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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 1685-1688

www.thepharmajournal.com
Received: 12-08-2023
Accepted: 17-09-2023

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Efficacy of bone substitute (β tricalcium phosphate and hydroxyapatite) and osteoinductive platelet rich plasma (PRP) alone and in combination for healing of periodontium in dogs

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Abstract
For the present study, 16 canine cases with stage III and IV of periodontal disease, presented to Teaching Veterinary Clinical Complex, Jabalpur were randomly divided into group I (dental scaling + gingival flap surgery + doxycycline deposition in periodontal pocket, n=4), group II (group I treatment + β-TCP and HA, n=4), group III (group I treatment + PRP, n=4) and group IV (group I treatment + β-TCP and HA + PRP, n=4). Oral cavity examination and radiographic examinations of the animals were done on 0 day before treatment and on 5th, 10th, 15th, 20th, 30th and 60th day post treatment.
Significant reduction in probing depth was found in group IV followed by group II, III and I. Periodontal probing depth was recorded (6.00±0.41), (6.75±0.25), (6.50±0.29) and (6.75±0.48) in group I, II, III and IV respectively at day 0. It reduced significantly at day 5 after treatment in all the groups and recorded (2.50±0.50), (1.50±0.29), (2.25±0.48) and (1.00±0.00) mm in group I, II, III and IV respectively at day 60. On the basis radiographic examination, healing was fast and complete in group IV, followed by group II. In group I and III almost comparable healing was observed and in both the groups radiolucent furcation area was observed upto day 60.

Keywords: Periodontal disease, β-tricalcium phosphate and hydroxyapatite, platelet rich plasma (PRP), gingival flap surgery and periodontal probing depth

Introduction
The periodontium is the set of adjacent structures to the teeth that provides them support and protection. These structures are: gingiva, cementum, periodontal ligament and alveolar bone (Nair and Anoop, 2012) [17]. Periodontal disease refers to a group of inflammatory diseases caused by bacterial plaque in the periodontium (Pieri *et al*, 2012) [21]. Periodontal disease includes both gingivitis and periodontitis.
The primary causes of periodontal disease is bacterial plaque and tartar or calculus. Plaque is a soft, sticky, white material that adheres over the tooth surface and is composed of oral micro-organisms, salivary glycoproteins, extracellular polysaccharide like glucans and fructans, and exfoliated epithelial cells. (Kinane, 2001) [13]. The bacterial metabolic byproducts from plaque diffuse into the epithelium of the gingiva and causing gingivitis. Periodontitis is caused by the host's response to subgingival plaque. Inflammatory mediators produced by the host directly result in bone and tissue damage around the root. The bacteria themselves and their metabolic products also contribute to the bone damage (Dupont, 1997) [9]. In severe periodontitis, bacteria may pass through blood into various organs like heart, kidneys, liver and lungs and cause systemic diseases such as endocarditis, nephritis, hepatitis and pneumonia as complication of tooth loss (Albandar, 2005 and Rawlinson *et al.*, 2011) [1, 23].
Diagnosis of periodontal disease includes oral and dental examination, dental radiograph, microbiological culture and hematological examination. Treatment of periodontal disease depends upon the severity of the disease and ranges from conventional/nonsurgical procedures to various surgical procedures. In grade I and II periodontal diseases nonsurgical treatment is used. Nonsurgical treatment includes scaling, tooth polishing, close root debridement, sulcular lavage and systemic and local antibiotics along with oral hygiene and diet change. For grade III and IV periodontal disease, there are several surgical treatments which include open root planing and subgingival curettage, periodontal debridement, gingivectomy, periodontal surgery, special therapeutics and tooth extraction. The selection of appropriate treatment option can be done by evaluation of patient's overall health, cost of treatment and owner's

willingness to provide oral hygiene at home (Pihlstrom *et al.*, 2005 and Albuquerque *et al.*, 2012) [22, 3].
Latest therapeutic procedures in treatment of periodontal disease are laser surgery, stem cell therapy, guided tissue regeneration, use of platelet rich plasma (PRP), enamel matrix derivatives and fibroblastic growth factors (Carranza, 1990) [5].

