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Application of Platelet Rich Plasma (PRP) with β-Tricalcium Phosphate (β-TCP) and Demineralized Bone Matrix (DBM) in Healing of Bone Tissues in Rabbits

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ABSTRACT

In the present study, bone healing efficacy of autologous Platelet Rich Plasma (PRP) with β-Tricalcium Phosphate (β-TCP) and Demineralised Bone Matrix (DBM) was evaluated in induced radial defect in rabbits. Eighteen rabbits divided equally into three groups, where PRP with β-TCP and DBM were applied in Group II & III respectively; while Group I was kept as control. The haematological parameters showed non-significant variation within physiological limit. The serum biochemistry analysis revealed significant elevation of Serum Alkaline Phosphatase on, 15th and 30th day; Serum Creatinine Kinase on 5th day; Serum Calcium on 30th day and Serum Phosphorus level in entire period in all the groups. Radiographs taken on 20th day revealed feathery/ cloudy bony growth in Group I and radio-opaque dense granular density was observed in Group II & III. On 40th day, Group I revealed even bony proliferation; while in Group II & III, large amount of radio-opaque granular tissue was observed along the bony defect. On 60th day radiographs showed incomplete bridging of fractured ends in Group I & III; however complete union was observed in Group II. Histopathological examination of the bone tissues revealed prominent osteoblastic activity in both treated groups. Based on the results it is concluded that application of PRP + β-TCP and DBM had little haematological changes; biochemical alterations were indicative of healing of bone. Radiological and histopathological evaluations were suggestive of better result with PRP + β-TCP when compared to DBM application in surgical reconstruction of radial bone defects.

HIGHLIGHTS

- PRP with β-TCP) and DBM application encouraged long bone healing.
- PRP with β-TCP) and DBM application in the long bone fracture site does not alter adversely the haemato-biochemical profile of blood.

Keywords: Platelet Rich Plasma, β-Tricalcium Phosphate, Demineralized Bone Matrix, Bone healing, Rabbits

Complication in bone healing viz delayed healing, non-union, malunion and bone infections can have a highly negative impact on life and society, therefore knowledge towards the management of fracture is important (Deschaseaux *et al*., 2009). In modern days, though various improved technologies have been routinely used for surgical reconstruction of long bone defects with loss of bone fragments needs; but still it is remains a big clinical challenge for the orthopaedic surgeons due one of

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both failure of biological and mechanical support in bone defects. Long bone fracture with application of autologous cancellous bone grafting is regarded as gold standard biological method of healing of bone defect due to local components such as osteoconduction, osteoinduction and osteogenic cells (Pountos, 2010). Therefore, the suitable alternatives could be various biomaterials and synthetic bone substitutes currently used as scaffolds, including collagen, hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and calcium-phosphate cements, and glass ceramics (Giannoudis *et al*., 2005; Finkemeier, 2002). The clinical applications of Platelet Rich Plasma (PRP) have been reported in a wide variety hard tissue as well as soft tissues surgeries including burn tissues, and tissue engineering and organ implantation (Lubkowska *et al*., 2012). Simultaneous application biomaterials such as β-tricalcium phosphate (β-TCP) enhance the effectiveness of Platelet Rich Plasma (PRP) in osteoinductive and osteroconductive properties (Oryan *et al*., 2015). Demineralised Bone Matrix (DBM) is derived from allogeneic bone by extensive decalcification procedures and retain both osteoinductive and osteoconductive (Gruskin *et al*., 2012). Due to evidence of literature available on use of above-mentioned biomaterials along with PRP the present experiment was undertaken to evaluate the efficacy of autologous Platelet Rich Plasma (PRP) with β-Tricalcium Phosphate (β-TCP) and Demineralised Bone Matrix (DBM) in healing effect of ostectomized radial defects in rabbits.

MATERIALS AND METHODS

Study was carried out in the Department of Surgery & Radiology, Faculty of Veterinary Sciences, Assam Agricultural University, Khanapara, Guwhati, Assam and was in full compliance with the Institutional Animal Ethics Committee. In the present study, eighteen (18) adults healthy rabbits (*Oryctolagus cuniculus*) of either sex with average body weight of 1.5-2.0 kg were divided equally into three groups viz. Group I, Group II and Group III was used.

Preparation of platelets rich plasma (PRP)

Platelet Rich Plasma (PRP) was prepared prior to creating bone defects. Samples were collected in sterile tubes containing sodium citrate (3.8% w/v) in the ratio of 9:1. Tubes were submitted to the double centrifugation protocol, with lid closed and 1600 revolutions per minute (rpm) for 10 minutes then the plasma was pipetted out and transferred into another sterile tube. Plasma was centrifuged again at 2000 rpm for 10 minutes to separate the platelet rich portion in the bottom leaving platelets poor plasma (PPP) on the top of the tube. The top part of the PPP was discarded and the remaining PRP was used for the experiment. The gaps between the bones were filled with the combination of Platelet Rich Plasma (PRP) with β-Tricalcium Phosphate (β-TCP) (B-OstIn^{TM})$^{(1)}$ in Group II followed by external immobilization.

