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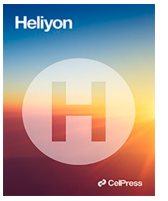

Research article

An evaluation of osseous regeneration capability of novel autogenous tooth graft along with orthobiologics for long bone segmental defects

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ABSTRACT

Extracted tooth, is predominantly considered a medical waste but tooth and bone evince similitude in biochemical composition, so tooth may be considered as bone graft material. We selected twenty-four adult rabbits with age and body weight ranges of 1–3 years and 2–4 kg respectively, regardless of sex and breed. These rabbits were allocated into four groups i.e., J, K, L, and M. Autogenous tooth graft was acquired from the individual's incisor. In group J (control), tooth graft alone was used at the mid shaft radius fractured site. For group K, tooth and bone marrow aspirate (BMA) were applied. In group L, tooth-platelet rich plasma (PRP) was administered while for group M, tooth-decellularized fish scale (DFS) was engrafted at the location. The research was conducted for 4 months and parameter evaluation was done on 0, 1st, 7th, 15th, 30th, 45th, 60th, 75th, 90th, 105th and 120th days. The therapeutic regimens were extensively appraised in terms of physiological vitals, hematology, serology, bone biomarkers, mechanical assessment, radiography and histomorphometric parameters. We noticed appropriate osteointegration of autologous tooth with the fractured site, good healing and bone remodeling in all groups with superior to lower trends in Tooth-BMA, Tooth-PRP, Tooth-DFS, and Tooth-solo groups respectively. Though usage of aforementioned regimens *in-vivo* needs further trials but overall, we may suggest that autogenous tooth is not only a novel and viable graft in solo but its healing capacity, osteointegration and firm callus formation can be augmented with appropriate orthobiologic materials and in future may be useful for bone defect treatments, not only in animals but humans as well.

1. Introduction

Lost parts of skeleton and complicated fractures are commonly regenerated with fresh autogenous bone grafts, as a gold standard. To obviate associated graft harvesting problems, additional surgeries, bone resorptions, infections, etc., bone substitute materials are in need [1]. In general, extracted tooth is considered medical waste, but its components (organic and inorganic) are in proximity to bone tissue. Tooth main constituents include various calcium types, collagen-I, polypeptides, signaling molecules, and numerous growth factors (GFs) that can promote bone healing [2,3]. All these show osteoinductive, osteoconductive, and biocompatible properties as bone graft material [4]. The afore-factors have drawn researchers' attention that tooth is a viable bone graft alternative

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and in need to establish evidence of its regenerative potential, which may lead tooth transformation from medical waste to innovative biomaterial, as effective and safe.

Usually, the healing of complicated bone anomalies is a slow process, regulated by numerous bio-mechanical stimuli. Recovery speed can be escalated by several biological materials and substrates called orthobiologics, mostly derived from natural body substances including body grafts, autogenous blood, bone marrow aspirate, platelet rich plasma, etc. [5].

Bone marrow aspirate (BMA) is affluent with mesenchymal stem cells (MSCs) having multipotent plasticity, to differentiate into diverse cell lineages of bone, cartilage, tendon, nerves, etc. This omnificence exhibits a substantial therapeutic role in damaged bone repair, by demonstrating osteoblasts proliferation with appropriate growth factors and vasculogenesis [6].

Platelet rich plasma (PRP), with supraphysiological levels of platelets, has manifested thrombocyte-derived factors that activate proliferation and chemotaxis of MSCs, osteoblasts, numerous GFs (PDGF, TGFs, IL-1, VEGF, etc.) and trigger angiogenesis for speedy regeneration of injured tissue [7].

Among natural orthobiologics, various materials including collagen, hydroxyapatite (HA), cellulose, etc. are of substantial significance. Among them, hydroxyapatite and collagen are considered a better choice, as these are also major bone tissue components [8]. Inter alia, natural sources to replete damaged bone, decellularized fish scale (DFS) may be a good consideration as it is highly enriched with HA and collagen type-I [9]. Marine collagen and HA exhibit bioactive properties like structural strength, biological activity, osteoconduction, homeostasis, and neo-angiogenesis rendering it an appropriate choice to be considered as a novel orthobiologic [10].

Autogenous bone is a well thought-of benchmark for bone grafting but with limitations and complications. Though, the extracted tooth is considered a biological waste, but bone and tooth have biochemical similarities, leading to the idea of its use as bone regenerative material. Moreover, orthobiologics are substances that enhance cure of musculoskeletal injuries by divergent mechanisms. Thus, keeping these all-in view, current study is designed to develop an understanding of the usage of the tooth as autograft with various orthobiologics (BMA, PRP, DFS) as adjunct regimens to treat long bone fractures, as very limited literature to assess their efficacy is available to-date.

