study aimed to determine the effect of the administration of bovine xenografts on accelerated healing in domestic dogs with femoral fractures. This study used a total of 4 dogs (two individuals as the treatment group and other two as the control group) diagnosed with diaphyseal femoral fractures. The bovine xenograft in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)$) was synthesized in this study. The femur fracture surgery was carried out using the open reduction and internal fixation (ORIF) method, followed by the administration of 10 grams of Hydroxyapatite powder from the bovine bone in the fracture area, in the treatment group. The control group was not treated with Hydroxyapatite. Radiographic examinations were carried out on days 0, 14, and 45 post-operation. The parameters observed included the formation of radiopaque mass on the radiographic imaging, indicating the formation of calli around the fracture area. The radiographic examination showed that in the treatment group, the radiopaque mass was apparent on day 14 post-operation while it was not observable in the control group. On day 45 post-operation, the mass was more clearly visible in the treatment group, while it was barely visible in the control group. This research indicated that bovine xenograft has the potential to accelerate the fracture healing process in domestic dogs

Keywords: bovine xenograft, dog, fracture, hydroxyapatite

INTRODUCTION

A fracture is a discontinuity of a bone. It potentially caused damage to the surrounding soft tissue (Witoko et al., 2021). The causes of fractures in animals are varied and include trauma due to accidents, animal activities, osteoporosis, and gunshot wounds. Additionally, bone abnormalities can occur due to pathological conditions such as neoplasia, tumours or metastases (Abd El Raouf et al., 2017; Aminatun et al., 2019; Tercanlioglu & Sarierler, 2009). Characteristics of fractures can be affected by animal factors, such as age, body weight, breed, sex and activity (Asma et al., 2014; Talaat et al., 2022). Fractures in small animals, such as dogs and cats, are often reported to occur in the long bones, and almost half of these fractures occur in the femur area (Asma et al., 2014). Piermattei et al. (2017) reported that fractures in the femur area was one-fifth of all fracture cases and 45-47%of all long bone fractures in small animals. Femur fractures often occur at a young age in the distal and proximal areas, while in adult dogs, fractures occur in the metaphysis and diaphysis areas of the femur (Simon et al., 2010). German Shepherd breed was at the largest proportion of fracture incidence, at 85%, followed by the German Pinscher breed (5%) and the White Griffon breed (5%) (Abd El Raouf et al., 2017).

Animal fractures are a problem not only for the animal itself but also for the owner (Talaat et al., 2022). Methods have been developed to treat fractures in animals. Reconstruction methods include the use of intramedullary pins, bone plates, external skeletal fixation (ESF), screws, and interlocking nails (ILNs) (Abd El Raouf et al., 2017). The choice of method is influenced by factors, such as equipment availability, fracture location, age, fracture configuration, soft tissue attachment around the fracture site and the condition of soft tissue around the fracture area (Asma et al., 2014; Das et al., 2020). The aim of fracture treatments is to restore bone continuity, alignment and functionality in a shorter time (Tercanlioglu & Sarierler, 2009).

A method to improve the healing process of bone fractures is by using bone graft. Bone grafting is a technique in which parts of bone are transferred from a donor to a recipient (Joshi et al., 2010). Bone grafts not only support accelerated healing of fractures but can also be used in treating other orthopaedic disorders such as arthrodesis, osteomyelitis, delayed union, nonunion, and comminuted fractures (Joshi et al., 2010). A good bone graft needs to meet the criteria of mechanical, osteo inductivity, osteo genicity, and osteo conductivity stand points (Joshi et al., 2010). One bone graft that potentially meets these criteria could be the bovine xenograft that contains 93% hydroxyapatite compounds ($Ca_{10}(PO_4)6(OH)2$) (Budiatin et al., 2022). Bovine xenografts were reported to contain hydroxyapatite compounds similar to that contained in human bones. It is also reported to have suitable osteoconductive properties, accelerate the process of osteoblast cell colonization and facilitate as a medium for osteoblast cell attachment, thereby accelerating the growth of new bone cells and fracture healing (Budiatin et al., 2022; Marzook et al., 2022; Sudimartini et al., 2019).

