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# β tricalcium phosphate as a bone substitute for fracture healing in goats

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**Abstract**
The study was conducted on twelve clinical cases of goats having long bone fracture, presented at Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Jabalpur, during the study period. In group I the fracture segment was immobilized by dynamic compression plate whereas, in group II after immobilization with dynamic compression plate gap at fracture site was filled with β tricalcium phosphate. There was a gradual decrease in exudates and pain at fracture site in both the groups from 2nd postoperative day onwards. A significant increase in weight bearing was observed from 7th post-operative day in both the groups of goat. Complete weight bearing by all the animals was observed on 30th day in group II. Radiographic examination on 15th post-operative day, depicted evidence of periosteal reaction on both the fracture segment, almost in all the cases of group II. Radiograph taken on 60th post-operative day, exhibited radiodense area at the fracture site, indicative of extensive periosteal and endosteal callus. The values of alkaline phosphatase increased non-significantly in both groups of goat up to 15th post-operative day. After reaching its maximum values, a decline in the values was observed in both the groups.

**Keywords:** β tricalcium phosphate, dynamic compression plate, fracture, radiograph, weight bearing

## 1. Introduction
Bone, one of the most important connective tissue of our body is responsible for providing it a structure. This rigid tough tissue protects our visceral organ from external injury by serving as protective gear. Besides protecting our body from an injury it has to perform various works. It acts as a production site for haemopetic and immune cells, it is a warehouse for calcium and phosphorous and is responsible for mobility of an individual (Caplan, 1991 and Hing, 2004) [3, 9]. Fracture is break in the continuity of hard tissue. Whenever there is a fracture it heals on its own if congenial environment is provided. Hence, it is the prime and bounden duty of a surgeon to provide the requisite environment for its proper healing and regaining its original entity. β-tricalcium phosphate (β-TCP) is a calcium phosphate ceramic used in bone grafting as an alternative bone substitute to autograft. It has been reported that β-TCP has excellent osteoconduction and resorbability when filling a bone defect (Tanka *et al*., 2014) [18]. Improper immobilization of fractured bones often leads to implant failure, nonunion, loss of limbs and mobility of these animals. Moreover, a lame animal loses its economic value; because of it's under performance. Internal immobilization, along with use of bone substitute, may lead to healing of bone fracture without any complication and may prove a milestone for establishment of an effective technique for fracture management. Keeping in view the above facts present research work was designed to evaluate β tricalcium phosphate as a bone substitute for fracture healing of long bones in goats.

## 2. Material and Methods
The work was approved by institutional ethical committee. The study was conducted on twelve clinical cases of goats having long bone fracture, presented at Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Jabalpur, during the study period. Goats of either sex, aged between one to six years having long bone fracture were selected for present study. The goats selected were randomly divided into two groups. In group I the fracture segment was immobilized by dynamic compression plate whereas, in group II after immobilization with dynamic compression plate gap at fracture site was filled with β tricalcium phosphate.

The surgical procedure was performed under general anaesthesia using Diazepam hydrochloride @ 0.5 mg/Kg body weight intravenous, followed by Ketamine hydrochloride @ 5 mg /Kg body weight intravenous till effect. Maintenance of anaesthesia was done by repeated intravenous administration of Ketamine hydrochloride as and when required. The animals were administered amoxicillin sulbactam @ 10 mg /kg intramuscular twice a day and continued up to 7th post-operative day. Similarly, Meloxicam @ 0.3 mg /Kg b. wt. intramuscular was administered for four postoperative days to take care of pain. Bone marrow aspirates were collected from the epiphyseal region of fractured long bone of goats undergoing internal immobilization. Fracture healing was evaluated on the basis of inflammation, exudation, pain, weight bearing by the animals, and radiographic evaluation and haemato-biochemical studies at different time intervals. Inflammation, exudation, and pain at the fracture site was observed on 2nd, 4th, 7th, 10th and 15th post-operative day, Assessment of weight bearing was done on 2nd, 7th, 10th, 15th, 30th, 45th, 60th and 90th postoperative day, as per modification done in the method of Aithal (1996) [1].