Creation of full thickness radial ostectomy and surgical procedure

Full thickness 1.0 cm radial ostectomy was created under general anaesthesia of Xylazine Hydrochloride (Xalyxin$^{(2)}$) @ 5 mg/kg IM and Ketamine Hydrochloride (Ketamax$^{(3)}$) @ 35 mg/kg IM in all the animals. The site of incision was selected on the basis of exposure of radial bone. An incision was made in the cranial aspect of the radial forearm, from which the skin, subcutaneous tissue and deep fascia was incised. After dissecting the muscles and exposing the radius, a small bone saw was used to create full thickness segmental bone defects of 1.0 cm in length. The bone defect was washed multiple times with normal saline and afterwards the fascia, subcutaneous tissues and skin was closed in routine manner. Group I animals were kept as control, the osteotecmized bone was kept as such and external immobilization was applied; the animals of Group II subjected to application of PRP with β-TCP to fill the bone defect, while in Group III DBM was used to fill the gap followed by routine external immobilization. In all the animal's standard post-surgical management was followed till the external wound is healed.

Haemato-biochemical, radiographic and histopathological examination

Haemato-biochemical analysis of whole blood and serum was carried out on 0th 5th, 10th, 15th, 30th day following

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surgical reconstruction. Blood Haemoglobin (Hb), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Packed Cell Volume (PCV) were estimated as per the method described by Schalm *et al*. (1975); while Serum alkaline phosphatase (ALP), Serum creatine kinase (CK), Serum Calcium (Ca) and Serum Phosphorus (P) were estimated as per the method described using standard commercial kit. On rendering the corrective treatment to all the groups, plain X rays were taken on Carestream Dental CS 2200 (Potential Difference, Voltage: DC- 60kV; Current, Ampere: 7mA; Exposure Time, sec: 0.238s) of the operated bones and were taken in standard projections preoperatively and postoperatively. Subsequent examinations to monitor the formation of bone union were performed at 0^{th} (after surgical reconstruction), 20^{th}, 40^{th} and 60^{th} post-surgical days. The radiographs were studied and the status of fracture healing was recorded for comparison between the groups. Histopathology of the operated bone was examined at 0^{th} (after surgical reconstruction), 20^{th}, 40^{th} and 60^{th} post-surgical days. The bone was collected and decalcified for further processing of bony tissues and other routine protocol was followed to prepare the slides and staining was done Haematoxylin and Eosin (H & E) stain as per protocol described by Culling (1974). The histopathological slides were analysed and recorded for comparing between the groups for further interpretation.

STATISTICAL ANALYSIS

The recorded data of the present study "Platelet Rich Plasma (PRP) with β-Tri Calcium Phosphate (β-TCP) and Demineralised Bone Matrix (DBM) in healing of bone tissues in rabbits" were analysed as per the method described by Snedecor and Cochran (1994) with the help of Statistical Analysis System 9.3 (SAS 9.3).

RESULTS AND DISCUSSION

Haematological examination

The haematological changes of whole blood examination have been depicted in Table 1. The variation of Hb % in all the groups showed non-significant decrease ($P>0.05$) till 10^{th} day followed by non-significant increase ($P>0.05$) in Group I to 30^{th} day; however, in Group II and III, there was a non-significant increase ($P>0.05$) on 15^{th} day and non-significant decrease ($P>0.05$) on 30^{th} day. The mean values of Hb % in all the three groups however, returned to its base level by the end of experiment. The declined trend of Hb% on 5^{th} day in all the groups might be attributed to presence of more inflammation, physical stress at the time of creating bone defects, loss of blood during surgery, as well as haemodilution and anaesthesia during reconstructive surgery procedure. The above findings were in accordance with the findings of Tembhurne *et al.*

Table 1: MEAN ± S.E. of the Haematological parameters of experimental animals in different treatment groups on various days of study

Parameters		0^{th} day	5^{th} day	10^{th} day	15^{th} day	30^{th} day
Haemoglobin (g/dL)	G I	13.05 ± 0.48^{A}	12.52 ± 0.49^{A}	12.20 ± 0.57^{A}	12.55 ± 0.69^{A}	13.14 ± 0.47^{A}
	G II	12.83 ± 0.46^{A}	12.47 ± 0.46^{A}	12.05 ± 0.48^{A}	12.74 ± 0.48^{A}	12.58 ± 0.48^{A}
	G III	12.98 ± 0.52^{A}	12.44 ± 0.51^{A}	12.17 ± 0.52^{A}	12.59 ± 0.52^{A}	12.19 ± 0.51^{A}
Total Erythrocyte Count ($x10^6/m^3$)	G I	6.52 ± 0.26^{A}	6.35 ± 0.23^{A}	6.07 ± 0.29^{A}	6.19 ± 0.37^{A}	6.70 ± 0.26^{A}
	G II	6.23 ± 0.13^{A}	6.05 ± 0.15^{A}	5.83 ± 0.15^{A}	6.24 ± 0.17^{A}	6.12 ± 0.15^{A}
	G III	6.49 ± 0.26^{A}	6.22 ± 0.40^{A}	6.08 ± 0.26^{A}	6.30 ± 0.26^{A}	6.09 ± 0.26^{A}
Total Leucocyte Count ($x10^3/mm^3$)	G I	8.66 ± 0.44^{A}	9.75 ± 0.41^{A}	8.46 ± 0.45^{A}	8.38 ± 0.45^{A}	8.33 ± 0.44^{A}
	G II	9.15 ± 0.57^{A}	9.06 ± 0.58^{A}	8.98 ± 0.58^{A}	8.88 ± 0.58^{A}	8.41 ± 0.58^{A}
	G III	8.92 ± 0.37^{A}	8.83 ± 0.36^{A}	8.27 ± 0.52^{A}	7.80 ± 0.35^{A}	7.83 ± 0.36^{A}
Packed Cell Volume (%)	G I	39.10 ± 1.53^{A}	38.10 ± 1.38^{A}	36.39 ± 1.74^{A}	36.82 ± 1.95^{A}	40.18 ± 1.54^{A}
	G II	37.35 ± 0.80^{A}	36.27 ± 0.91^{A}	34.95 ± 0.92^{A}	37.44 ± 0.99^{A}	36.73 ± 0.92^{A}
	G III	38.95 ± 1.57^{A}	37.32 ± 1.54^{A}	36.50 ± 1.55^{A}	37.78 ± 1.55^{A}	36.56 ± 1.54^{A}