However, whether the administration of bovine bone graft in dog fractures resulted in similar healing properties as that in humans remained unknown. The aim of this research, therefore, was to determine the effect of the administration of bovine xenografts on the fracture healing of the femoral bone of domestic dogs. This study specifically looked at the callus growth and the acceleration of fracture healing.

MATERIALS AND METHODS

Animals

This research was conducted from September 2017 to December 2018. A total of four village dogs were used in this study. These dogs consisted of two controls (K1 and K2) and two treatments (P1 and P2). The dog used in this study was approximately one year old and was diagnosed by a veterinarian as having a fracture in the diaphysis of the femur. Supporting radiographs of the fracture area were obtained to determine a definitive diagnosis of the fractured animals.

Bonegraft Synthesis

The source of the bone graft used in this research was bovine bone powder. The beef bones used were long bones, namely, the humerus, femur, radius-ulna and tibia-fibula. The manufacturing process begins with washing and boiling as a step to remove fat and remaining meat that is still attached. Boiling was carried out for 7 hours at a temperature above 90°C. After boiling, the beef bones were cleaned and dried using sunlight for 14 days. The dried beef bones were cut into smaller pieces and ground using a grinder machine.

In order to synthesize the hydroxyapatite compound ($Ca_{10}(PO_4)_6(OH)_2$), a calcination process was performed at temperatures of 900°C and 1000°C for 5 and 6 hours so that the carbonate ions were removed and did not interfere the synthesis process. The resulting a(OH)2 compound was mixed with a phosphoric acid solution (H3PO4) using a magnetic stirrer at 300 rpm/minute (Suryadi, 2011). The homogenized solution was heated to a temperature above 90°C for one hour and cooled for 24 hours at room temperature to produce a precipitate. The hydroxyapatite precipitate was filtered using filter paper and dried in an oven at 120°C for 5 hours, the latter process was repeated until the weight did not change.

Bone Graft Iimplantation

The fractured animals underwent surgery using the open reduction and internal fixation (ORIF) technique. After the animal had fasted for approximately 12 hours, the hair was shaved in the area around the fracture. An intravenous catheter was installed to administer Ringer's lactate fluid. Atropine sulphate was used as premedication at a dose of 0.04 mg/kg bw (body weight) subcutaneously. Anaesthesia was induced with 1-3 mg/kg bw of Xylazine and 10-15 mg/kg bw Ketamine given intramuscularly. A 2% Isoflurane inhalation was used to maintain anaesthetic states.

The animals were incised on the cranioventral of femur to expose the subcutaneous area and superficial fascia. The superficial fascia was retracted, and the fascia lata was explored. The biceps femoris was pulled caudal to the femur, while the vastus lateralis, intermediate muscle and fascia latawere pulled toward the cranial of femur so that the fracture area of the femur was exposed. The pin with a diameter of 2 mm was used, installed via the retrograde method, followed by the administration of 5 grams of calcium hydroxyapatite powder, which was poured into the space between the fractures in the diaphyseal area of the femoral bone. Following the completion of the treatments, the fascia lata and biceps femoris muscles were sutured using a 2-0 chromic catgut with a simple continuous pattern. The subcuticular suture was performed using2-0 chromic catgut suture. After surgery, the animals were orally administered with Clavulanic acid (12.5 mg/kg bw) for 7 days and Ketoprofen (2 mg/kg bw) for 5 days. Hematodin™ was administered at a dose of 0.5-2 ml/kg bw for 3 days. The post operative observation was carried out to ensure the animals recovered from anaesthesia uneventfully.

Radiographic examinations

Radiographic examinations were carried out on day-0, day-14 and day-45 after surgery. The parameters observed included callus formation around the fracture area in the control and treatment groups, indicated by the presence of radiopaque mass observable on the radiographic imaging (Budiatin et al., 2022).