2. Methodology

2.1. Animals' selection

A total of twenty-four adult rabbits with age and body weight range, 1–3 years and 2–4 kg respectively, regardless of sex and breed were selected. Ten days before trials, they were accommodated at the experimentation center of Surgery Department, for detailed health checks and to attune with the environment. Access to feed and water was ad-lib.

2.2. Experimental design and treatments

Twenty-four rabbits were allocated into four groups i.e., J, K, L and M. Autogenous tooth graft was acquired from the individual's incisor. In group J, tooth graft in solo, was used at fractured site. Tooth-bone marrow aspirate, tooth-platelet rich plasma, and tooth-decellularized fish scale were applied in groups K, L, and M respectively at the defect location. The study was conducted for four months and each parameter was evaluated on the 0,1st,7th, 15th,30th,45th,60th,75th,90th,105th, and 120th days with minor variations for serum analysis.

During preoperative evaluation, a thorough physical and clinical examination (CBC, LFT, RFT) was done to debar health issues in all animals. For parasitic infestation, rabbits were given ivermectin (Ivomec®, Merial) @ 0.2 mg/kg (SC). Three days preceding surgeries, prophylactic antibiotics enrofloxacin @10 mg/kg (Enroxsel®, Selmore) was administered (IM), as previously described [11].

2.3. Premedication and anesthesia

Sedation was done by Xylazine HCl @ 3 mg/kg (Xylaz®, Farvet) and Ketamine @ 20 mg/kg (Ketasol®, Indus Pharma) combination (IM). Propofol bolus @ 5 mg/kg (Pofol®, Dongkook Pharma) (IV), was used for induction purposes. Anesthesia was maintained by Isoflurane gas @ 3 % (Forane®, Abbott) as previously described [48].

2.4. Surgical procedures and graft-orthobiologics collection

Standard aseptic measures regarding equipment, surgical team and animal preparation were adopted as previously described [12]. After anesthesia, the radius was exposed via a small incision (2–3 cm) through the skin, sub-cut tissue and muscles. At the mid-shaft radius, an osteoperiosteal defect was induced, which was repleted with autogenous tooth and orthobiologics as per assigned groups. Standard procedures were opted to close incision site as previously described [13].

Fresh autogenous tooth graft was collected from rabbit's incisors with a tooth cutter, pulverized sufficiently and to prevent graft cells modifications, it was employed at defect site without chemical or radiation treatments. The remnant tooth part was filled with dental cement to prevent pain and post-operative complications.

Bone marrow aspirate was obtained from the iliac crest with a marrow biopsy needle (18-gauge). Penetration within the bone cortex via skin and muscles was achieved by slow needle rotation. After stylet removal, 5 mL BMA was garnered in an anticoagulant-

containing syringe, to be used at fractured site [14].

Blood in anticoagulant tube was collected to prepare platelet rich plasma, by double centrifugation method. First spin (@ 300g) was given for 5 min, which resulted in 3 layers i.e. top (Platelets and WBCs), central (WBCs), and bottom coating (RBCs). The upper- and middle-layer's superficial portions were decanted in a new sterile tube. The second spin was executed (@ 240g) for 8 min, resulting in platelet pellet formation at bottom of the tube. Upper layer was discarded and pure PRP was obtained after pellets were homogenized [15,16].

Fish grass carp was procured from the local fish supermarket. It was stored at 4 °C, after instant transportation to lab. Descaling was done and scales were rigorously cleansed with distilled water. Decellularization sol. [0.1M Triton x-100 & 0.05M tris-buffer (Sigma-Aldrich®)] was used to remove cellular components at 4 °C, for 3 days. Then, 70 % ethanol was used to rinse scales and were reposited in a phosphates-buffer at 4 °C [17].

2.5. Post-op management

Infection prevention was achieved by antibiotics, enrofloxacin @ 15mg/kg for 5 days (Enroxsel®, Selmore) (IM). To relieve pain, flunixin meglumine @ 2.2 mg/kg was administered for 5 days (Loxin®, Selmore) (IM) [18].

2.6. Parameters evaluated

2.6.1. Physiological indexes

Clinical vitals including temperature, pulse and respiration were recorded from rectum, femoral artery, and lungs respectively, on stipulated days [19,20].

2.6.2. Hematology indexes

Blood (1 mL) was collected from jugular vein and added to vacutainer with anticoagulant (K2 EDTA). Hematological analyzer (Abbott Cell-Dyn 4000) was used to perform a complete blood count (CBC) to exclude various abnormalities like infections, blood loss, anemia, dehydration, etc.

2.6.3. Serum analysis

To analyze calcium and phosphates, 5 mL jugular blood was collected in an additive-free vacutainer. It was stored to allow clot formation for 30 min at environment temperature, then centrifuged (@3000g) for 20 min to separate serum from blood cells [21]. Plastic freezing vials were used to store serum at −20 °C. Onwards, it was transferred to the University Diagnostics Lab (ISO: Certification No-17025 & lab 033), to analyze Ca and PO_4.

