



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2026; 11(4): 240-246

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www.veterinarypaper.com

Received: 21-02-2026

Accepted: 24-03-2026

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Clinical efficacy of demineralised bone matrix as xenograft for healing of comminuted long bone fracture in dog

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DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i4d.3214>

Abstract

The present clinical study was conducted to evaluate the efficacy of demineralised bone matrix (DBM) xenograft in enhancing fracture healing in dogs with comminuted long bone fractures. Out of 56 dogs presented with fractures to the Veterinary Clinical Complex, Post Graduate Institute of Veterinary and Animal Sciences, Akola, 16 dogs confirmed with comminuted long bone fractures were selected for the study and randomly divided into two equal groups. Dogs in Group I were treated with appropriate internal fixation techniques alone, whereas dogs in Group II received internal fixation along with implantation of DBM xenograft at the fracture site.

Physiological assessment, including rectal temperature, heart rate and respiration rate, were elevated preoperatively and gradually returned to normal physiological limits postoperatively in both groups. Clinical evaluation such as degree of lameness, swelling over fractured limb, pain sensation and weight-bearing improved progressively during the study period, with comparatively better clinical improvement observed in DBM-treated dogs. Haematological estimations, including haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count, remained within normal physiological limits, indicating the biocompatibility of DBM xenograft.

Biochemical estimations, including serum calcium, phosphorus and total protein, showed non-significant variations. However, serum alkaline phosphatase activity was significantly higher in Group II, indicating enhanced osteoblastic activity and accelerated fracture healing. Radiographic evaluation revealed earlier fracture line obliteration and restoration of cortical continuity in DBM-treated dogs compared to the control group.

The study concluded that demineralised bone matrix xenograft is a safe and effective adjunct to internal fixation and enhances fracture healing in dogs with comminuted long bone fractures.

Keywords: Demineralised bone matrix, xenograft, fracture healing, comminuted fracture, dogs, internal fixation.

1. Introduction

Dogs have been closely associated with humans for over 30,000 years, serving various roles ranging from working animals to companions, thereby strengthening the human-animal bond across diverse sociocultural settings (Elias Plata, 2019) [5]. In modern society, dogs are valued not only for their utility but also for their emotional companionship. Consequently, conditions causing pain and disability in dogs, such as fractures, significantly impact both animal welfare and owner wellbeing.

The skeletal system provides structural support, facilitates locomotion, and serves as an attachment site for muscles. Bones are subjected to physiological forces during normal activity; however, exposure to excessive forces such as automobile accidents or falls may exceed the structural strength of bone, resulting in fractures (Yadav *et al.*, 2016) [18]. Long bone fractures are among the most commonly encountered orthopaedic conditions in veterinary practice, with the femur being the most frequently affected bone, followed by the tibia, radius-ulna, and humerus (Kushwaha *et al.*, 2011; Bishnoi *et al.*, 2013; Muhamad *et al.*,

2021) [10, 3, 11].

The primary objective of fracture treatment is to restore anatomical alignment, structural stability, and functional limb use (Johnson *et al.*, 1998) [9]. Various fracture management techniques, including internal fixation, external skeletal fixation, and external coaptation, are employed depending on fracture type and patient factors. Internal fixation techniques such as intramedullary pinning, plate fixation, and locking compression plates are widely used for fracture stabilization (Harasen, 2003) [8].

Complex comminuted fractures face complications such as delayed healing or non-union. These issues often arise due to infection, instability, poor fragment alignment, and insufficient blood supply—challenges that are well-recognized in veterinary studies. A comminuted fracture includes multiple fracture lines, producing at least one completely separated medium-sized fragment (Candela *et al.*, 2025).

Management of comminuted fractures of long bones requires not only effective internal fixation but also the preservation of the bone's structural integrity. The loss of bone fragments or the gap formation at the fracture site is undesirable, as it may result in complications such as mal-union, delayed union, or non-union. While fixation method alone may provide stabilization, they are often insufficient for complete fracture repair. To promote proper healing, bone grafts are frequently utilized. These grafts serve as a structural support and a scaffold, facilitating the growth of new bone tissue and bridging the gap or defect at the fracture site.