| Weight bearing by the animal | Score |
|---|---|
| Carrying the limb away from ground | 0 |
| Touching the toe on the ground | 1 |
| Touching the sole on the ground | 2 |

Radiographs were taken prior to surgery and subsequently on 7th, 15th, 30th, 45th, 60th and 90th postoperative day, and the evaluation was done as per modification done in the method of Hammer *et al*. (1985) [8].

| Callus formation | Fracture line | Stage of union | Grade / Score |
|---|---|---|---|
| No callus | Distinct | Not achieved | 0 |
| Trace: No bridging of fracture line | Distinct | Not achieved | 1 |
| Apparent: Bridging of fracture line | Discernible | Uncertain | 2 |
| Massive: Bone trabeculae crossing fracture line | Barely discernible | Achieved | 3 |
| Homogenous bone structure | Obliterated | Achieved | 4 |

## 3. Results and Discussion

### 3.1 Exudates and Pain

There was a gradual decrease in exudates at fracture site in both the groups from 2nd postoperative day onwards. Complete absence of exudates was noticed in group II on 7th post-operative day whereas, slight exudates were present in group I even on 15th post-operative day. Steady decrease in degree of pain was observed in both the groups from 2nd post-operative day. Throughout the observation period, a lower value of pain was observed in group II in which fracture gap was filled with β tricalcium phosphate as compared to group I where only plating was done. Progressive decrease in exudation and pain at fracture site was too observed by De' Souza (2012) [4], Gupta (2015) [7] and Singh (2015) [16]. Early paucity of exudates and decrease in pain in group II might be accredited to proper immobilization of fractured segments and filling of gaps with β tricalcium phosphate scaffold which provided a podium for migration of inflammatory and reparative cells and reduction in inflammation.

### 3.2 Weight bearing

A significant increase in weight bearing was observed from 7th post-operative day in both the groups of goat. Complete weight bearing by all the animals was observed on 30th day in group II however, complete weight bearing by all the animals of group I was observed on 60th post-operative day. These findings were similar to the findings of Kushwaha *et al*. (2011) [11], Avasthi *et al*. (2012) [2] and Vinit (2018) [19] who reported gradual increase in weight bearing from 7th day onwards. An early weight bearing by all the animals of group II can be explained by the fact that there was early alleviation of pain in group II as compared to group I.

### 3.3 Radiographic examination

Post-operative radiograph of both the group revealed proper apposition of fracture segment with distinct fracture line. Bone graft substitute (β-tricalcium phosphate) was visible as a radiodense material at the fracture area in group II. Radiograph of all the goats on 7th post-operative day, exhibited proper apposition of fracture segments without any incidence of implant failure. Fracture line as well as bone substitute (β-tricalcium phosphate) were evident. Radiographic examination on 15th post-operative day, depicted evidence of periosteal reaction on both the fracture segment, almost in all the cases of group II. β-tricalcium phosphate was well appreciated and there was no evidence of implant failure in any animal of the group. Fracture line at this interval was well appreciated and the score for callus formation achieved by this group, was 0.50 ± 0.22, which was graded as nil to trace. Radiographic score of group I at this stage was $0.17^{a}$± 0.17 and was too referred to as nil to trace.
Interpretation of radiograph on 30th postoperative day of group II animal revealed moderate callus at the fractured site. Discernible fracture line without any incidence of implant failure and nonunion. There was a clear substantiation of β-tricalcium phosphate near the fracture site almost in all goats. Score for callus formation achieved by the goats of this group was 2.17 ± 0.31, which was graded as trace to apparent. whereas, in group I bridging of fracture line was incomplete. Massive callus with increased radio density at the fracture site was visible on 45th post-operative day. Bone trabeculae crossing the fracture line for bridging of the fracture segments were visible at this interval. β-tricalcium phosphate was well identifiable at this interval, however initiation of remodeling of the bone, had not yet started in any goat of this group. The score achieved by this group was, 2.83 ± 0.40 and the callus was graded as apparent to massive. In group I fracture line was still discernible and the callus formation was graded as apparent. Radiograph taken on 60th post-operative day, exhibited radiodense area at the fracture site, indicative of extensive periosteal and endo steal callus. Fracture line at this time interval was completely obliterated. An initiation of homogenesity was well appreciated in most of the case of this group, which was indicative of start of remodeling; however patency of medullary cavity couldn't be achieved up to this time interval. The score achieved by the goats of this group was 3.33 ± 0.21, which was graded as massive to homogenous. On the other hand complete union with radiodense callus between fractured segments was observed in group I. Radiograph of the animals of group II on 90th post-operative day, revealed almost homogenous bone, without any indication of fracture line. Patency of the bone marrow was not completely achieved. The score achieved by the animals of this group was 4.00 ± 0.00, indicative of nearly completion of remodeling stage. Radiograph of group I animal at this stage revealed union of fractured segment with an initiation of remodeling stage which was indicated by start