2.6.4. Bone turnover markers

Bone markers i.e., osteocalcin (OC) and alkaline phosphatase (ALP) were analyzed from the collected serum. For OC assay, procedures were opted as per maker's directions (BT-laboratory, Bio-assay Tech Lab, Birmingham, UK). For ALP, serum was transported to University Diagnostic Lab.

2.6.5. Mechanical assessment

Pain score and limb deformities were appraised by visual analogue scale (VAS) as previously described [22]. To assess pain scores in bio-medical research, "Colorado-University institutional animal care committee", directions were followed. Pain scores, for orthopedic manipulations, were counted from 3-0 (3 = Severe, 2 = moderate, 1 = mild, 0 = no) [23]. Limb deformities were earmarked 3-1 (3 = severe, 2 = serious, 1 = minor) as previously described [24]. To reduce grade 2 and 3 deformities, suitable support was furnished.

2.6.6. Radiographic evaluation

Radiographs were taken at days 30, 60, 90, and 120 by a digitalized unit (collimator: R = 20j, 2015; Shimadzu corps.) at the Department of Small Animal Clinical Sciences.

For the radius union score (RUSS Score), radiographs were allocated to a panel of 3 radiologists and 2 surgeons with diverse training backgrounds, who were blinded to data. RUSS system, designated scores based on callus formation, to anteroposterior and lateral views of radiographs. Each view was allotted score of 0–2, that were summed for a total scoring of 0–8 [25].

2.6.7. Histomorphometry

Histomorphometry was performed to appraise bone reconstruction site healing efficacy and cellular changes, at project completion. Bone samples were stored in formalin solution (10 %) [26] and shifted to histo-pathology laboratory. To score them: anew bone, cartilaginous tissue, fibrous part, and residue defect was estimated. The scoring was ranked 1–10 for osseous and cartilaginous tissue, whereas for fibrous part and residue defect, it was numbered 10-1 [27].

2.7. Statistical analysis

GraphPad Prism 6 (La Jolla, USA) was used to perform graphical analysis. Data analysis was done by two-factorial ANOVA with

repeated measures. Tuckey's test (Post Hoc test) was used to figure out significance among groups. Non-parametric, Friedman test was used to statistically analyze VAS, while significant difference was assessed via Dunn's test. The significance of values was manifested as "**P ≤ 0.01, *P ≤ 0.05".

3. Results

3.1. Vital clinical parameters

Temperature, pulse, and respiration (TPR) as clinical vitals were monitored on stipulated days. At day 1, an increase in temperature above normal physiological limits (n = 101.5–104.2 °F) was seen for each group. On day 7, a slight decline was observed but values remained at upper normal range. For the rest of the trial, temperatures further decreased and were noted fluctuating within normal limits. On statistical analysis, significance (P ≤ 0.01) between groups K-L and K-M at day 7 was noted (Fig. 1). An initial rise in pulse rate above physiological ceilings (n = 180–350 per min.) was seen on day 1, for all groups. Afterwards, it was observed within normal limits from day 7 till end of studies. Statistics manifested a significant difference (P ≤ 0.05) between group K and M at day 7 (Fig. 1). Respiration rate (n = 30–60 per min.) slightly elevated to upper normal limits at day 1, then petite decrease to mid-ranges was noted for rest of period, for each group. Statistically non-significance was noted among groups during studies [Fig. 1(A–C)].

3.2. Hematological parameters

At day 1, white blood cells slightly elevated above normal range (n = 5.2–12.5 × 10^{13}μl), in all groups. Then WBCs were noted within physiological limits, from day 7 to the end of trials. No significant difference was observed for groups during the experiment. Platelet number was observed within physiological limits (n = 250–650 × 10^{3}μl) for whole period. Though at day 1, a minor decline than initial was noted but overall values were observed within normal, for each group. No statistical differences were present among groups during studies. Red blood cells and hemoglobin minutely decreased below normal range (RBCs: n = 5–8 × 10^{13}μl; Hb: n = 10–17 g/dl) at day 1. On day 7, an uptrend in both parameters manifested the values in normal limits. From day 15 to the trial conclusion, RBCs and Hb with slight fluctuations, were observed within physiological range. Non-significance was noted among groups at stipulated times [Fig. 2(A–D)].

3.3. Serum analysis

Serum calcium manifested a slight increase at day 1, within normal ranges. Then a downtrend below physiological levels (n = 11–14 mg/dl) was seen at day 3, in all groups. On day 7, a sharp rise above normal limits was noted. From day 15 to the end of trials, the values with slight variation were noted within normal range. Statistical analysis showed a significant difference between groups J-K, K-L (P ≤ 0.01) and K-M (P ≤ 0.05) on day 3 and groups J-K, J-L, J-M, and K-L (P ≤ 0.05) at day 7. For the remaining studies, no significant difference was observed among groups.