Means with similar superscripts and different superscript within column (lowercase) and within rows (uppercase) showed non-significant ($P>0.05$) and significant ($P<0.05$) respectively; GI- Group I; GII- Group II; GIII-Group III.

(2010); Rajhans (2013); Singh *et al*. (2017) and Chaurasia *et al*. (2019) in canine; Gabriel *et al*. (2014) and Gupta (2015) in goat. The variation TEC revealed non-significant (P>0.05) decline in all the groups till 10th day, followed by non-significant increase (P>0.05) from 10th in Group I; while the same was seen in Group II & III on 15th day onwards. The transient fall in TEC may be due to mild haemorrhages during surgical reconstruction procedure or sequestration of RBC to spleen during early post-surgical periods. Similar findings were also reported by Aithal *et al.* (1998) and Tembhurne *et al.* (2010) in dog; Kumar *et al*. (1999) in calves. There was a non-significant (P>0.05) elevation of TLC in Group I within 5th day followed by declining trend towards normal; while Group II & III animals exhibited non-significant decrease of TLC till 30th day of observation. This fluctuation might be attributed to the inflammatory reaction, surgical intervention and tissue reaction and normal physiological response of the body. Author's observations were in agreement with the findings of Singh *et al*. (2017) in dog in management of femur fracture; however, in contrast to present findings, Aithal *et al*. (1998) recorded marked increase in TLC up to 15th day following management of supracondylar fracture management in dog. There was a non-significant variation of PCV % in all the groups during the entire experimental period which might be due to the blood losses during surgical reconstruction and the values returned to normal once the animals recovered from the stresses. Above findings were in accordance with Gabriel *et al.* (2014) in goat and Kumar (2016) in dog during femoral fracture management.

Biochemical evaluation

The biochemical changes of blood serum following surgical reconstruction for different time interval were presented in Table 2. Post-surgical values of ALP was recorded elevated significantly (P<0.05) in Group I within the 5th day followed by non-significant decrease (P>0.05) till 15th day and again significant increase (P<0.05) on 30th day; on the other hand in Group II & III animals showed significant (P<0.05) increase till 30th day of observation. The changes of values of ALP might be due to implantation of osteo-inductive biomaterials at bone defect site and osteoblastic activity during the healing process of bone matrix formation and mineralization. Similar observation was also recorded by Chaurasia *et al*. (2019); Pardeshi and Ranganath (2008) in correction of tibial fracture in canine. In all the groups exhibited significant (P<0.05) elevation of CK within 5th days followed by declining trend till 30th post-surgical days, which might be attributed to trauma to the skeletal muscle during creation of radial bone osteotomy. These findings corroborate with the findings of Laurence (2000), who observed increased activity of CK after surgery and receded back to its normal value after healing. Oni *et al*. (1989) and De'Souza (2012) observed

Table 2: MEAN ± S.E. of the Biochemical parameters of experimental animals in different treatment groups on various days of study