Autografts are considered the gold standard for bone grafting due to their osteogenic, osteoinductive and osteoconductive properties. However, limitations such as donor site morbidity and limited availability necessitate alternative grafting options (Ragety *et al.*, 2011) [14]. Demineralised bone matrix (DBM), first described by Urist in 1965 [17], has demonstrated significant osteoinductive potential by stimulating differentiation of mesenchymal stem cells into osteoblasts. Demineralization enhances the release of bone morphogenic proteins (BMPs) which play a crucial role in bone healing. (Riley *et al.*, 1996) [15]. BMPs stimulate the transformation of local undifferentiated mesenchymal cells into osteoblasts (osteoiduction), while the collagenous framework of DBM provides a scaffold, allowing newly regenerated tissue to migrate into the site (osteoconduction). In addition to promoting osteogenesis, certain BMPs and DBM particles have shown potential in aiding the repair of osteochondral defects. (Amin Bigham-Sadegh *et al.*, 2013) [2].

Although DBM has been extensively used in human orthopaedic surgery, its application as a xenograft in veterinary fracture management remains limited. Therefore, the present study was undertaken to evaluate the clinical efficacy of demineralised bone matrix xenograft in enhancing healing of comminuted long bone fractures in dogs.

1.1 Objectives of the study

1. To evaluate the clinical efficacy of demineralised bone matrix xenograft in healing comminuted long bone fractures in dogs.
2. To assess haemato-biochemical changes and graft-related complications during the study period.

2. Materials and Methods

The study was conducted at the Department of Veterinary Surgery and Radiology and Veterinary Clinical Complex, Post Graduate Institute of Veterinary and Animal Sciences,

Akola, following approval from the Institutional Ethical Committee for Veterinary Clinical Research.

Sixteen dogs diagnosed with comminuted long bone fractures were selected irrespective of breed, age, sex and body weight and were randomly divided into two groups:

- Group I (n=8): Long bone fracture treated using appropriate internal fixation techniques without the use of DBM.
- Group II (n=8): Long bone fracture treated using appropriate internal fixation techniques along with the use of DBM at fracture site for enhancing the fracture healing.

2.1 Physiological assessment

The physiological assessment includes rectal temperature (°F), heart rate (beats/min.) and respiration rate (breaths/min.) were recorded on day '0' (zero), 7th, 14th, 30th and 45th postoperative days.

2.2 Clinical evaluation

Clinical evaluation for crepitation, degree of lameness, swelling over the fractured limb, weight bearing and pain sensation were determined and evaluated on the intervals of day '0' (zero), 7th, 14th, 30th and 45th postoperative day using standardized scoring systems.

2.3 Radiographic evaluation

Orthogonal radiographs were obtained preoperatively and on postoperative days 7, 14, 30, and 45 to assess fracture alignment, callus formation and healing progression.

2.4 Haematological and biochemical analysis

Blood samples were collected at predefined intervals of day '0' (zero), 7th, 14th, 30th and 45th postoperative day to assess haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, differential leukocyte count, serum calcium, phosphorus, alkaline phosphatase and total protein levels.

2.5 Surgical procedure

Fractures were stabilized using intramedullary pinning or locking compression plate fixation, depending on fracture configuration in clinical cases of both the groups. In Group II dogs, demineralised bone matrix xenograft was implanted at the fracture site following reduction and fixation.

Routine aseptic surgical protocols, anaesthetic management and postoperative care were followed.

2.6 Statistical analysis

Data were analysed using ICAR WASP 2.0 software employing two-way factorial ANOVA.

3. Results and Discussion

Sixteen dogs with comminuted long bone fractures were successfully treated using internal fixation techniques, with DBM xenograft implanted in Group II dogs (n=8 dogs).

3.1 Signalment and fracture characteristics

Fractures were more common in dogs aged between 6–12 months (56.25%), followed by dogs older than 12 months (31.25%). Male dogs (81.25%) were affected more frequently than females. The femur was the most commonly fractured bone (68.75%), followed by tibia, humerus, and radius.

Automobile accidents were the primary cause of fractures (62.5%).

These findings are consistent with previous studies reporting higher fracture incidence in young, active dogs due to increased susceptibility to automobile trauma (Kushwaha *et al.*, 2011; Gopinathan *et al.*, 2023) ^[10, 7].

3.2 Physiological parameters

Rectal temperature, heart rate, and respiration rate were elevated preoperatively due to fracture-associated stress and returned to normal physiological limits postoperatively. The results are in accordance with Patil *et al.*, (2017) ^[13]. No significant differences were observed between groups.