Phosphates revealed an increase above normal (n = 4–6.5 mg/dl) on day 1, for each group. At day 3 a minor decrease in values, still above normal was noted. Onwards, an up peak till day 30 was observed. From day 45 to day 120, a gradual decline within physiological range was noted. Statistics revealed non-significance between groups throughout the trial [Fig. 3(A and B)].

3.4. Bone biomarkers

Alkaline phosphatase revealed slight ascension above normal range at day 1 (n = 12–96 U/L). A sharp surge peak was seen at day 7, for all groups. On day 15, a minor decline was noted but the values were sufficiently above physiological limits. From day 30–75, a steady gradual downtrend was observed for all groups, but ALP levels were still above normal. From day 90 to the end of studies, the values were noted within normal ranges. Statistical significance (P ≤ 0.01) was observed among all groups except K-L on day 7 and J-M, K-L on day 15. At day 30, significance (P ≤ 0.01) was seen for J-K, K-L and K-M groups. At 45th day, it (P ≤ 0.01) was only present

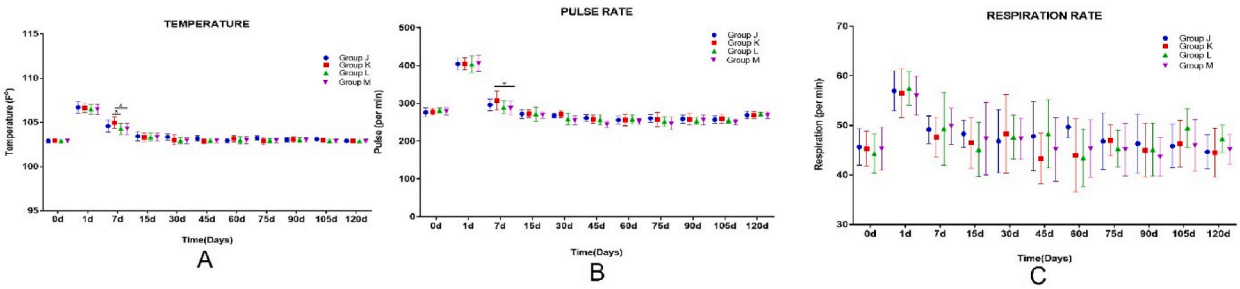

Fig. 1. Time-point changes in clinical vitals: (A)-temperature, (B)-pulse (C)-respiration among groups. Significance * (P ≤ 0.05) for K, L and M groups recorded on assigned days. For respiration, non-significance was seen among groups at designated time slots.

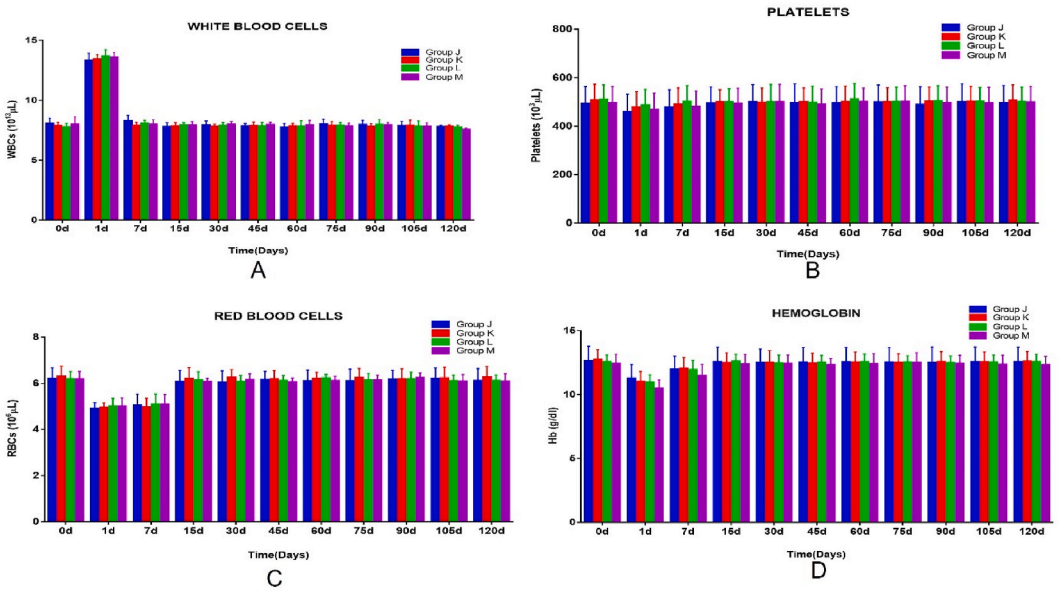

Fig. 2. Time-point changes in hematology: (A)-WBCs, (B)-platelets, (C)-RBCs and (D)-Hb among groups. For all parameters, non-significance was seen for all groups at designated time slots.