Parameters		0th day	5th day	10th day	15th day	30th day
Serum Alkaline Phosphatase (IU/L)	G I	63.98±1.90^{Aa}	101.92±0.82^{Ba}	98.59±0.70^{Ba}	95.73±0.92^{Ba}	137.75±4.82^{Ca}
	G II	68.98±4.85^{Aa}	134.19±4.68^{Bb}	149.44±4.9^{Cb}	169.22±5.03^{Db}	193.07±5.13^{Eb}
	G III	70.12±3.81^{Aa}	129.20±3.76^{Bb}	142.28±3.85^{Cb}	154.79±3.81^{Dc}	173.72±3.93^{Ec}
Serum Creatine Kinase (IU/L)	G I	175.06±10.62 ^{Aa}	401.26±11.32^{Ba}	365.94±11.02^{Ca}	345.21±11.18^{Ca}	311.73±10.76^{Da}
	G II	187.71±13.14^{Aa}	393.66±13.32^{Baa}	346.66±12.90^{Ca}	309.19±12.57^{Db}	255.63±12.06^{Eb}
	G III	177.91±8.22^{Aa}	380.61±8.51^{B}	338.91±7.99^{Ca}	311.26±7.89^{Cb}	267.98±7.32^{Db}
Serum Calcium (mg/dL)	G I	11.60±0.39^{Aa}	10.09±0.40^{Ba}	9.90±0.40^{Ba}	9.66±0.40^{Ba}	11.59±0.41^{Aa}
	G II	10.87±0.42^{Ab}	10.11±0.46^{Aa}	9.04±0.47^{Ba}	7.85±0.47^{Cb}	10.32±0.46^{Ab}
	G III	11.37±0.35^{Aa}	10.24±0.34^{ABa}	9.77±0.34^{BCa}	8.96±0.34^{Ca}	10.17±0.32^{Bb}
Serum Phosphorus (mg/dL)	G I	5.47±0.14^{Aa}	6.02±0.10^{Ba}	5.46±0.10^{Ba}	4.08±0.11^{Ca}	5.60±0.10^{Aa}
	G II	5.32±0.10^{Aa}	5.46±0.10^{Bb}	5.58±0.10^{Ca}	6.13±0.10^{Db}	6.40±0.10^{Bb}
	G III	5.56±0.09^{Aa}	5.80±0.09^{Bc}	5.97±0.09^{Cb}	6.57±0.10^{Dc}	6.91±0.10^{Bc}

Means with similar superscripts and different superscript within column (lowercase) and within rows (uppercase) showed non-significant (P>0.05) and significant (P<0.05) respectively; GI- Group I; GII- Group II; GIII-Group III.

elevated serum CK in closed tibial fractures in dogs prior to fracture repair followed by its gradual decrease and reached its normal value on 45th post-operative day. The changes of Serum Ca in all the groups showed significant decrease ($P<0.05$) till 15th day followed by significant increased ($P<0.05$) till 30th day. The decrease in serum Ca might be due to deposition of the excessive calcium at the fracture site and urinary excretion of calcium after creation of bone defect as well as remodelling of healed bone. Author's findings were in agreement with observations of Rani and Ganesh (2003) and Chaurasia *et al*. (2019). However, the serum Ca fluctuation was recorded higher in Group II & III than Group I. The serum phosphorus estimation revealed significant increase ($P<0.05$) in Group I on 5th day followed by significant decrease ($P<0.05$) on 15th day and then significant increase ($P<0.05$) on 30th day; while in Group II and III, there was significant ($P<0.05$) increase of serum phosphorous was observed till 30th day. The increase in phosphorus level in all the groups might be due to osteoblastic activity and collagen synthesis at defect site. The rate of bone remodelling also influenced the changes of serum phosphorous level and increased mineralization resulted its declining trend. A similar observation in changes of serum phosphorous level was also recorded by Goretti and Alon (2012); Julie (2005); Mahendra *et al*. (2007); Mathew (2009) and Manjunath (2010). In contrast to the present findings Pandey and Udupa (1980) and Rajhans (2013) observed decrease in serum phosphorus level in post-operative days in dogs.

Radiographical interpretation

The radiograph taken immediately operation in Group I showed sharp and pointed bony edges in the proximal and distal part of the ostectomized radial bone (Fig. 1).

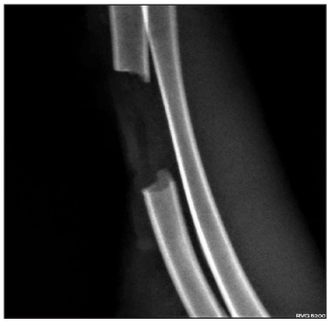

Fig. 1: Radiograph on 0th Day in Group I

On 20th post-operative day, it revealed irregular border and feathery/ cloudy appearance of bony growth either ends of the bone defect (Fig. 2).

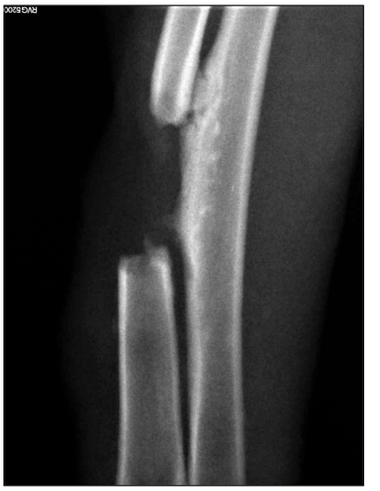

Fig. 2: Radiograph on 20th Day in Group I

Mild periosteal reaction in the cranial border of ulna was visible and similar observation also recorded by Bishnoi (2013) within 2-4 weeks for surgical immobilization of fracture in dogs. Towards the end of 40th post-operative day, even bony formation and union from proximal to distal ends at the caudal aspect of the radial bone defect was visible (Fig. 3).

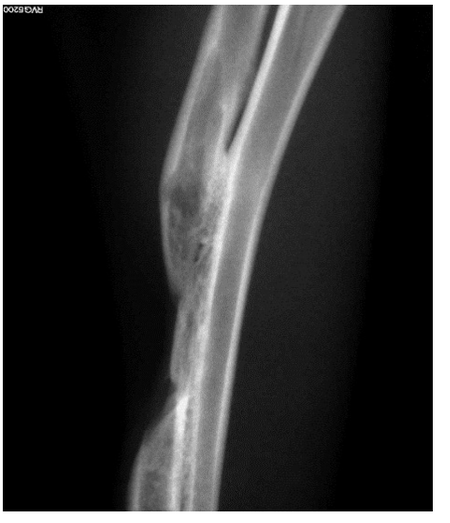

Fig. 3: Radiograph on 40th Day in Group I

Incomplete bridging of the proximal and distal ends of the radius on the posterior aspect of radius could be detected on 60th day radiograph (Fig. 4).