Table 1: Mean $\pm$ SE values for respiratory rate (breaths/min), heart rate (beats/min) and rectal temperature ($^{\circ}$ F) of dogs

Rectal temperature ($^{\circ}$ F)						
	Day 0	Day 7	Day 14	Day 30	Day 45	Pooled mean
Group I	102.79 $\pm$ 0.05	101.95 $\pm$ 0.13	101.73 $\pm$ 0.14	101.70 $\pm$ 0.09	101.63 $\pm$ 0.07	101.96 $\pm$ 0.08
Group II	102.84 $\pm$ 0.07	101.80 $\pm$ 0.06	101.64 $\pm$ 0.08	101.53 $\pm$ 0.09	101.48 $\pm$ 0.18	101.84 $\pm$ 0.09
Pooled mean	102.81 $\pm$ 0.04 ^a	101.88 $\pm$ 0.07 ^b	101.68 $\pm$ 0.08 ^c	101.62 $\pm$ 0.06 ^c	101.55 $\pm$ 0.10 ^c	
Heart rate (beats/min.)						
Group I	104.63 $\pm$ 0.78	79.75 $\pm$ 2.48	85.88 $\pm$ 0.72	85.75 $\pm$ 0.82	85.00 $\pm$ 0.65	88.20 $\pm$ 1.47
Group II	105.63 $\pm$ 0.60	85.13 $\pm$ 0.77	85.00 $\pm$ 0.46	85.00 $\pm$ 0.89	85.38 $\pm$ 0.60	89.23 $\pm$ 1.34
Pooled mean	105.13 $\pm$ 0.49 ^a	82.44 $\pm$ 1.43 ^c	85.44 $\pm$ 0.43 ^b	85.38 $\pm$ 0.59 ^b	85.19 $\pm$ 0.43 ^b	
Respiration rate (breaths/min.)						
Group I	37.63 $\pm$ 0.53	26.88 $\pm$ 0.52	25.50 $\pm$ 0.42	24.88 $\pm$ 0.48	25.50 $\pm$ 0.33	28.08 $\pm$ 0.80
Group II	37.25 $\pm$ 0.53	26.75 $\pm$ 0.53	26.25 $\pm$ 0.37	26.25 $\pm$ 0.41	25.13 $\pm$ 0.40	28.33 $\pm$ 0.75
Pooled mean	37.44 $\pm$ 0.36 ^a	26.81 $\pm$ 0.36 ^b	25.88 $\pm$ 0.29 ^c	25.56 $\pm$ 0.35 ^c	25.31 $\pm$ 0.25 ^c	

Means bearing different superscripts are significant at 1% level of significance

3.3 Clinical evaluation

Significant improvement in degree of lameness, swelling over the fractured limb, pain sensation and weight-bearing was observed in both groups. However, DBM-treated dogs demonstrated comparatively faster clinical recovery, likely due to enhanced osteoinductive activity. Similar findings were recorded by Farooq *et al.* (2019) ^[6] and Singh *et al.* (2025) ^[16].

A significant decrease in lameness score on day 45th when compared to days 0 and 7th in both the groups suggests that the use of internal fixation techniques and DBM xenograft as osteoinducer was beneficial for long bone fracture management. However, the dogs in group II demonstrated a greater decrease in lameness score as compared to group I. This was attributed to the early resolution of the inflammatory phase, which occurred as a result of osteoinductive activity at the fracture site following implantation of the DBM xenograft. Similar findings were recorded by Farooq *et al.* (2019) ^[6] and Singh *et al.* (2025) ^[16] who also found lameness score to be better in DBM implanted dogs.

A significant decrease in swelling observed on day 30th and 45th when compared to days 0 and 7th in both groups, suggesting that the use of internal fixation techniques and DBM xenograft as osteoinducer was beneficial for resolution of inflammation and promoting healing of long bone fracture in dogs. However, the dogs in group II demonstrated a greater decrease in swelling as compared to those of group I, suggestive of faster resolution of inflammation and promoting earlier fracture healing in dogs implanted with DBM. Similar observations were reported by Farooq *et al.* (2019) ^[6], who noted a significant reduction in postoperative swelling in all dogs included in their study; however, the swelling scores were comparatively lower in dogs that received DBM implantation. They attributed this finding to the additional healing response provided by DBM, which facilitated faster resolution of inflammation and promoted earlier fracture healing.