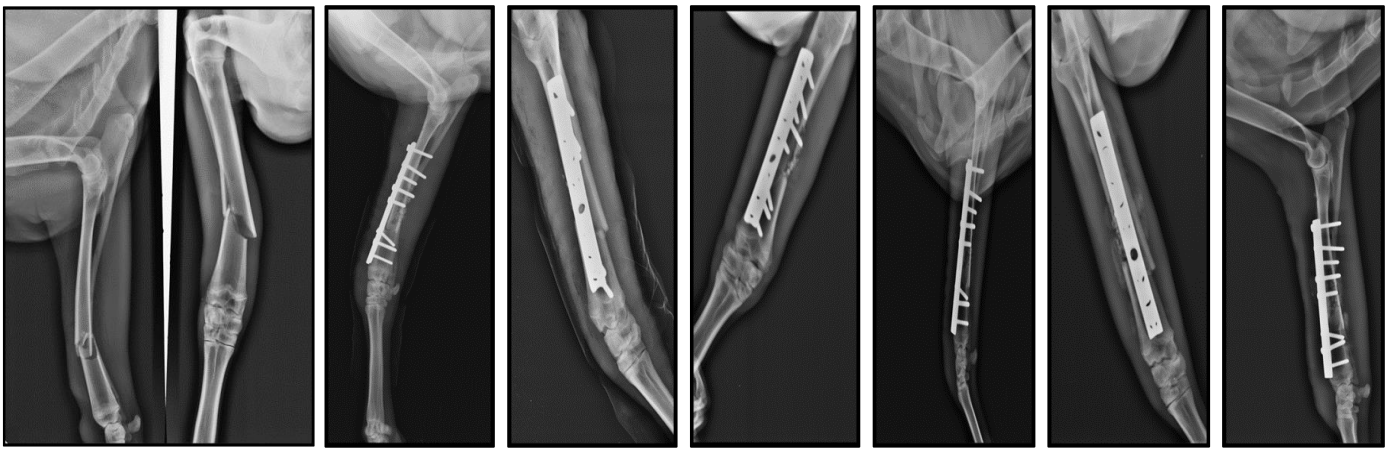
of homogenesity of bone. These finding were in accordance with the findings of Ohura *et al*. (1996) [13], Kondo *et al*. (2005) [10] and Goel *et al*. (2013) [6] and Singh (2015) [16], who reported that β – tricalcium phosphate when used at fracture site acts as an osteoconductive agent, it enhances fracture healing process. Moreover, it gets absorbed in about 90th post-operative day, from the site of implant. In partial agreement to this, Erbe *et al.* (2001) [5] reported, resorption of the implant as early as in 3 weeks throughout the bone defect in canine and noticed complete remodeling in 6–12 weeks with bone density in the normal range. Early healing in case of group II, might be due to osteoconductive property of β – tricalcium phosphate, which enhances the process of fracture healing.

### 3.4 Biochemical changes

The values of alkaline phosphatase increased non-significantly in both groups of goat up to 15th post-operative day, with maximum value at this interval. After reaching its maximum values, a decline in the values was observed in both the groups and minimum values were seen on 60th day in group I and 45th day in group II. Decrease in the value of alkaline phosphatase might be indicative of cessation of osteoblastic activity and receding of the values towards its base value may be due to ossification and consolidation of fractured bone. Higher activity of alkaline phosphatase on 15th postoperative day and its gradual decrease was too supported by the findings of radiographs. After 45th postoperative day interval, there was a gradual increase in the values of alkaline phosphatase till 90th postoperative day, in group II and from 60th postoperative day till 90th postoperative day in group I, which might be due to the fact that remodeling phase may have started after these time intervals, which correlates with the radiographic findings. The above observations are in agreement with the findings of Rani and Ganesh (2003) [15], Phaneedra *et al*. (2016) [14], Szponder (2018) [17], Vinit (2018) [19] and Yadav *et al*. (2020) [20] who reported that alkaline phosphatase increases in initial days of fracture healing and then its value gradually decreases till 60th post-operative day.