Materials and Methods

For the study, all the dogs suffering with periodontal disease were placed into four different stages of periodontal disease (Colmery 2005) [6] and 16 dogs suffering from stage III and IV of periodontal disease were randomly divided into 4 equal treatment groups having four animals each.
In group I supra and subgingival scaling and gingival flap surgery were performed and slow release antibiotic doxycycline was deposited in subgingival periodontal pockets. In group II, along with group I treatment synthetic bone substitute "G-Bone" containing ceramic mixture of β tricalcium phosphate (TCP) and hydroxyapatite (HA) was filled in periodontal pockets. In group III, along with group I treatment platelet rich plasma (PRP) was instilled in periodontal pockets. In group IV, along with group I treatment a combination of β tricalcium phosphate and hydroxyapatite and platelet rich plasma was filled in periodontal pockets. General anaesthesia was given in all the animals for supra and subgingival scaling, flap surgery, oral cavity examination and radiography. Oral cavity examination including evaluation of periodontal probe depth using graduated periodontal probe and radiographic examinations of the animals were done on 0 day before treatment and on 5th, 10th, 15th, 20th, 30th and 60th day post treatment. The data thus obtained were statistically analyzed using one way ANOVA.

Results and Discussion

Periodontal probing depth was recorded (6.00±0.41), (6.75±0.25), (6.50±0.29) and (6.75±0.48) in group I, II, III and IV respectively. It reduced significantly at day 5 after treatment in all the groups and recorded (2.50±0.50), (1.50±0.29), (2.25±0.48) and (1.00±0.00) mm in group I, II, III and IV respectively at day 60 (Table 1). Significant reduction in periodontal probing depth was found in group IV followed by group II, III and I from day 5 onwards and remain continued upto day 60 (Figure 1). On the basis radiographic examination, healing was fast and complete in group IV, followed by group II. In group I and III almost comparable healing was observed and in both the groups radiolucent furcation area was observed upto day 60.
Similarly Kovacs *et al.* (2003) [14] reported that use of PRP accelerates the remodelling of new bone created by β-TCP in comparison to β-TCP alone in tooth extraction cavity in beagle dogs. Harnack *et al.* (2009) [11] and Ozdemir *et al.* (2012) [20] found no additional statistically significant improvements in intrabony defects when PRP was used alone. The finding of Dori *et al.* (2008) [8]; Piemontese *et al.* (2008) [24] and Dori *et al.* (2009) [7] also coincide with the finding of present study that the use of PRP alone failed to enhance the healing but gives good results when combined with the graft material.
In group IV early reduction in periodontal probing depth indicates rapid healing of periodontal tissue which occurred due the combined effect of bone substitute (G-Bone), Platelet Rich Plasma (PRP) and antibiotic deposited locally and supra and subgingival debridement. G-Bone contains β tricalcium phosphate and hydroxyl apatite. Calcium Phosphate biomaterials have similarity in composition to bone mineral, have ability to form bone apatite like material on their surfaces, osteo conductivity i.e. ability to provide scaffold for bone formation, ability to bind and concentrate endogenous bone morphogenetic proteins in circulation, and may become osteoinductive i.e. capable of osteogenesis. Therefore, these biomaterials may be useful as grafts for bone repair, augmentation, or substitution for regeneration of hard tissues (Legeros, 2002) [15].
The PRP reduces bleeding and enhancing soft tissue healing and bone regeneration. During wound healing, platelets are among the first cells to respond at a wound site, being critical to the initiation of this process (Albanese *et al.*, 2013) [2]. Besides their procoagulant effects, platelets form a rich source of important growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-b (TGF-b) 1 and 2, and vascular endothelial growth factor (VEGF) and (EGF) all of these are assist in hard and soft tissue wound healing (Anitua *et al.*, 2004; Marx, 2004; El-Sharkawy *et al.*, 2007; Nikolidakis and Jansen, 2008) [4, 16, 10, 8].
In group II healing in furcation defect was good due to the osteoconductive property of bone substitute that enhance the bone repair. In group III where PRP was used alone, healing was not as good (Figure 4) as in group IV (Figure 5) and II (Figure 3) may be due to the limited role of PRP in absence of bone substitute as well as leakage and loss of PRP from the periodontal pockets as no barrier was filled there. Group I also showed less healing (Figure 2) as compared to other treatment groups. Though, debridement and antibiotic deposition further controlled infection but healing was slow in the absence of any osteoconductive or osteoinductive material.