A significant increase in weight bearing observed on days 14th, 30th and 45th when compared to days 0 and 7th in both groups suggests that the use of internal fixation techniques and DBM xenograft as osteoinducer was beneficial for the management of long bone fracture in dogs. However, the dogs in group II demonstrated a greater weight bearing as compared to those of group I, suggestive of beneficial effects of DBM on fracture healing. Similar observations we noted by Farooq *et al.* (2019) ^[6] and Singh *et al.* (2025) ^[16] who found the weight bearing score to be significantly increased in the dogs on subsequent post-operative days. They opined that this may be due to decrease in pain due to healing of the fractured bone. Moreover, they also observed the weight bearing scores to be better in dogs implanted with DBM. Singh (2025) ^[16] suggested that this could be attributed to the faster fracture healing observed in dogs as a result of the DBM xenograft implanted at the fracture site.

A significant decrease in pain scoring was observed on post-operative days 7th, 14th, 30th and 45th when compared to day 0 in both groups suggests that the use of internal fixation techniques and DBM xenograft as osteoinducer was beneficial for the management of long bone fracture in dogs. However, the dogs in group II demonstrated decreased pain scoring as compared to those of group I, suggestive of faster fracture healing in the DBM treated dogs. Similar observations were noted by Farooq *et al.* (2019) ^[6] and Singh *et al.* (2025) ^[16] who found the pain score to be significantly reduced in the dogs on subsequent post-operative days. According to them the elevated pain scores observed during the initial period may be attributed to the onset of inflammatory responses associated with the fracture. They also observed lower pain scores in dogs implanted with DBM and attributed this improvement to faster fracture healing in the DBM treated dogs.

Crepitus was present preoperatively in all dogs and was absent postoperatively, indicating effective fracture stabilization.

Table 2: Mean $\pm$ SE values for lameness scoring, pain assessment, degree of swelling and weight bearing of dogs

	Day 0	Day 7	Day 14	Day 30	Day 45	Pooled mean
Lameness scoring						
Group I	4 $\pm$ 0	2.75 $\pm$ 0.16	2.25 $\pm$ 0.25	1.13 $\pm$ 0.35	0.63 $\pm$ 0.38	2.15 $\pm$ 0.22
Group II	4 $\pm$ 0	2.5 $\pm$ 0.19	1.75 $\pm$ 0.25	0.88 $\pm$ 0.40	0.5 $\pm$ 0.38	1.93 $\pm$ 0.23
Pooled mean	4 $\pm$ 0 ^a	2.63 $\pm$ 0.13 ^b	2.00 $\pm$ 0.18 ^c	1.00 $\pm$ 0.26 ^d	0.56 $\pm$ 0.26 ^e	
Degree of swelling						
Group I	3 $\pm$ 0	1.25 $\pm$ 0.16	0.63 $\pm$ 0.26	0 $\pm$ 0	0 $\pm$ 0	0.98 $\pm$ 0.19
Group II	3 $\pm$ 0	1.13 $\pm$ 0.13	0.5 $\pm$ 0.19	0 $\pm$ 0	0 $\pm$ 0	0.93 $\pm$ 0.18
Pooled mean	3 $\pm$ 0 ^a	1.19 $\pm$ 0.10 ^b	0.56 $\pm$ 0.16 ^c	0 $\pm$ 0 ^d	0 $\pm$ 0 ^d	
Degree of Weight bearing						
Group I	0 $\pm$ 0	1.63 $\pm$ 0.18	2.63 $\pm$ 0.18	3.25 $\pm$ 0.16	3.75 $\pm$ 0.16	2.25 $\pm$ 0.22
Group II	0 $\pm$ 0	2 $\pm$ 0.19	2.75 $\pm$ 0.25	3.38 $\pm$ 0.26	3.88 $\pm$ 0.13	2.40 $\pm$ 0.23
Pooled mean	0 $\pm$ 0 ^e	1.81 $\pm$ 0.14 ^d	2.69 $\pm$ 0.15 ^c	3.31 $\pm$ 0.15 ^b	3.81 $\pm$ 0.1 ^a	
Pain assessment						
Group I	4 $\pm$ 0	2.63 $\pm$ 0.18	2 $\pm$ 0.19	1 $\pm$ 0.27	0.25 $\pm$ 0.16	1.98 $\pm$ 0.22
Group II	4 $\pm$ 0	2.38 $\pm$ 0.18	1.63 $\pm$ 0.18	0.75 $\pm$ 0.31	0.25 $\pm$ 0.25	1.83 $\pm$ 0.23
Pooled mean	4 $\pm$ 0 ^a	2.50 $\pm$ 0.13 ^b	1.81 $\pm$ 0.14 ^c	0.88 $\pm$ 0.20 ^d	0.25 $\pm$ 0.11 ^e	