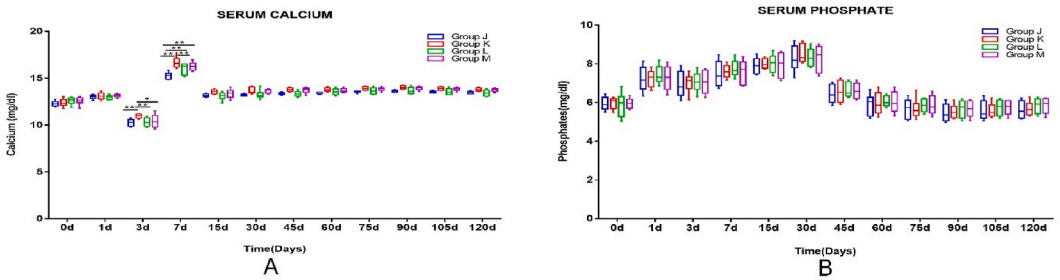

Fig. 3. Time-point changes in biochemical markers: (A)-calcium, (B)-phosphates among groups. For calcium, significance * ($P \leq 0.05$), ** ($P \leq 0.01$) for K, L and M groups recorded on assigned days. For phosphates, non-significance was seen among groups at designated time slots.

for groups J-K and K-M. Non-significance was recorded among all groups for the rest of trials.

Osteocalcin (OC) showed peak levels at day 7 after an initial slight surge at day 1, for each group. From day 15, a gradual and consistent decline in OC was noted that continued to the end of trials. Significance ($P \leq 0.01$) for groups J-K, J-L and K-M at day 7 was seen. At day 15, a significant difference was noted between groups J-K, J-L, J-M, K-M ($P \leq 0.01$) and K-L ($P \leq 0.05$). On day 30, statistical difference was noted for groups J-K, J-L, J-M and K-M ($P \leq 0.01$). A difference was observed between groups J-K, J-L, and K-M ($P \leq 0.01$) on day 45. At day 60, statistical difference was noted for groups J-K ($P \leq 0.01$), J-L and K-M ($P \leq 0.05$), while at day75 significance ($P \leq 0.05$) was observed only for groups J and L. Non-significance was recorded among groups for rest of trial period

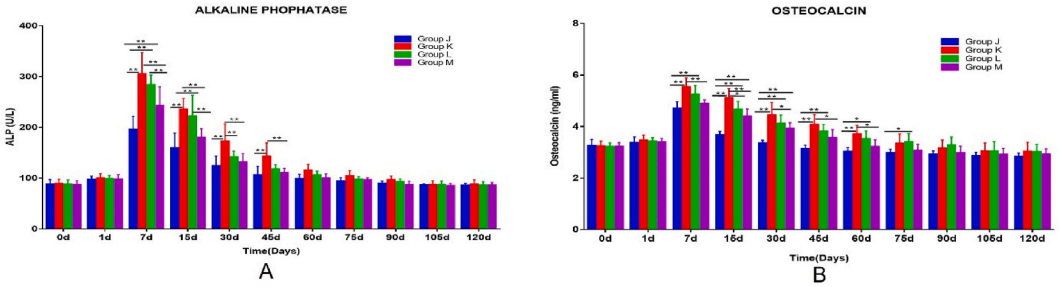

Fig. 4. Time-point changes in bone-markers: (A)-alkaline phosphatase, (B)-osteocalcin among groups. Significance * ($P \leq 0.05$), ** ($P \leq 0.01$) for J, K, L and M groups recorded on assigned days.

[Fig. 4(A and B)].

3.5. Mechanical assessment

Pain scores were at peak level on day 1 for each group, with gradual down-trend to end of studies. The least pain was observed in group K, while an increasing tendency was noted for groups L, M, and J, respectively. Statistical difference ($P \leq 0.01$) was noted between groups J and K (Fig. 5).

Limb deformity was observed in all groups. Rabbit J6, showed grade-III deformity on day 30, which was noted at grade-II from day 45–75. Onwards, for remaining trial it further reduced to grade-I. For animal L2, the deformity level was grade-II on day 45 and day 60, that reduced to grade-I afterwards. In M3 rabbit, it was noted at grade-III on days 30 and 45, then declined to grade-II at days 60–75. It further demoted to grade-I from day 90 to day 120. For the M6 animal, grade-II deformity was noted at days 60 and 75, that reduced to grade-I for remaining period. Statistical difference ($P \leq 0.01$) was seen only between groups K-M [Fig. 5(A and B)].