Data Analysis

The results of the radiographs of the femoral bone area were subjected to descriptive analysisofthe callus growth around the fracture area.

RESULT AND DISCUSSION

The study investigated the effect of the administration of Hydroxyapatite from bovine bone on the healing process of fractured femurs in dogs. The analysis was performed using radiographic imaging. The first radiographic examination (**Figure 1**) was carried out on the day zero after surgery, to determine the position of the intramedullary pin and bone repositioning. It indicated that the positions of the pin and the bone repositions were correct. No healing process in the form of callus formation was observable. (Harwood et al., 2010) reported that an inflammatory process began to occur only 48 hours after the fracture, characterized by the presence of a hematoma, which would disappear after a week.

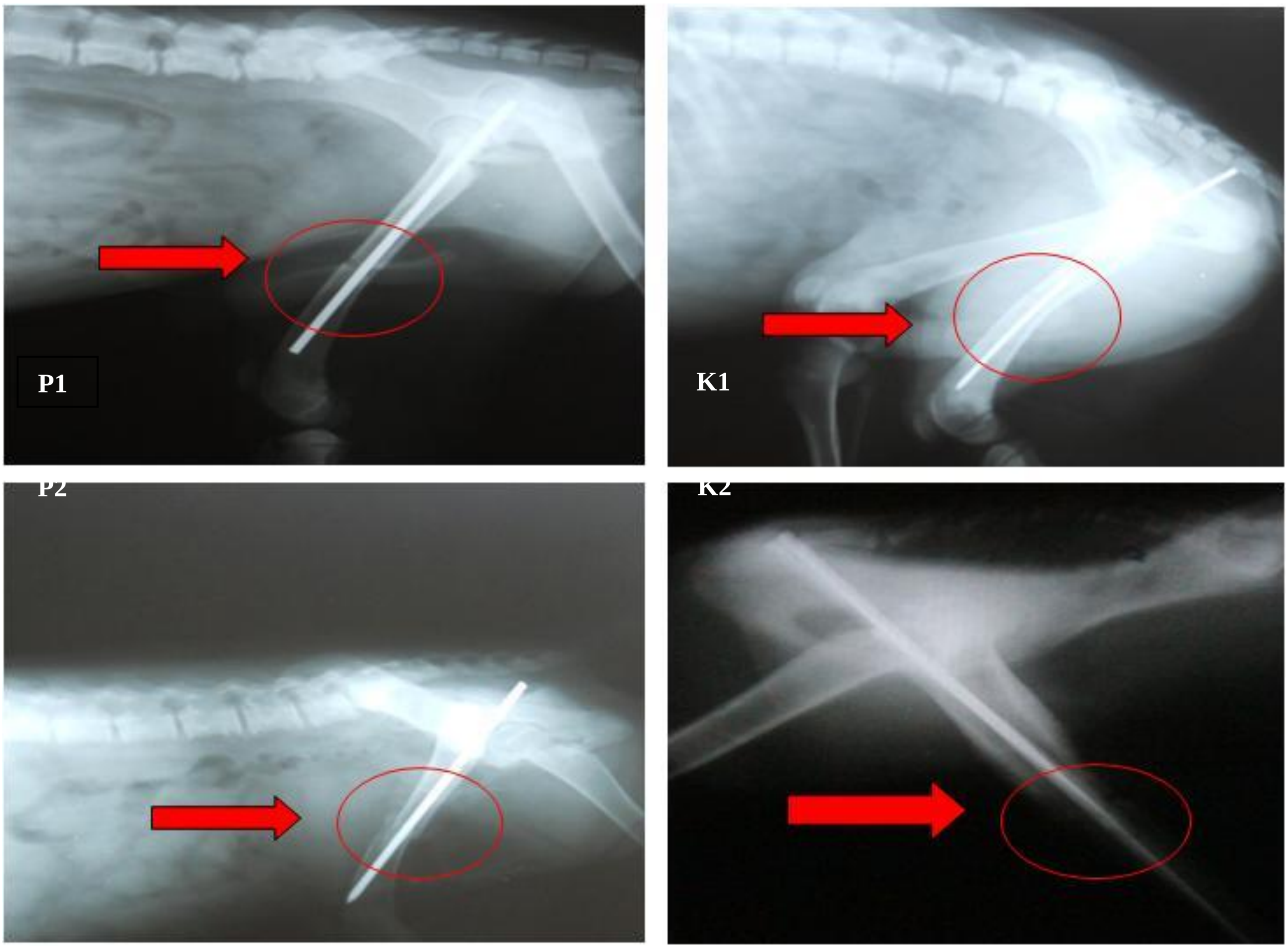

Figure 1. The radiographic imaging on day 0 of the fractured femoral area of dogs treated with Hydroxyapatite (left, P1 and P2) and the control group without the treatment (right, K1 and K2), showingradiotranslucencyin the area of the fractured space and no visible callus formation in both groups.

The second radiographic examination (**Figure 2**) was taken on day 14 post-operation to determine the fracture healing process, which was characterized by the growth of callus around the area of the fractured femur. Radiographic imaging in the treatment group showed that a radiopaque mass began to be observable in the area around the fractured bone indicating the callus grow thin the area affected. The radiopaque mass, however, was not apparent in the control group. This finding indicated that the healing process could have