Means bearing different superscripts are significant at 1% level of significance

3.4 Haematological findings

All the dogs included in this study were subjected to haematological estimations i.e., Hb, PCV, TEC, TLC, DLC on day 0, 7th, 14th, 30th and 45th post operatively. The non-significant variation in haematological parameters indicated that the DBM xenograft did not cause any detrimental effect on the haemogram. Similar findings were noted by Singh *et al.* (2025) [16] in their research. The stability of PCV, TEC and TLC suggested minimal systemic blood loss and satisfactory post-operative healing. Similar findings were observed by

Amal *et al.* (2025) [1].

A gradual decrease in neutrophil count and increase in lymphocyte count suggested resolution of inflammation and progression toward healing. Patil *et al.*, (2017) [13] suggested that neutropenia with relative lymphocytosis indicated gradual decrease of inflammatory reaction. The normal ranges of eosinophils may suggest the absence of hypersensitivity reactions or implant-related rejection, as also found by Amal *et al.*, (2025) [1].

Table 3: Mean $\pm$ SE values for haematological parameters of the dogs in both groups

	Day 0	Day 7	Day 14	Day 30	Day 45	Pooled mean
Haemoglobin (gm/dl)						
Group I	12.26 $\pm$ 0.13	12.42 $\pm$ 0.13	12.48 $\pm$ 0.13	12.56 $\pm$ 0.15	12.54 $\pm$ 0.14	12.46 $\pm$ 0.06
Group II	12.28 $\pm$ 0.13	12.45 $\pm$ 0.13	12.53 $\pm$ 0.11	12.59 $\pm$ 0.12	12.69 $\pm$ 0.13	12.51 $\pm$ 0.06
Pooled mean	12.27 $\pm$ 0.09	12.44 $\pm$ 0.09	12.50 $\pm$ 0.08	12.58 $\pm$ 0.09	12.61 $\pm$ 0.09	
Packed cell volume (PCV%)						
Group I	39.65 $\pm$ 0.43	40.18 $\pm$ 0.43	40.29 $\pm$ 0.39	40.55 $\pm$ 0.41	40.34 $\pm$ 0.35	40.20 $\pm$ 0.18
Group II	38.88 $\pm$ 0.40	39.85 $\pm$ 0.26	40.20 $\pm$ 0.41	40.40 $\pm$ 0.31	40.61 $\pm$ 0.46	40.15 $\pm$ 0.16
Pooled mean	39.66 $\pm$ 0.23	40.01 $\pm$ 0.25	40.24 $\pm$ 0.27	40.48 $\pm$ 0.25	40.48 $\pm$ 0.28	
Total erythrocyte count (TEC) (X10⁶/cu mm)						
Group I	5.94 $\pm$ 0.08	6.01 $\pm$ 0.08	6.06 $\pm$ 0.12	6.11 $\pm$ 0.13	6.10 $\pm$ 0.05	6.07 $\pm$ 0.04
Group II	5.95 $\pm$ 0.05	6.07 $\pm$ 0.05	6.08 $\pm$ 0.07	6.10 $\pm$ 0.05	6.11 $\pm$ 0.05	6.06 $\pm$ 0.03
Pooled mean	5.94 $\pm$ 0.04	6.04 $\pm$ 0.05	6.07 $\pm$ 0.07	6.10 $\pm$ 0.06	6.11 $\pm$ 0.03	