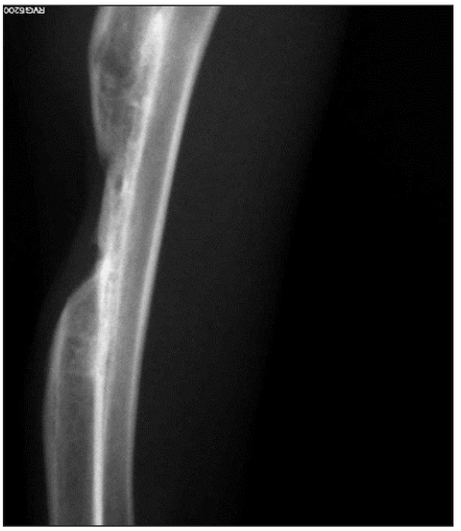

Fig. 4: Radiograph on 60th Day in Group I

The post-operative radiograph in Group II revealed dense radio-opaque materials at the site of the defect extending from the proximal to the distal ends of radial bone defect (Fig. 5).

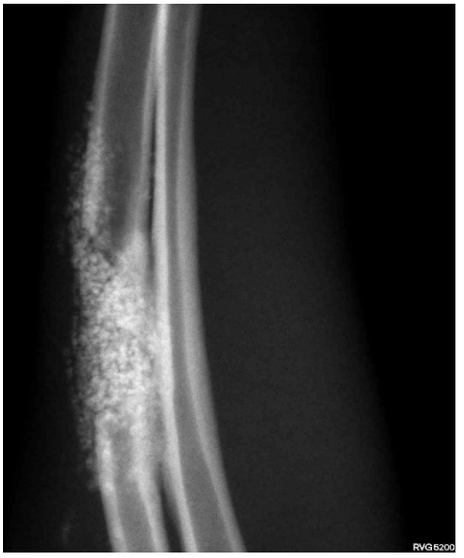

Fig. 5: Radiograph on 0th Day in Group II

The radio-density of the bone defect on 20th day in Group II appeared denser than its 0th day which may be attributed to the osteogenic property of PRP + β-TCP (Fig. 6).

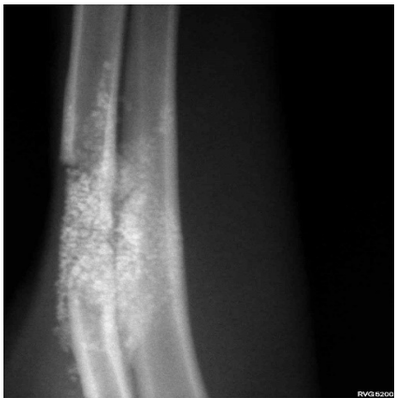

Fig. 6: Radiograph on 20th Day in Group II

Nandi *et al.* (2008) observed that porous β-TCP implants stimulate extensive bone formation over the bone defect suggestive of its osteoconductive property. In contrary to author's findings Van Hamert *et al.* (2004) recorded that the appearance of β-TCP was similar to the density of bone in early phase with resorption of β-TCP and bone remodelling occurred thereby density decreased more. On 40th day in Group II, radiograph showed a large amount of radio-opaque granular tissue extending from cranial to caudal aspect of the proximal and distal ends of the radius. The radio-density was more in comparison to that of 20th day and the width of the implants reduced comparatively (Fig. 7).

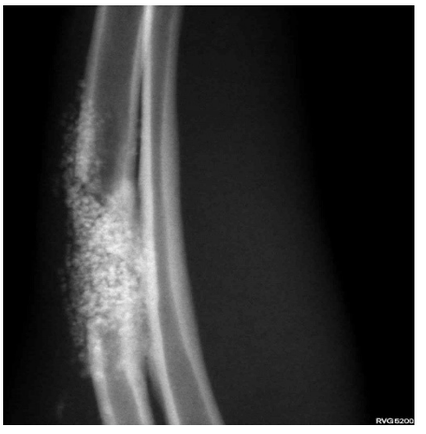

Fig. 7: Radiograph on 40th Day in Group II

On 60th day radiograph revealed complete bridging the gap with density almost as dense as before showing the initiation of the resorption phase of the healing process and defect space resembles the shape of normal bone (Fig. 8).

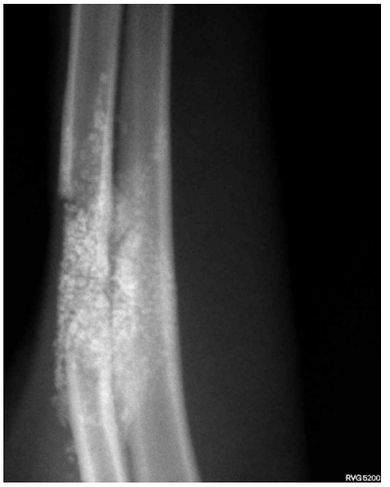

Fig. 8: Radiograph on 60th Day in Group II

In Group III the radiograph revealed radio-opaque materials at the site of the defect extending from the proximal to the distal ends of radial bone on 0th day (Fig. 9).

