Original Article

Evaluation of the effect of moringa oleifera gel and autologo platelet-rich fibrin in the treatment of rabbit intra bony defects. (Radio graphic and Histological study)

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Abstract

Purpose: Periodontitis is the most common condition, which causes bony defects; the ultimate goal of periodontal therapy is the regeneration of the destroyed tissues. There is always a need to search for better biomaterials that can be used for the treatment of intrabony defects. This study evaluated the effect of Moringa oleifera (MO) gel and platelet-rich fibrin (PRF) in the treatment of bone defects.

Hypothesis: We hypothesized that MO gel may increase the bone mineral contents and density of bone.

Methods: The study was conducted on 16 defects in 8 adult male rabbits divided into 2 groups; group (1) buccal bone defect treated with moringa hydrogel and PRF (right site), group (2) buccal bone defect treated with PRF (left site). Computed tomography (CT) radiography and histological examination were assessed at baseline, 14 and 28 days. The defects were induced in the form of one osseous wall defect between the 1st and the 2nd molars. Comparisons between groups were done using an unpaired t-test. For comparison within each group, analysis of variance (ANOVA) was used.

Results: CT radiograph results showed there was a significant increase in bone density at 28 days in group 1 than in group 2 (843.13 ± 97.82 to 713.0 ± 51.09). The *histological result revealed t*he defect area on the (PRF + Moringa) was almost filled completely by newly formed bone with few spots of retarded calcification. While (PRF) showed complete filling of the defect area by more fibrous tissue. The healing score showed a significant elevation of bone defect healing score in (PRF + Moringa group) when compared to (PRF group) at both times of evaluation.

Conclusion: Radiographical examination, and histological and healing scores confirmed the superiority of Moringa + PRF results in an increase in bone fill and density in induced periodontal intrabony defects regeneration. Clinical trials should be considered to detect the effectiveness of MO in intrabony defects.

Keywords: Intrabony defects, moringa oleifera gel, periodontal regeneration, platelet-rich fibrin

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INTRODUCTION

Periodontitis is a common cause of tooth loss in adults, and it is characterized by bacterial-induced inflammation and degradation of periodontal supporting tissue, which frequently leads to intrabony defects.[1] The fundamental goal of periodontal therapy is to not only stop the progression of periodontal disease but also to regenerate the periodontal architecture and function which includes biological events such as cell adhesion, migration, proliferation and differentiation in a coordinated sequence leading to the formation of new cementum on the tooth root and new periodontal attachment.[1]

The use of filler materials to regenerate periodontal deficiencies is the traditional method of periodontal regeneration. Many procedures involving autografts, allografts, xenografts and various man-made bone substitutes have been developed in the quest for efficient defect filling materials.[2]

Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are the two generations of platelet concentrates (PCs) obtained after centrifugation of autologous whole blood samples, respectively. Anti-coagulants are required for the production of PRP (the first generation of PCs) at the time of blood collection; when utilized in the gel form; bovine thrombin and calcium chloride are required to be added. On the other hand, PRF (second PCs generation), which is easier to make, is simply centrifuged blood with no additives.[3] Many growth factors such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epithelial growth factor (EGF) and fibroblast growth factor β (FGF-β) are all highly concentrated in both PRP and PRF, which have the ability to improve wound healing and periodontal regeneration by influencing neo-angiogenesis, cell proliferation, migration, differentiation and other cellular activities.[4]

Herbal extracts from several plants have been employed in bone healing. Among herbal extracts, Moringa oleifera (MO) leaf, which contains amino acids, fatty acids, beta carotene, minerals, vitamin E and flavonoids, is one of the natural components that offers many benefits.[5] These flavonoids have anti-inflammatory, anti-cancer, antibacterial, antiviral, immunomodulatory, antithrombotic and osteoprotection properties.[6,7]

Moringa leaf extract has been demonstrated to suppress the inflammatory pathway by inhibiting carrageenan in rats induced by oedema in prior studies. Bone resorption is inhibited by suppressing the inflammatory pathway, which may also promote the proliferation and differentiation of osteoblast cells.[8-10]

Bone deficiency is a major concern in all branches. Unfortunately, systematic reviews performed by Trombelli *et al*.[11] and Reynolds *et al*.[12] reported that there is insufficient evidence to support the clinical use of bone replacement graft materials in intrabony defects due to significant heterogeneity among included studies, and the use of bone grafts for the treatment of intrabony pockets does not show complete agreement because the inclusion criteria were not similar.