Total Leucocyte count (TLC) (X10³/cu mm)						
Group I	10.97 $\pm$ 0.19	10.99 $\pm$ 0.77	10.86 $\pm$ 0.52	10.85 $\pm$ 0.56	10.59 $\pm$ 0.42	10.85 $\pm$ 0.22
Group II	10.84 $\pm$ 0.30	10.74 $\pm$ 0.57	10.73 $\pm$ 0.66	10.92 $\pm$ 0.69	10.70 $\pm$ 0.41	10.78 $\pm$ 0.23
Pooled mean	10.90 $\pm$ 0.17	10.86 $\pm$ 0.46	10.80 $\pm$ 0.41	10.88 $\pm$ 0.43	10.64 $\pm$ 0.28	
Neutrophil count (%)						
Group I	73.75 $\pm$ 0.80	69.75 $\pm$ 0.41	65.63 $\pm$ 0.56	62.38 $\pm$ 0.71	62.38 $\pm$ 0.71	66.78 $\pm$ 0.76
Group II	73.50 $\pm$ 0.50	67.75 $\pm$ 0.59	65.88 $\pm$ 0.48	63.00 $\pm$ 1.02	63.25 $\pm$ 0.98	66.68 $\pm$ 0.69
Pooled mean	73.63 $\pm$ 0.46 ^a	68.75 $\pm$ 0.43 ^b	65.75 $\pm$ 0.36 ^c	62.69 $\pm$ 0.60 ^d	62.81 $\pm$ 0.59 ^d	
Lymphocyte Count (%)						
Group I	16.63 $\pm$ 0.50	20.00 $\pm$ 0.73	23.63 $\pm$ 0.78	26.63 $\pm$ 0.56	26.50 $\pm$ 0.46	22.68 $\pm$ 0.67
Group II	16.75 $\pm$ 0.45	22.13 $\pm$ 0.67	23.50 $\pm$ 0.80	25.88 $\pm$ 0.81	25.75 $\pm$ 0.73	22.80 $\pm$ 0.61
Pooled mean	16.69 $\pm$ 0.33 ^d	21.06 $\pm$ 0.55 ^c	23.56 $\pm$ 0.54 ^b	26.25 $\pm$ 0.49 ^a	26.13 $\pm$ 0.43 ^a	
Monocyte Count (%)						
Group I	5.38 $\pm$ 0.73	5.63 $\pm$ 0.50	4.88 $\pm$ 0.35	5.38 $\pm$ 0.50	5.50 $\pm$ 0.46	5.35 $\pm$ 0.23
Group II	4.50 $\pm$ 0.53	5.00 $\pm$ 0.63	5.63 $\pm$ 0.42	5.50 $\pm$ 0.57	5.38 $\pm$ 0.53	5.20 $\pm$ 0.24
Pooled mean	4.94 $\pm$ 0.45	5.31 $\pm$ 0.39	5.25 $\pm$ 0.28	5.44 $\pm$ 0.36	5.44 $\pm$ 0.34	
Eosinophil Count (%)						
Group I	4.13 $\pm$ 0.48	4.13 $\pm$ 0.40	5.25 $\pm$ 0.70	5.00 $\pm$ 0.63	5.00 $\pm$ 0.63	4.70 $\pm$ 0.26
Group II	4.88 $\pm$ 0.44	4.88 $\pm$ 0.55	4.50 $\pm$ 0.60	5.00 $\pm$ 0.60	5.00 $\pm$ 0.60	4.85 $\pm$ 0.24
Pooled mean	4.50 $\pm$ 0.33	4.50 $\pm$ 0.34	4.88 $\pm$ 0.46	5.20 $\pm$ 0.40	5.00 $\pm$ 0.42	

Means bearing different superscripts are significant at 1% level of significance

3.5 Biochemical findings

Serum calcium, phosphorus and total protein levels were non-significant and were within normal physiological limits. Nangi (2025) ^[12] also reported non-significant variation in calcium and phosphorus observed in fracture healing. Singh *et al.* (2025) ^[16] observed that total protein values remained within physiological limits and exhibited non-significant difference.