Table 1: Values of periodontal probing depth (mm) in dogs suffering with periodontal disease

Intervals (Days)	Group I	Group II	Group III	Group IV
0	6.00^{a}±0.41	6.75^{a}±0.25	6.50^{a}±0.29	6.75^{a}±0.48
5	5.25^{b}±0.48	6.00^{b}±0.41	5.50^{b}±0.29	5.75^{b}±0.48
10	4.50^{c}±0.29	5.00^{c}±0.41	5.00^{bc}±0.41	4.75^{c}±0.48
15	3.75^{d}±0.25	4.25^{d}±0.25	4.50^{c}±0.29	4.00^{d}±0.41
20	3.25^{d}±0.48	3.75^{d}±0.48	3.75^{d}±0.25	3.25^{e}±0.25
30	2.50^{e}±0.50	2.75^{e}±0.48	2.75^{e}±0.25	2.25^{f}±0.25
60	2.50^{e}±0.50	1.50^{f}±0.29	2.25^{e}±0.48	1.00^{g}±0.00

ABC values with different superscript between interval differs significantly (p<0.05).

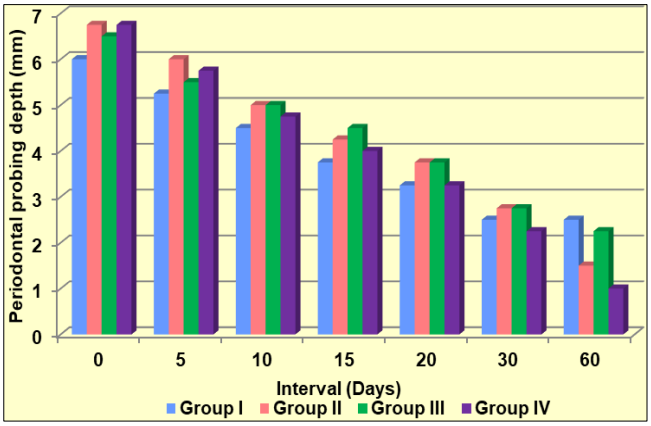
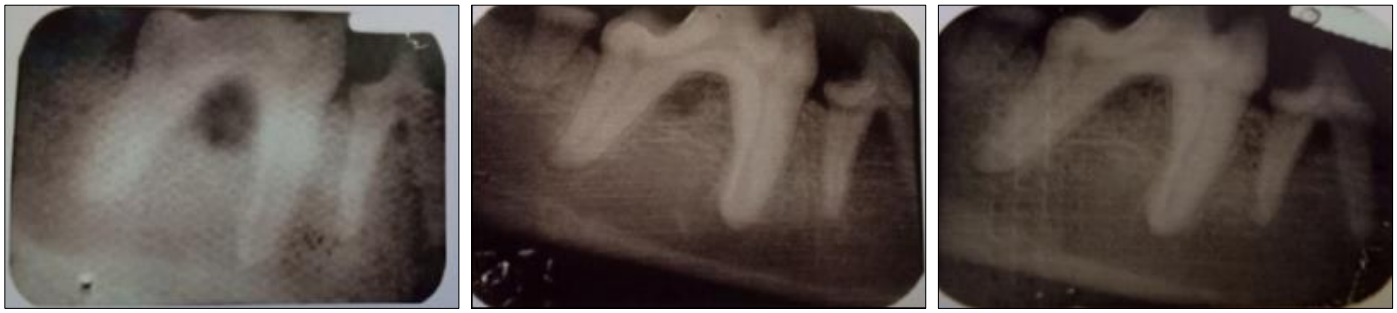
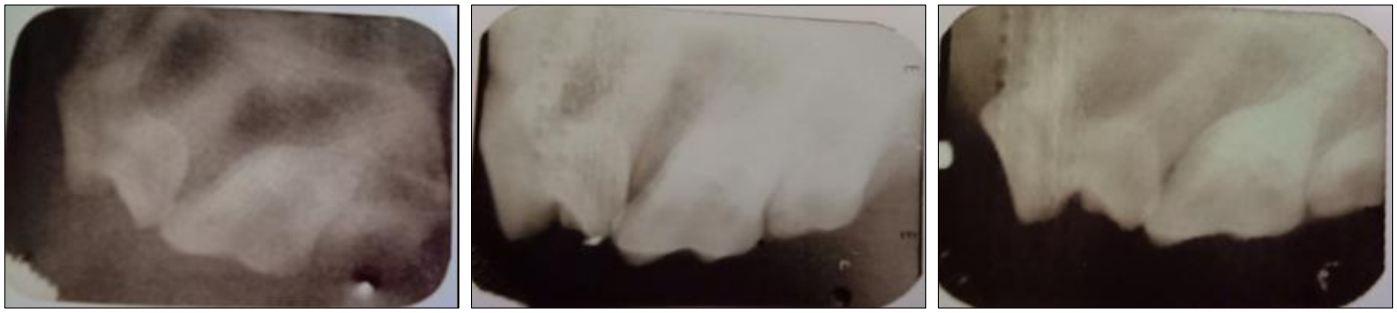