Thus, we hypothesized that MO with PRF could improve bone healing, which may, in turn, enhance the regeneration of bone tissue.

So, the aim of the present study was the histological and radiographic comparison of the osseous regeneration in the rabbit intrabony defect treated with autologous PRF and MO gel.

MATERIALS AND METHODS

The present study was carried out after the approval of the Research Ethics Committee (REC), Faculty of Dentistry Suez Canal University. The study was established according to the principles of the WHO standards 2011. Serial number: 384/2021.

Materials

preparation of Moringa

Cold aqueous extracts of fresh and dried leaves:[1] Fresh and dried MO Lam. leaves were weighed (100 g) and crushed straight in a grinder, then dipped in 400 ml cold distilled water into a conical flask stoppered with rubber corks and left for 7 days with intermittent shaking. Using sterile filter paper (Whatman no. 1, Sigma Aldrich,USA), the aqueous solvent was filtered into a clean conical flask and evaporated in a water bath at its boiling temperature of 100°C.[13]

Moringa gel preparation

Polyethylene glycol (PEG)[2], a synthetic substance that is non-toxic and inert and can be used in medicinal applications, was used to prepare a synthetic hydrogel. After making an aqueous PEG solution, the aqueous plant extract was dissolved in it and thoroughly mixed to achieve a homogenous solution. The hydrophilic gel's thixotropic behaviour guarantees that it remains stable when applied to the specimen.

The vial inversion method was used to assess the gel's thixotropic behaviour. Using a vortex genie (Scientific Industries, Inc, USA), the hydrogel in the vial was shaken and mechanically compacted for many seconds. The gel that had been converted to sol was left to set at room temperature until it returned to gel condition, which was assessed by visual examination of the bottle inverted.[14]

PRF preparation

Before sedation, 5 ml of blood was collected from each rabbit using capillary tubes from the inner canthus of the eye into syringes without anti-coagulants and centrifuged at 30.000 RPM for 15 min. To make a thin membrane, PRF was picked up and squashed between two sterile glass slides.

Methods

Animals, anaesthesia and surgery

Eight male New Zealand white rabbits (3.5–4 kg) were used. Animals were divided into two groups: group (1) the induced buccal bone defect treated with moringa hydrogel and PRF in the right site, group (2) the induced buccal bone defect treated with PRF in the left site. Before the start of the study, all rabbits were given a week to acclimate to the experimental circumstances. Animals were fed a conventional rodent meal and had unrestricted access to water, as well as be subjected to a 12-h light/dark cycle at room temperature of 18–22°C and relative humidity of 55–65%. For the duration of the trial, all animals were kept in their assigned cages.[3]

For general anaesthesia, 0.15 ml/kg xylazine hydrochloride and 0.35 ml/kg ketamine hydrochloride were injected intramuscularly. On the first day of the study, the surgical field was meticulously shaved in preparation for the surgical intervention, and then sterilized with 70% ethanol. An extraoral buccal approach was created through a full-thickness incision in the skin and underlying muscles.

In the mandibular alveolus, the distal and buccal roots of the first molar, and the mesial root of the second molar are then exposed via a flap raised without vertical incisions.

With the use of a stopper-premeasured tapered FG drill Azdent, China connected to a high-speed motor with copious physiological saline irrigation, the intrabony defect was then produced. The surgical defect measured 10 mm corono-apical (from the cementoenamel junction to the most apical edge of the defect) and 4 mm deep (buccolingual direction) from the surface of the alveolar bone to the lingual surface of the defect.