3.6. Radiographic evaluation

Radius union score was observed highest for group K, while down-trends were noted in the L, M, and J groups, respectively. Only significance ($P \leq 0.05$) was observed between J and K groups [Fig. 6(A)][Fig. 7(A–D)].

3.7. Histomorphometry

During histomorphometric analysis, maximum bone formation and cartilage conversion with the least fibrous tissue and residual size was noted in group K, followed by groups L, M, and J, respectively. Non-significant difference was seen among groups for the whole trial period [Fig. 6(B)]. [Fig. 8(A–D)].

4. Discussion

Clinical vitals of temperature, pulse and respiration were assessed to monitor rabbit's physiological conditions post-surgery. At day1, increase in all parameters may be associated with a 'physiological fever response' [28] due to elevated thermostatic set point, owing to surgically induced inflammation, post-operative stress factor, foreign object exposure during operations etc. [29]. From day 7 to the end of trial, the values were seen within the normal range. On initial days, significant difference was noted between groups for temperature and pulse but not evident for respiratory rates [30,31].

Hematological evaluation depicted an increase in WBCs at day1 above the normal range, for each group, which may be related to stress (physiological, perioperative, postoperative), generalized surgical inflammation response, extrinsic object hazard amid operations, anesthetic drugs, etc. [32]. From day 7, WBCs levels were observed within normal range. Non-significance was noted among groups during trials [33]. Platelet levels manifested an initial decrease, within normal range, at day1 that may be associated with platelet migration to the damaged site, their aggregation and plug formation, perioperative fluid administration, post-anesthesia delayed resumption of hepatic functions, etc. [34]. From day 7, the values were seen fluctuating within normal ranges. Non-significance was noted among groups for the trial span [35]. RBCs and Hb declined towards lower physiological levels on initial days, in each group. For the rest of the period, both values were noted within median normal limits. Nagra et al. [36] reported that the initial decrease may be associated with vasculature damage and blood loss during surgery, perioperative manipulations reduced erythropoiesis due to surgical inflammation, etc. Non-significance was observed among groups for entire term [37].

Serological studies for calcium and phosphates revealed calcium values below the normal range at day 3 after a slight increase at day1, in each group. This initial hypocalcemia may be associated with post-fracture negative balance due to increased urinary excretion of Ca and is also linked with absorption of fractured site trabeculae. Ca peak was noted at day 7 in each group, indicating glutted Ca blood levels, supporting Ca-ion deposition at the fractured site and new callus formation. Ca levels were near upper ranges for each group, from day 15 to end of the trial, revealing that soft callus is being replaced by hard callus due to its deposition at the site

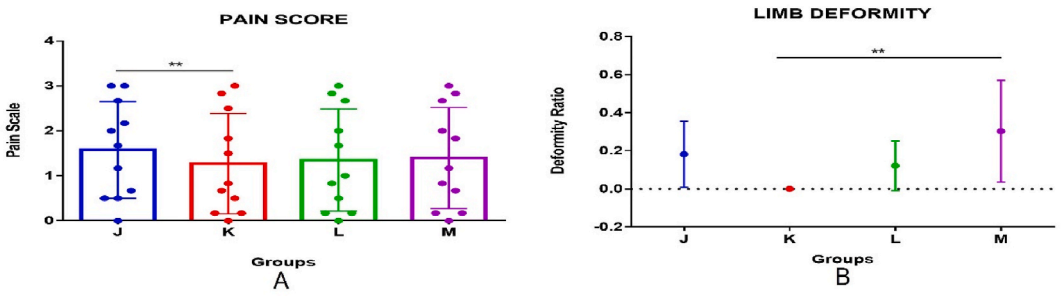

Fig. 5. Time-point changes in (A)-pain score, (B)-limb deformity among groups. Significance* ($P \leq 0.01$) for J, K and M groups recorded on assigned days.

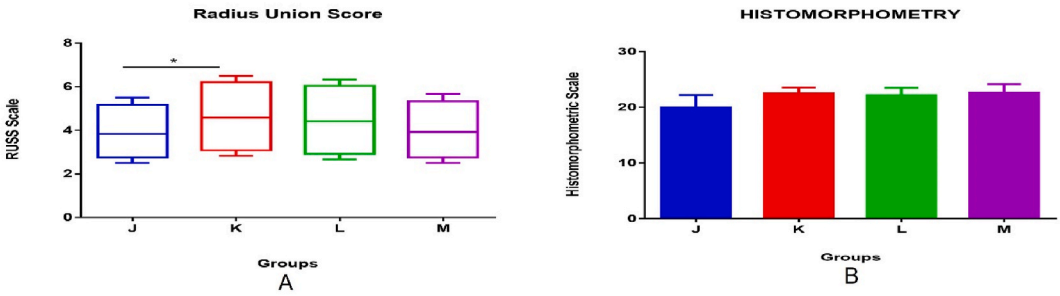

Fig. 6. Time-point changes in (A)-radius union score, (B)-histomorphometry among groups. For radius score, significance*($P \leq 0.05$) for J and K groups recorded on assigned days. For histology, non-significance was seen among groups at designated time slots.