Fig 1: Values of periodontal probing depth (mm) in dogs suffering with periodontal disease



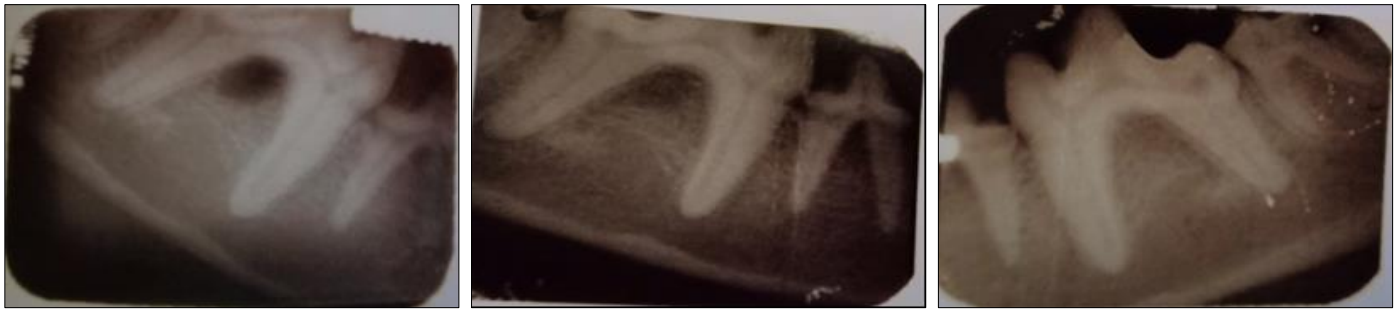
Day 0 Day 30 9 Day 60

Fig 2: Radiographs showing bone healing at furcation area of affected tooth at day 0, 30 and 60 in group I



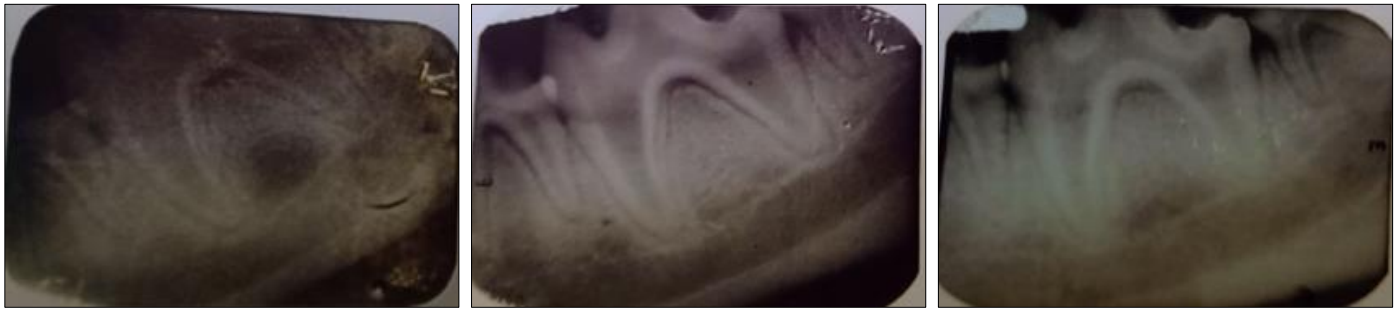
Day 0 Day 30 Day 60

Fig 3: Radiographs showing bone healing at furcation area of affected tooth at day 0, 30 and 60 in group II



Day 0 Day 30 Day 60

Fig 4: Radiographs showing bone healing at furcation area of affected tooth at day 0, 30 and 60 in group III



Day 0 Day 30 Day 60

Fig 5: Radiographs showing bone healing at furcation area of affected tooth at day 0, 30 and 60 in group IV

Conclusion

Significant reduction in probing depth was found in group IV followed by group II, III and I. radiographic examination also revealed rapid and complete bone fill in group IV followed by group II. Combination of doxycycline, bone substitute (β tricalcium phosphate and hydroxyapatite) and platelet rich plasma (PRP) in group IV showed early and complete healing of periodontal tissues.

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