The mean ALP values exhibited an increasing trend up to the 14th postoperative day, followed by a gradual decline by the 30th day, and were significantly reduced to within the normal physiological range by the 45th postoperative day. The values remained above the normal physiological limits from the 7th to the 30th postoperative day. ALP was observed as highly significant for group as well as interval. According to Amal *et al.* (2025) ^[1], the elevated ALP levels in the healing phase

reflect the osteogenic response at the fracture site, while the subsequent decline indicates callus maturation and cortical bridging. Preethi *et al.* (2022) and Nangi (2025) ^[12] suggested that this transient elevation of ALP reflects heightened chondroblastic activity and early bone formation associated with the repair process, which by day 30, returned to baseline. The values of alkaline phosphatase of group II were significantly elevated than that of group I. These observations suggested an improved osteoblastic activity in group II dogs. These findings are in accordance with Singh *et al.* (2025) ^[16] who also observed the values of alkaline phosphatase were significantly higher in dogs implanted with DBM as compared to the control group. They opined that findings may be due to the implantation of the osteoinductive biomaterial (DBM xenograft) at the fracture site, leading to enhanced osteoblastic activity.

Table 4: Mean $\pm$ SE values for biochemical parameters of all the dogs

	Day 0	Day 7	Day 14	Day 30	Day 45	Pooled mean
Calcium (mg/dL)						
Group I	9.83 $\pm$ 0.13	9.75 $\pm$ 0.11	9.79 $\pm$ 0.18	9.85 $\pm$ 0.13	10.08 $\pm$ 0.11	9.86 $\pm$ 0.06
Group II	9.84 $\pm$ 0.08	9.89 $\pm$ 0.19	9.83 $\pm$ 0.19	9.89 $\pm$ 0.23	10.09 $\pm$ 0.12	9.91 $\pm$ 0.07
Pooled mean	9.83 $\pm$ 0.07	9.82 $\pm$ 0.11	9.81 $\pm$ 0.12	9.87 $\pm$ 0.13	10.08 $\pm$ 0.08	
Phosphorous (mg/dL)						
Group I	3.88 $\pm$ 0.12	3.89 $\pm$ 0.06	3.93 $\pm$ 0.06	3.85 $\pm$ 0.06	3.83 $\pm$ 0.05	3.87 $\pm$ 0.03
Group II	3.800 $\pm$ 0.05	3.83 $\pm$ 0.08	3.95 $\pm$ 0.07	3.81 $\pm$ 0.06	3.87 $\pm$ 0.07	3.85 $\pm$ 0.03
Pooled mean	3.84 $\pm$ 0.06	3.86 $\pm$ 0.05	3.94 $\pm$ 0.04	3.83 $\pm$ 0.04	3.85 $\pm$ 0.04	
Alkaline Phosphatase (ALP) (IU/L)						
Group I	107.63 $\pm$ 1.48	144.13 $\pm$ 2.39	154.25 $\pm$ 2.02	137.25 $\pm$ 1.83	93.75 $\pm$ 1.61	127.40 $\pm$ 3.75 ^b
Group II	110.00 $\pm$ 1.71	168.38 $\pm$ 3.09	187.00 $\pm$ 2.52	193.88 $\pm$ 2.12	111.75 $\pm$ 2.96	154.20 $\pm$ 5.92 ^a
Pooled mean	108.81 $\pm$ 1.13 ^e	156.25 $\pm$ 3.65 ^c	170.63 $\pm$ 4.51 ^a	165.56 $\pm$ 7.43 ^b	102.75 $\pm$ 2.84 ^d	
Total protein (g/dl)						
Group I	6.33 $\pm$ 0.02	6.59 $\pm$ 0.11	6.51 $\pm$ 0.11	6.54 $\pm$ 0.16	6.39 $\pm$ 0.20	6.469 $\pm$ 0.06
Group II	6.31 $\pm$ 0.05	6.59 $\pm$ 0.05	6.56 $\pm$ 0.12	6.59 $\pm$ 0.12	6.48 $\pm$ 0.17	6.506 $\pm$ 0.05
Pooled mean	6.32 $\pm$ 0.02	6.59 $\pm$ 0.06	6.53 $\pm$ 0.08	6.56 $\pm$ 0.10	6.43 $\pm$ 0.13	

Means bearing different superscripts are significant at 1% level of significance

3.6 Radiographic evaluation

On the 7th postoperative day, radiographic evaluation showed satisfactory alignment of the fracture fragments with implants maintained in their normal positions in the majority of cases across both groups. No radiographic evidence of callus formation was observed in any of the cases in either group at this stage.

On 14th post-operative day, radiographs revealed presence of complete fracture lines in most of the cases of group I but fracture lines were slightly visible in most of the cases of group II dogs, where partial filling of voids left out in comminuted fracture repair could be seen. Soft callus formation was seen in most of the cases of both the groups.