been accelerated by the Hydroxyapatite in the treatment group, while in the control group, the process was slower and had not been observable radiographically. Studies have reported that the fracture healing process, mineralization and calli formation occur between the first and third weeks (Graham, 2007; Uddin et al., 2017; Sudimartini et al., 2019; Das et al., 2020).

The third radiographic examination (**Figure 3**) shows that on day 45 post-operation, a clear radiopaque mass was apparent to be concentrated around the fractured area of the femur in the treatment group, joining the two parts of fractured sites. On the contrary, in the control group, the appearance of radiopaque mass was minimal and comparable to that in the treatment group on day 14. In a normal process of fracture healing in dogs, Asma et al. (2014) reported that a radiographic imaging taken on day 35 post-operation showed only some callus formation, resembling what appeared in day 14 post-operation of the treatment group of the current study, indicating that the Hydroxyapatite could have played a role in accelerating the fracture healing in dogs. Sudimartini et al. (2019) used Hydroxyapatite from pig bone and reported that the accelerated healing of fracture in dogs in the treatment group began to be observable on week four post-operation, using radiographic imaging. Further, (Aminatun et al., 2019) reported a study that indicated that the Hydroxyapatite from bovine bone tended to be superior compared to that from fish bone, but both were able to accelerate the fracture healing process in rat models, compared to that in the control group. A comparison of the potential of Hydroxyapatite from bovine versus pigs in accelerating fracture healing warrants further study.