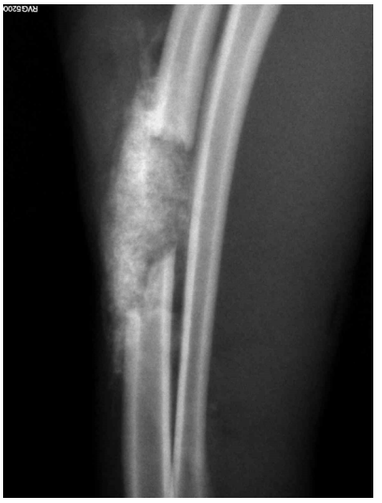

Fig. 9: Radiograph on 0th Day in Group III

By 20th day, radio-opaque dense granular density between the proximal and distal aspect of the radial bone defect (Fig. 10).

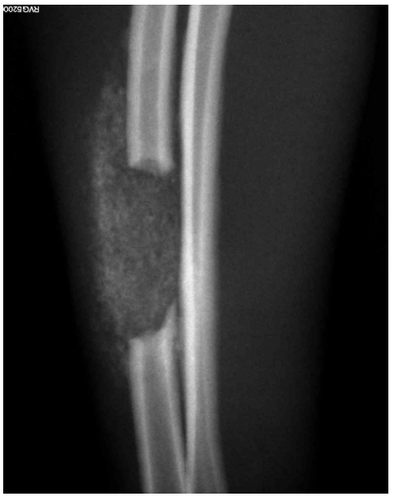

Fig. 10: Radiograph on 20th Day in Group III

On 40th post-operative day there was granular radio-opaque density extending from the proximal to distal aspect of radial bone defect which was looked denser than that of 20th day and the width of the implant was reduced comparatively without periosteal and soft tissue abnormality (Fig. 11).

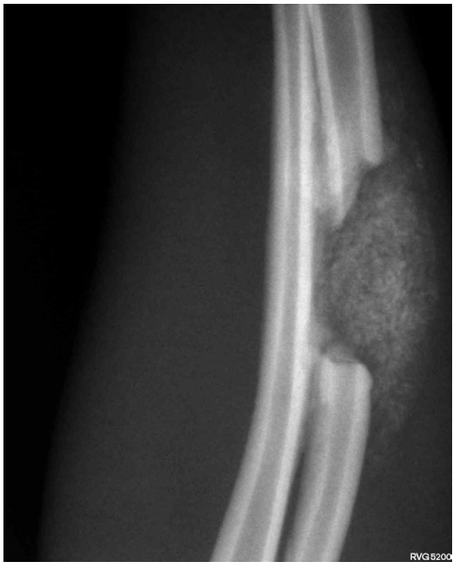

Fig. 11: Radiograph on 40th Day in Group III

Incomplete healing was noticed on 60th day radiographic observation (Fig. 12).

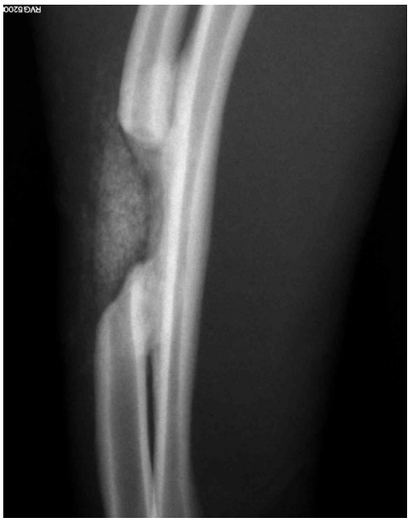

Fig. 12: Radiograph on 60th Day in Group III

The findings of various experimentation as recorded the role of DBM in new bone formation with complete resorption was in accordance with Frenkel *et al.* (1993) in canines; Bomback *et al.* (2004) in rats and Leupold *et al*. (2006) in rabbits. Furthermore, radiographs in Group I & III revealed incomplete bridging of the proximal and distal ends of the radius on the posterior aspect of radius but in Group II, large amount of granular radio-opaque material in between the fractured end as well as proximal and distal end of the radius suggestive of the proximal and distal end of fracture fragments appears to be united.

Histopathological interpretation

Photomicrograph of bone specimen in on 0th day (Fig. 13) showed the presence of osteoid (haversian canals), osteocytes found in lacunae in the bone matrix with Volkmann canal emerged from the haversian canals. Similar finding was also recorded by Kini and Naandesh (2012). In Group I, the microscopic evaluation on 20th day (Fig. 14), revealed extensive fibrous connective tissue in the defect area and development of bone marrow developed gradually. By 40th day (Fig. 15), microscopic examination revealed presence of fibroblasts and mild angiogenesis with infiltration of mononuclear phagocytic cells in the bone matrix. On 60th day (Fig. 16), microscopic evaluation presented bone marrow fibrosis with formation of haversian canal.