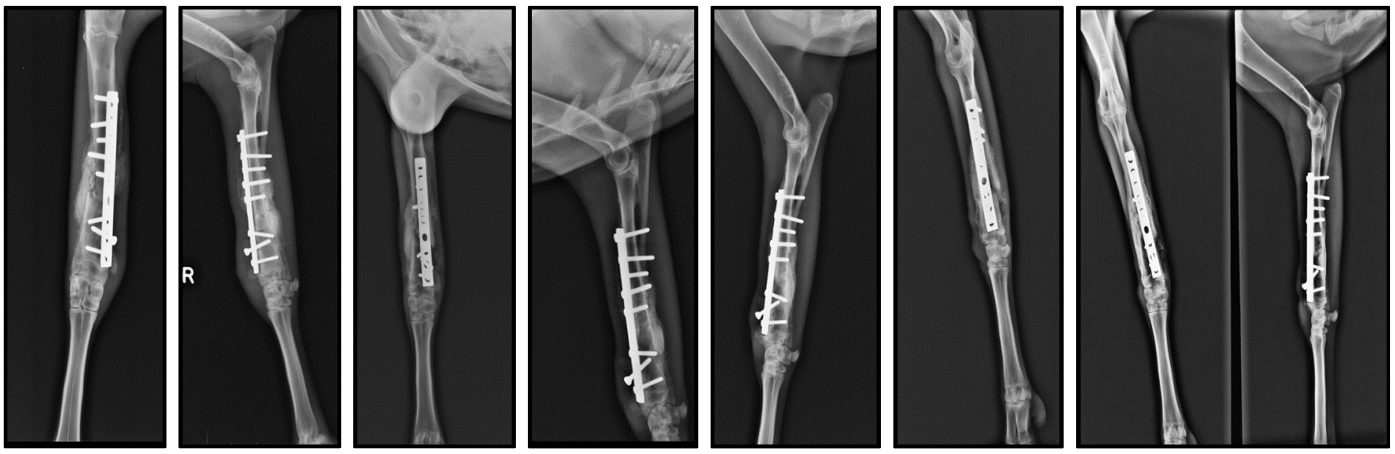
Creatinine kinase showed significant decrease from 7th day to 90th day interval in both the groups of goats. The present findings corroborate with the findings of Laurence (2000) [12], who observed increased activity of creatinine kinase after surgery, which receded back to its normal value after healing. Higher values of creatinine kinase at initial stage might be attributed it to the extent of muscle damage at this stage which decreased as the healing progressed.

There was a gradual decrease in the values of serum calcium till 45th post-operative day after which there was a gradual increase in these values up to 90th post-operative day. The above findings are in accordance with the findings of Rani and Ganesh (2003) [15] and Vinit (2018) [19], who observed an increase in the value of serum calcium on first day of fracture repair, followed by marked reduction in goats. Higher value of serum calcium in initial intervals can be attributed to increased osteoclastic activity, leading to resorption of dead bone in initial stage.



Pre-operative day | Post-operative day | 7th post-operative day | 15th post-operative day

**Fig 1:** Radiograph at different time intervals in group II (DCP + β -TCP)



30th post-operative day | 45th post-operative day | 60th post-operative day | 90th post-operative day

**Fig 2:** Radiograph at different time intervals in group II (DCP + β -TCP)

## 4. Conclusions

From the above discussion it can be concluded that β tricalcium phosphate can be used to accelerate the process of fracture repair since it acts as a scaffold and alleviate pain, early weight bearing by the animal, initiates early periosteal reaction, callus formation and its remodeling. Radiograph and biochemical parameters can be used as an indicator of fracture healing.

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