Finally, the flap was repositioned and sutured. Following surgery, each rabbit was placed on a heating blanket to help it recover from anaesthesia without becoming hypothermic, and then returned to its assigned cage. Carprofen (4 mg/kg) and (ceftriaxone 5 mg/kg) were given postoperatively for 7 days. After 7 days, the sutures were removed.

Animals were euthanized at 2 and 4 weeks to harvest the lower jaw. All surgery sites were visually evaluated before euthanasia to assess wound healing and detect any problems. Ketamine and xylazine were given intramuscularly as a premedication before the euthanasia.

Histological assessment and healing score

Immediately after euthanasia, the lower jaw was dissected and bone blocks containing the area of the osseous defect were removed. After fixation in buffered 4% formaldehyde solution for at least 2 weeks, bone blocks were dehydrated in ascending grades of alcohol. Histological sections were prepared perpendicular to the long axis of each defect in an anterior to posterior direction with a thickness of 5 mm. The specimens were routinely stained with haematoxylin and eosin stain.

Healing of bone defect was assessed on a scale of 1 to 10 based on the amount of new bone formed, quality of bone (woven vs lamellar), vascularization and presence or lack of inflammation as shown in Table 1.[15]

Radiographic examination

Computed tomography (CT) radiographs were taken for the live animals at baseline, 14 and 28 days later. Another monitor displayed cone-beam computer tomography (CBCT) images in the axial, sagittal, coronal and 3D views.

Bone density was assessed on a scale of 1 to 5 as shown in Table 2.

Statistical analysis

The statistical software for the social sciences (SPSS) version 26 was used to code and input the data (IBM Corp., Armonk, NY, USA). The mean and standard deviation were used to summarize the data. The unpaired t-test[16] was used to compare the groups. The repeated-measures analysis of variance (ANOVA) was used to compare serial measurements within each group.[17] Statistical significance was defined as a P value of less than 0.05.

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Table 1: The histopathological score of bone defect healing

Score	Bone defect coverage Percentage of defect bridged by bone	New bone type Nature of new bone within the defect	Vascularization Presence of vascularization within the newly formed bone	Inflammation Presence of inflammatory cells around the newly formed bone
0	0%	No new bone	No evidence of neovascularization	Abundant inflammation and evidence of encapsulation
1	1-24%	Predominantly woven	Few new vessels (<10)	Relative few (10–50) inflammatory cells present
2	25-49%	Predominantly lamella remodelled	Abundant neovascularization	No evidence of inflammatory cells present
3	50-74%	———	———————	———————
4	75-100%	———	———————	———————

Table 2: The radiographic score of bone defect

Type of bone	CT value	Comments
D1	>1250 HU	Homogenous and compact bone
D2	850-1250 HU	A thick layer of compact bone surrounding a core of dense trabecular bone
D3	350-850 HU	A thin layer of cortical bone surrounding a dense trabecular bone of favourable strength
D4	150-350 HU	A thin layer of cortical bone surrounding a core of low-density trabecular bone
D5	<150 HU	Very soft bone with incomplete mineralization

RESULT

Radiographic results

Both treated groups showed an increase in bone density after 14 and 28 days.

After 14 days: Group 1 (moringa hydrogel + PRF) showed that the area of interest/defect is totally surrounded by a bony edge being hazy anteriorly, well defined posteriorly, and group 2 (PRF) showed that the area of interest/defect is totally surrounded by a well-defined bony edge and ill-defined in the superior portion.

On comparison to baseline in both groups, sagittal cut showed an increase in the average and maximum density in the area of interest. Coronal cut also showed a newly formed focus of calcification in the superficial part of the area of interest.

After 28 days: Group 1 (moringa hydrogel + PRF): The area of defect is surrounded by well-defined bony edges, and group 2 (PRF) showed an area of interest/iatrogenic defect surrounded by bony.

On comparison to results after 14 days: There is an increase in the average and maximum density in the area of interest in both groups.