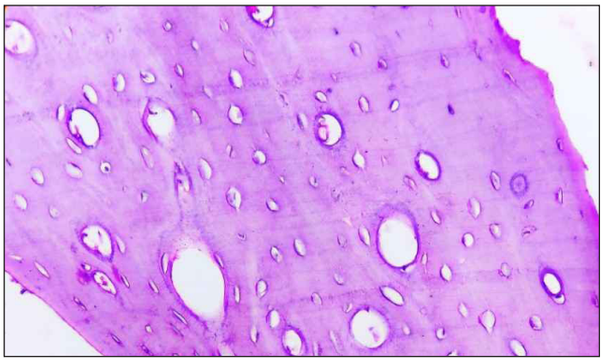

Fig. 13: Photomicrograph of the specimen at 0th at 10X (H&E)

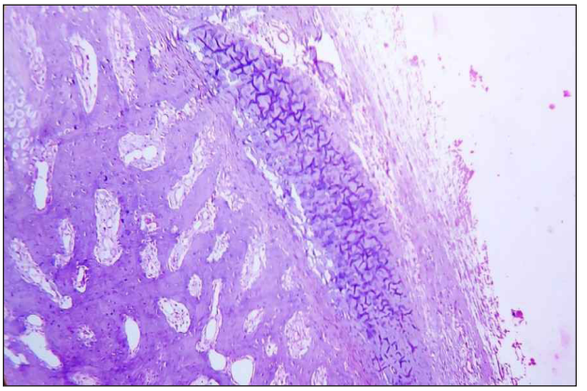

Fig. 14: Photomicrograph of the specimen at 20th at 10X (H&E) in Group I

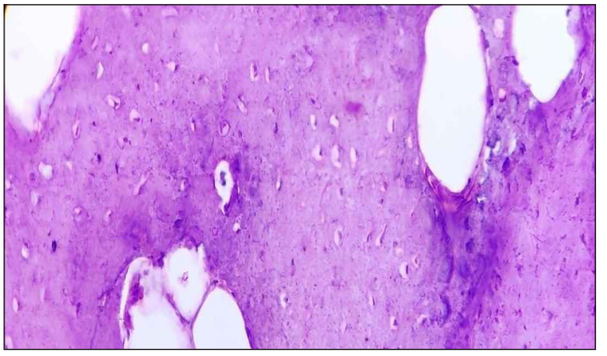

Fig. 15: Photomicrograph of the specimen at 40th at 10X (H&E) in Group I

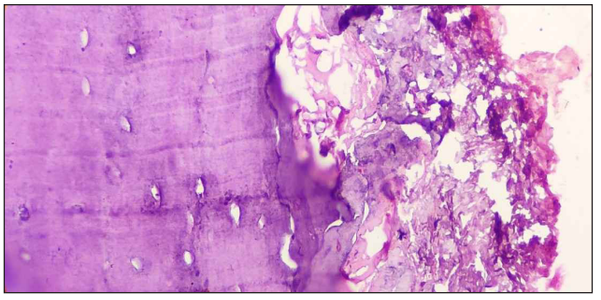

Fig. 16: Photomicrograph of the specimen at 60th at 10X (H&E) in Group I

In Group II, the photomicrograph taken on 20th day (Fig. 17) showed prominent osteoblastic and osteoclastic activity with presence of osteocytes dispersed over the collagen fibers. While by 40th day (Fig. 18), similar osteoblastic and osteoclastic activity compared to 20th day along with excessive vascularization. By the end of 60th day (Fig. 19), microscopic specimen showed prominent osteoblastic activity, neovascularization with formation of haversian canal. There was also evidence of fibro-cartilaginous activity.