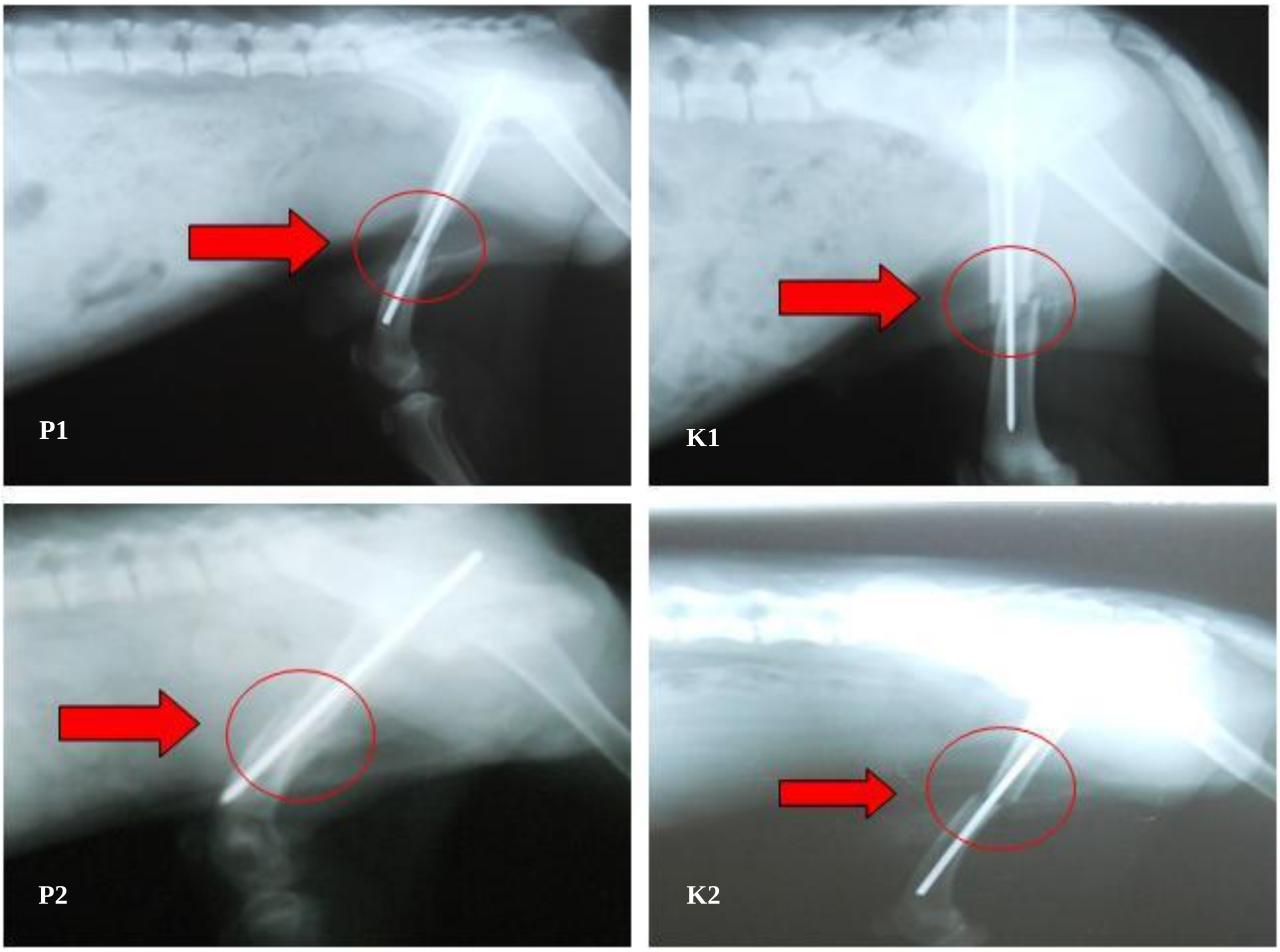

Figure 2. The radiographic imaging on the day14 post-operation in the treatment group (left, P1 and P2),showsradiopaque mass indicating callus formation around the fracture area, while the control group shows no visible radiopaque mass (right, K1and K2).

Pin migration was observable in this study in the second and the third radiographic examinations in both groups. Kreszinger et al. (2020) and Uddin et al. (2017) argued that it may occur due to inequalities between the diameter of the pin and the medullary canal or unstable fracture fragments. Additionally, excessive movement of the hip during the healing process was reported to cause pin migration in a fracture study in a dog (Witoko et al., 2021). These need to be taken into account when conducting research using intramedullary pin installation in animal models.