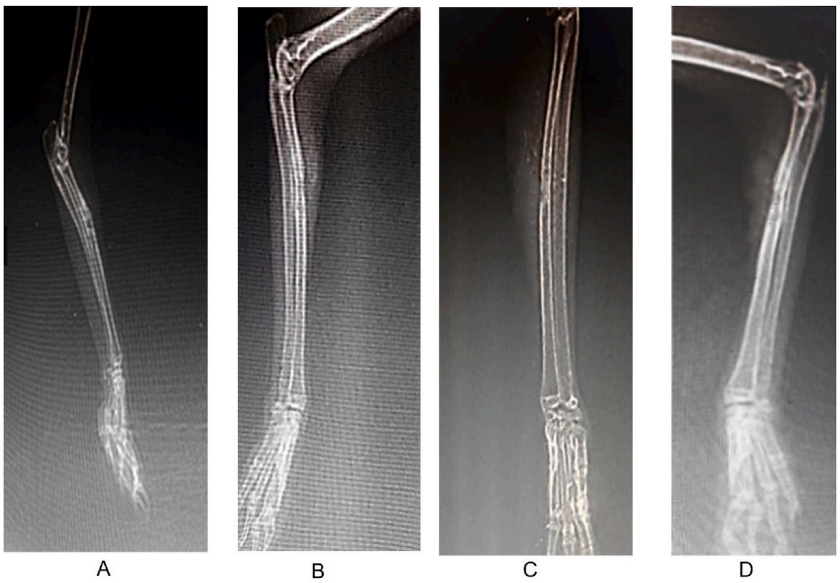

Fig. 7. Osseous healing/callus genesis in groups J (A), K (B), L (C) and M (D) at trial end. Best to poor rehab in groups K, L, M and J, respectively.

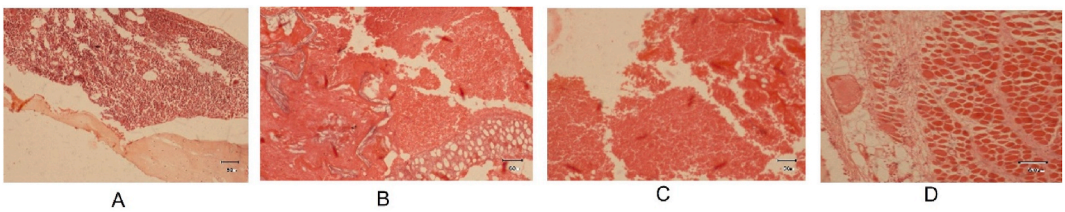

Fig. 8. Histomorphometry for groups J (A), K (B), L (C) and M (D) at trial end. Best to poor rehab in groups K, L, M and J, respectively.

[38]. A significant difference was noted between groups J-K, J- L, J-M, K-L, and K-M at day 3 and day 7 [39]. An increase above normal physiological limits was seen in phosphates from day 1 to day 45 except for a slight dip at day 3. Bauer et al. [40] reported that the hyperphosphatemia may be associated with bone and tissue trauma, fractured bone cell disintegration or necrosis, and increased renal phosphorous retention that invokes body responses to initiate resorption and remodeling of fractured site. Non-significance was observed among groups for trial duration. Mineralization (Ca/PO_4) of fractured site and solid callus formation was found excellent in K-group, due to presence of mesenchymal stem cells and numerous factors and cytokines (VEGF, FGF, IGF-I,GMCSF etc.). Group L, manifested very good ion deposition, due to its growth factors (TGF-B, bFGF, PDGF, EGF, CTGF), chemokines, proteins that stimulate chemotaxis and proliferation of cells/molecules in this regard. Group M, showed good Ca/PO_4 at site and robust callus strength due to sufficient presence of collagen and HA in DFS, while group J was noted inferior to all mentioned groups.

Bone turnover markers including alkaline phosphatase and osteocalcin are indicative of osteoblasts activity. Activated osteoblast's plasma membrane shows high ALP enzyme localized activity [1], which is later released in the vascular stream along with osteocalcin

protein formation, during its fracture healing activity [41]. Thus, ALP and OC help with HA-crystal deposition, bone matrix mineralization, and osseous regeneration [42,43]. In our experiment, ALP and OC values showed an incline at day 1 and were maximal at day 7, then a slow gradual decline was noted but both remained sufficiently above normal limits till late trials. A significant difference was noted between groups at variable days [44]. In group K, abundant cytokines and MSCs not only transformed stem cells to osteoblasts but also activated existing ones as well, manifesting superior results for bone-turn over markers. For group L, growth factors and proteins (fibrinogen, fibronectin, vitronectin) not only proliferated inflammatory responses but also helped in osteoblasts epithelial trigger, development of CT and matrix. Turnover rate was found reasonable in M group J.