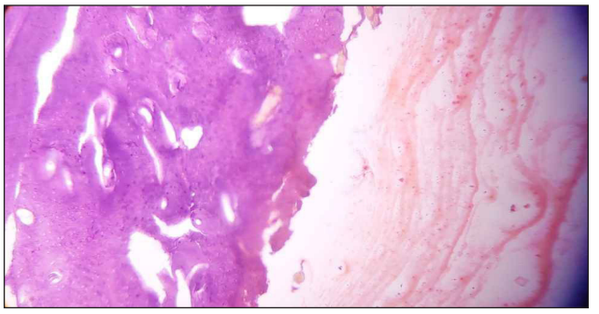

Fig. 17: Photomicrograph of the specimen at 20th at 10X (H&E) in Group II

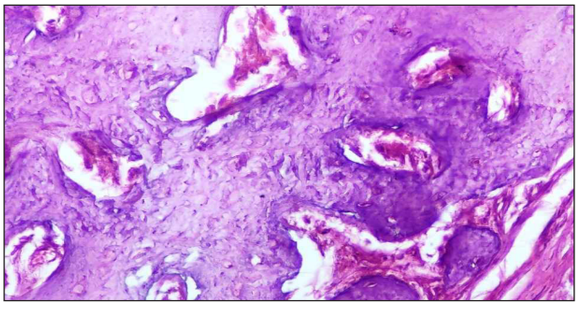

Fig. 18: Photomicrograph of the specimen at 40th at 10X (H&E) in Group II

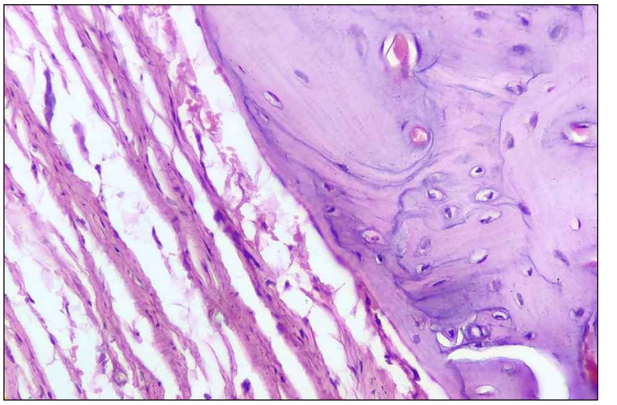

Fig. 19: Photomicrograph of the specimen at 60th at 10X (H&E) in Group II

The present study established a significant increase in histological bone area of Group II which is in agreement with the findings of Aghaloo *et al.* (2002). Kovacs *et al*. (2003) concluded that PRP accelerates the bone healing transformation of β-TCP more than β-TCP does when apply alone. Furthermore, the application of PRP in these treatments can increase the efficiency of new bone formation. Wiltfang *et al*. (2003) compared the use of β-TCP and β-TCP + PRP in and recorded average bone formation of 29% in the β-TCP alone group and 38% in combined group. In Group III, the photomicrograph taken on 20th day (Fig. 20) showed mild osteocytic activity along with the initiation of formation of haversian canals with some areas of infiltration of few mononuclear cells could be seen.

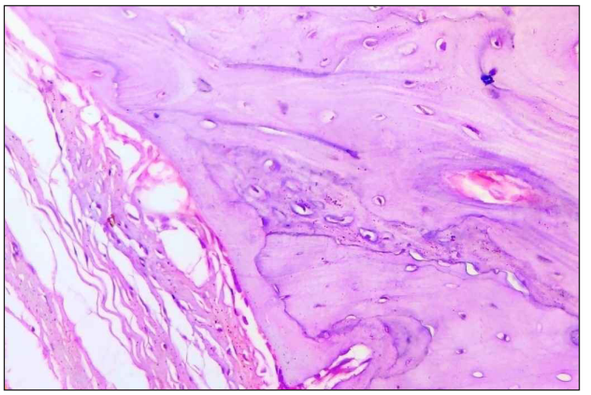

Fig. 20: Photomicrograph of the specimen at 20th at 10X (H&E) in Group II

Microscopic evaluation on 40th day (Fig. 21) showed extensive fibrosis, neovascularization and mild- to -moderate osteoblastic activity. By the end of 60th day in Group III photomicrograph (Fig. 22), showed mild-to-moderate osteoblastic and osteoclastic activity was evident with formation of haversian canals over the bone matrix. In agreement with the present findings Parizi *et al*. (2015) observed superiority in osteogenic properties with application of DBM when compared with control group. Martin *et al*. (1999) described that DBM appears to support new bone formation through osteoconductive mechanisms.

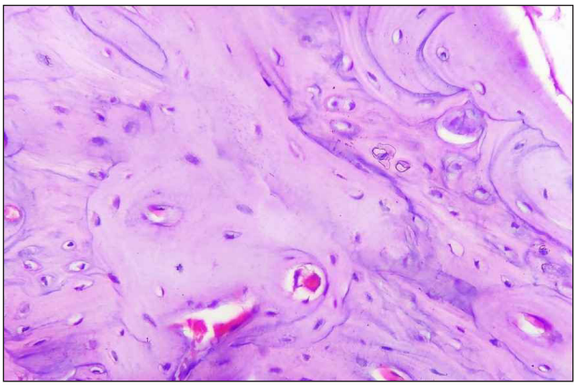

Fig. 21: Photomicrograph of the specimen at 40th at 10X (H&E) in Group II

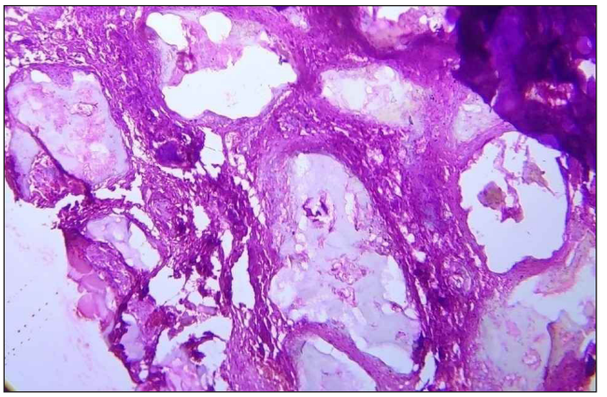

Fig. 22: Photomicrograph of the specimen at 60th at 10X (H&E) in Group II

CONCLUSION

From the above study it may be concluded that surgical reconstruction of radial bone defects in rabbits with application of PRP + β-TCP and DBM had little haematological changes. The biochemical alteration found during the study period was indicative of osteogenic activity healing bone. Radiographic study was suggestive of better long bone healing with application of PRP + β-TCP compared to DBM. Histopathological examination of the bone tissues revealed prominent osteoblastic activity in both treated groups; however the extent of same was more prominent in Group II which might be due to presence of PRP. The study promises clinical utility of both the treatments.

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