Comparison over time in each group

Group 1 showed a significant increase in bone density from baseline to 14 days (126.25 ± 11.2 to 611.5 ± 178.38) and between 14 and 28 days (611.5 ± 178.38 to 843.13 ± 97.82). *P* value is less than 0.05 as shown in Table 3 and Figures 1 and 3.

Group 2 showed a significant increase in bone density from baseline to 14 days (165.87 ± 69.29 to 504.25 ± 98.66) and between 14 and 28 days (504.25 ± to 713.0 ± 51.09) as shown in Table 4 and Figures 2 and 4.

Comparison between groups

There was a significant increase in bone density at 28 days' results in group 1 than in results of group 2 (843.13 ± 97.82 to 713.0 ± 51.09) as shown in Table 5 and Figure 5.

Histological and healing score results

After 14 days

On the Rt. side (PRF + moringa group), better healing signs were histologically observed, and the defect area was filled by organized non-inflammatory fibrous tissue with a higher percentage of defect bridging by newly formed bone. Regarding the defect area from the Lt. side (PRF), the defect area was partially filled by fibrous tissue with the presence of small fragments of newly formed bone. Most of the examined sections showed retarded healing signs as the defect area was filled by non-ossifying fibrous tissue; in which the characteristic storiform pattern was obvious with numerous multinucleated cells. Increased numbers of inflammatory cells were noticed within the fibrous tissue that fills the defect area. A small amount of newly formed woven bone was observed in a few sections [Figures 6 and 7].

After 28 days

The defect area on the Rt. side (PRF + moringa group) was almost filled completely by newly formed bone with few spots of retarded calcification in some instances. The surface of the bone facing the defect area was filled with an increased number of active osteoblasts. While Lt. side (PRF) showed complete filling of the defect area by more fibrous tissue with less inflammatory reaction and more vasculature. The defect gap was bridged by a moderate amount of newly formed bone [Figures 8 and 9].

Healing score

As shown in Table 6, a significant elevation of bone defect healing score was observed in Rt. side (PRF + moringa

Table 3: Bone density in Group 1 over time (baseline, 14 and 28 days)

	PRF + MORINGA		*P*-value between baseline and 14 days	*P*-value between baseline and 28 days	*P*-value between 14 days and 28 days
	Mean	Standard Deviation			
Baseline	126.25	11.20	< 0.001	< 0.001	0.002
14 days	611.50	178.38			
28 days	843.13	97.82			

Table 4: Bone density in Group 2 over time (baseline, 14 and 28 days)

	PRF		*P*-value between baseline and 14 days	*P*-value between baseline and 28 days	*P*-value between 14 days and 28 days
	Mean	Standard Deviation			
Baseline	165.87	69.29	< 0.001	< 0.001	0.004
14 days	504.25	98.66			
28 days	713.00	51.09			

Table 5: Comparison between groups over time

	PRF + MORINGA		PRF		*P*
	Mean	Standard Deviation	Mean	Standard Deviation	
Baseline	126.25	11.20	165.87	69.29	0.133
14 days	611.50	178.38	504.25	98.66	0.159
28 days	843.13	97.82	713.00	51.09	0.007

group) when compared to Lt. Side (PRF group) on both times of evaluation.

DISCUSSION

Periodontitis induces attachment and underlying bone loss, altering its architecture and leading to a variety of intrabony defects. Various clinical techniques have been developed to enhance periodontal regeneration, such as guided tissue regeneration (GTR), the use of bone grafts and the use of biologically active agents.[18] No biomaterial has been identified as the gold standard for the treatment of intrabony defects.[19]

The present study was conducted on 16 intrabony defects and assessed through histological and radiographic examination at 14 and 28 days. The methodology of the current study agreed with research conducted by *Guskuma*[20] that showed on day 7, bone defects are still inflamed and enter the early stage of resorption, while on day 30, bone defects begin in the early stage of bone formation. The results were also in line with research conducted by *Kresnoadi*[21] who reported that the number of osteoblasts on the 30th day increase, while osteoclasts decrease significantly compared to the previous day.