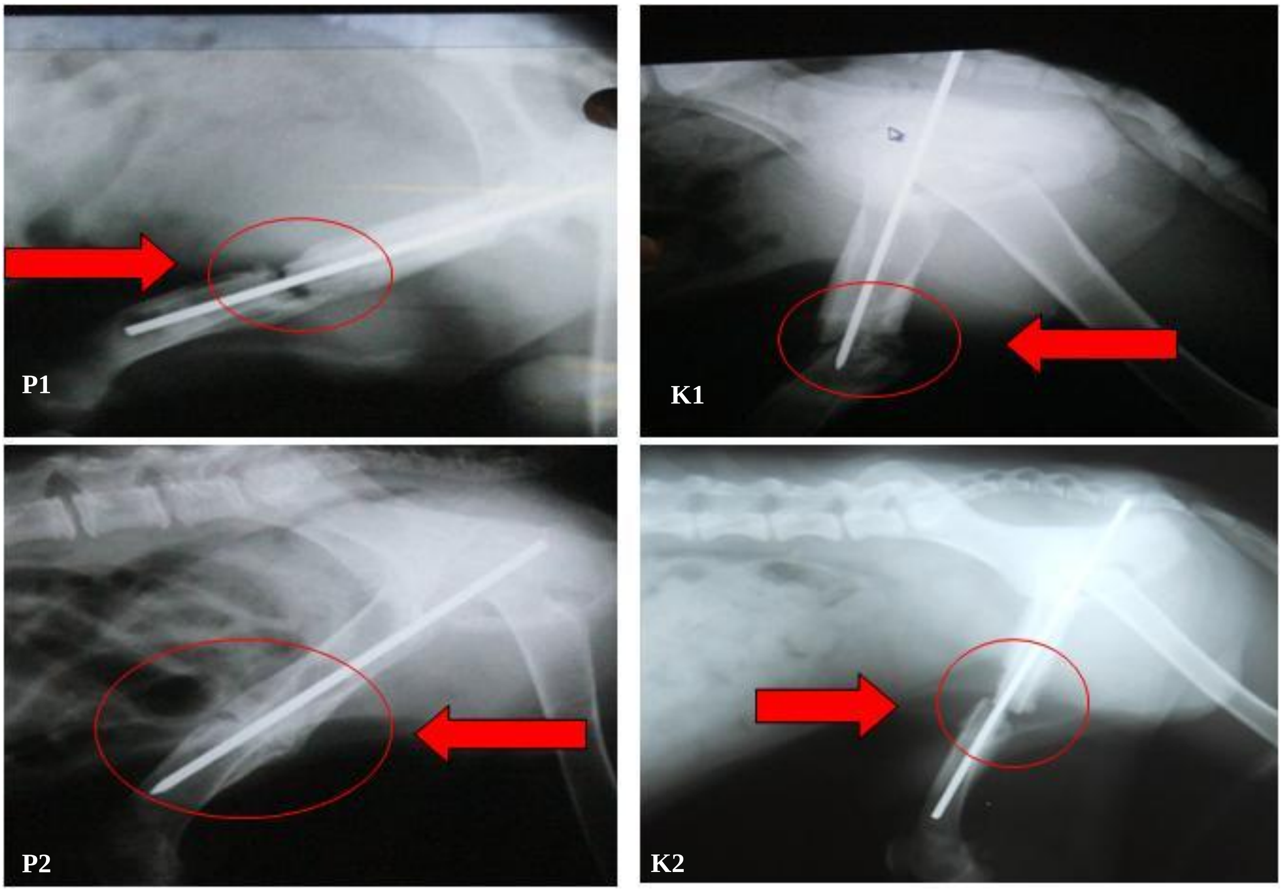

Figure3. The radiographic imaging on day 45 post-operation in dogs with femoral fractures treated with Hydroxyapatite (left, P1 dan P2) shows the clear visibility of radiopaque mass connecting two parts of the fractured bone. Contrarily, the radiographic imaging of the control group shows a barely visible radiopaque mass on the fractured area of the femur (right, K1 and K2).

CONCLUSION

The administration of bovine xenografts in the fracture treatment was able to accelerate the fracture healing process in dogs.

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