Visual analog scale (VAS) is a psychometric scale expressed by changed sensation and social behavior, which helps to understand varying pain degrees experienced by patients. Pain is a nervous system signal, indicating unpleasant body feelings, discomfort, and emotional distress. VAS is the most frequently used assessment tool to evaluate orthopedic pains and to measure the therapeutic effects of various procedures [22]. In our studies, pain scores were observed at peak at day1, in each group. Onwards, a gradual decrease was noted to the end of the study. Group K revealed the least pain intensity, while high to down trends were noted in groups J, M, and L, respectively. Statistical significance was recorded for the J and K groups, due to fast callus development and bone strength in group K than other therapeutic regimens [45]. Fracture-related limb deformities were noted in one rabbit of groups J and L, while in two animals of group M. The deformity was noted at grade-III in one rabbit of groups J and M each, while at grade-II in group L and 2nd rabbit of group M. An improvement was noted in all groups with time lapse. Significant difference was observed between groups K and M only [46,47].

Plain radiographs are considered the best single predictor of biomechanical strength during fracture healing, in vivo model. RUSS score provided a simple, dependable, and reproducible means to evaluate fracture healing at variable time intervals. Surgeons and radiologists demonstrated notable intra-viewer dependability, facilitating good comparison among various treatment regimens [25]. Our studies revealed that bone union was observed best for group K, and then a slower healing pattern was noted in the L, M, and J groups, respectively. Statistical significance was noted between groups J-K only. RUSS score demonstrated fractured gap fill and callus amount' that was best in K group than others.

Our histomorphometric analysis measured incipient cartilage, fibrous tissue, and residue bone defect size in addition to anew bone. These additional measures helped to identify various levels of new callus formation (soft or hard) and derived valuable information about the effects of various tissue-engineering regimens on the healing process [27]. Histological scoring in our studies revealed that group K showed maximum bone formation while a descending trend was observed in groups L, M, and J respectively. Mesenchymal stem cells of BMA, resulted in best in new bone formation, cartilage to bone conversion with least fibrous tissue and residual defect than PRP,DFS and solo groups.

5. Conclusion

Due to the proximity of tooth structure with bone tissue, our studies revealed that autogenous tooth as bone graft material showed good osteoinductive, osteoconductive, and bio-compatible characteristics with no immune reactions. Tooth-BMA graft with sufficient MSCs cells and growth factors, reasonably enhanced fracture healing with stable hard callus formation. The tooth-PRP graft (with more than 30 proteins and growth factors in platelets alpha-granules) also revealed promising results for bone healing, but the tooth-BMA graft was noted as worthier than the tooth-PRP one. Tooth-DFS (with HA and collagen-I) revealed many beneficial properties including non-toxicity, low-immunogenicity, osteoconduction, and biocompatibility but it lagged both regimens in recuperation speed and callus strength. Thus, our findings suggest that autologous tooth is a novel and viable bone graft material, and three therapeutic regimens are helpful to treat complicated bone issues regarding healing efficacy, osteointegration, callus robustness, etc. to declining orders as tooth-BMA, tooth-PRP, tooth-DFS, and tooth-solo. Furthermore, their in-vivo clinical relevance, indications, and advancement of these considerations to a greater extent may necessitate additional large-scale trials.

Limitations associated with the studies include, that if large bone defect size repair in need, only limited amount of autogenous tooth graft may be available.

CRediT authorship contribution statement

N. Hussain: Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M.A. Khan:** Writing – review & editing, Supervision, Project administration, Conceptualization. **A.K. Mahmood:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **M.Y. Tipu:** Writing – review & editing, Visualization, Supervision, Project administration. **S. Aslam:** Writing – review & editing, Visualization, Software, Formal analysis.

Institutional review board statement

The present study and all treatments were conducted as per directives assented to by the Ethical Review Committee (Ethical Approval No: DR-456, Dated: 17-05-2019), University of Veterinary and Animal Sciences, Lahore, PB, PK.

Data availability statement

Most data are available in manuscript figures/tables. Supplementary data files have been provided with the manuscript. Moreover,

the data will be available on request as well. This paper is a part of PhD studies. On paper publication, complete data set will be available in Higher Education Commission, Pakistan country directory database. Data can also be downloaded from link: https://drive.google.com/drive/folders/1OvymUJvjNyinOco16dmjgLCgBkRaJBlB?usp=sharing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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