On the 30th postoperative day, radiographic examination demonstrated incomplete bridging of the fracture lines in Group I, with the fracture lines remaining visible. In contrast, Group II showed organized primary callus formation at the fracture site, with fracture lines being completely absent in most cases. Both endosteal and periosteal callus bridging were evident in the dogs of Group II.

On the 45th postoperative day, complete obliteration of the fracture lines by newly formed osseous tissue and complete healing of the fractured bone were observed in Group II dogs, with restoration of cortical continuity in almost all cases. In Group I dogs, the fracture lines had nearly disappeared in most cases. However, evidence of superior healing was noted in Group II animals, where the use of DBM xenograft appeared to enhance the rate of fracture healing.

These findings are in concurrence with Farooq *et al.* (2019) ^[6] and Singh (2021) ^[16] who also found complete disappearance of fracture line by 45th day post-operatively.

4. Abbreviations

DBM - Demineralised bone matrix-
 BMP - Bone morphogenic proteins-
 Hb - Haemoglobin
 TEC - Total erythrocyte count
 TLC - Total leucocyte count
 DLC - Differential leucocyte count



Fig 1: Retrograde intramedullary pinning to stabilize the fracture in dog of group II.



Fig 2: Application of DBM xenograft after internal fixation of fracture fragments.



Fig 3: Application of 3.5 mm LCP to lateral surface of humerus of case 7 of group II



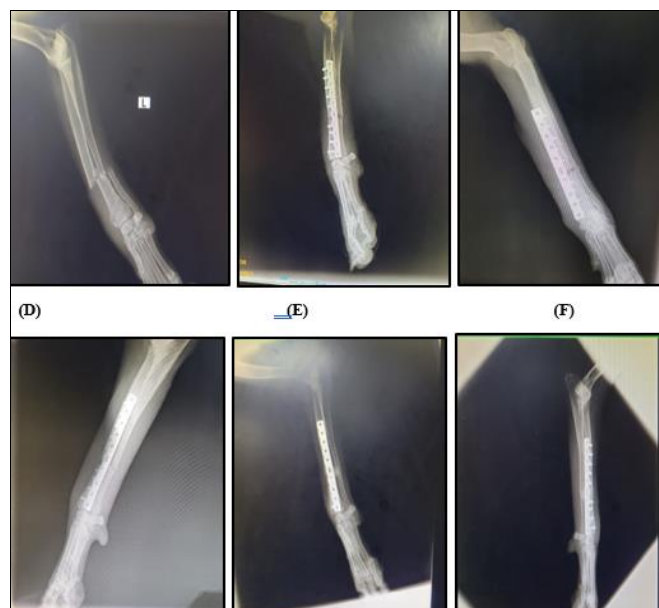
Fig 4: Preoperative non-weight bearing lameness



Fig 5: Fourteenth post-operative grade 1 lameness exhibited by dog of group II



Fig 6: 45th day post-op full weight bearing on operated limb



They depict callus formation with progressive filling of the voids that are typically present following internal fixation of a comminuted fracture, corresponding to grade 1 radiographic healing

Fig 7: Skiagrams depicting radiographic healing of day 0 (A) and postoperative 7th day (B), 14th day (C), 30th day (D) and 45th day (E) and (F) of Case 8 of Group II.

5. Conclusion

Demineralised bone matrix xenograft can be safely used as an adjunct to internal fixation in the management of comminuted long bone fractures in dogs without causing any local or systemic adverse effects. Better and earlier clinical improvement was observed in DBM-treated dogs. Serum alkaline phosphatase levels were significantly higher in DBM-treated dogs, reflecting enhanced osteoblastic activity and accelerated fracture healing. Overall, the incorporation of demineralised bone matrix xenograft along with internal fixation enhances the rate of fracture healing and is a valuable osteoinductive biomaterial for the treatment of comminuted long bone fracture in dog.

Conflict of Interest

The authors do not have any conflict of interest.

Financial Support

Not available

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How to Cite This Article

Choudhury AJ, Raulkar RV, Fani F, Thorat MG, Tarate PS, Moon N, *et al*. Clinical efficacy of demineralised bone matrix as xenograft for healing of comminuted long bone fracture in dog. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;11(4):240-246.

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