PRF has been the most intensively researched biomaterial and has been introduced as a biomaterial in France in 2001.[22] There have been several successful clinical studies using PRF in regenerative treatment published.[23,24]

PRF consists of many cells including platelets, leucocytes, macrophages, granulocytes and neutrophils. The bulk

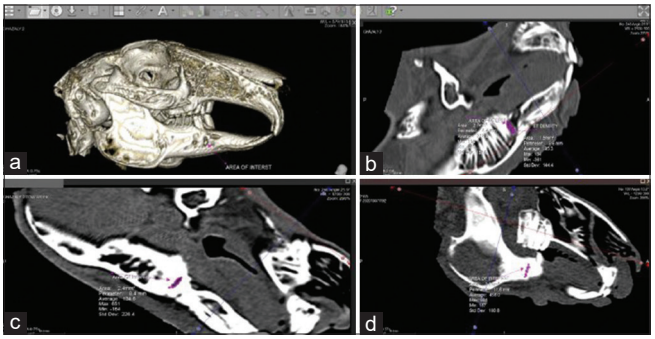

Figure 1: Showed rabbit 3D (a) and sagittal cut in right side group (1) PRF + Moringa at baseline (b), at 14 days (c) and 28 days (d)

of these cells are retained within the three-dimensional fibrin matrix after the centrifugation cycle[25] and the matrix proteins are released more slowly and consistently due to the three-dimensional architecture of the adhesive glycoproteins in the fibrin.[26] The concentrated leucocytes trapped in the fibrin mesh also give PRF antibacterial and anti-inflammatory capabilities.[4,27,28]

PRF includes natural growth factors, which are released slowly for 7 through 14 days[17] with the potential to promote wound healing and periodontal regeneration. As TGF-beta is a recognized agent responsible for the rapid proliferation of numerous cell types found in the oral cavity.[29,30] PDGF is a key regulator of mesenchymal cell migration, proliferation and survival. VEGF is a third key PRF growth factor that is essential for angiogenesis and future blood flow to injured tissues. Epidermal growth factor and insulin-like growth factor are two more growth factors that regulate cell proliferation and differentiation in a variety of cell types.[31]

PRF can be used as a regenerative material in intrabony defects because of its autologous origin, low cost and quick turnaround. *Pripatnanont et al.*[32] reported that PRF had a positive effect on bone formation when used alone or combined with autogenous bone. Also,

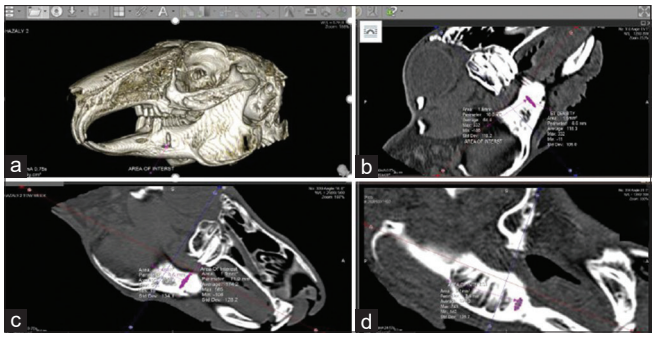

Figure 2: Showed rabbit 3D (a) and sagittal cut in left side group (2) **PRF only** at baseline (b), at 14 days (c) and 28 days (d)

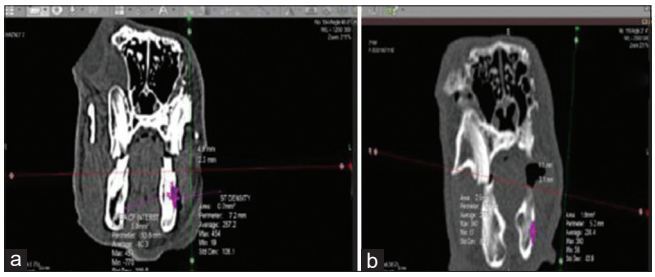

Figure 4: Showed coronal cut in left side group (2) PRF at baseline (a) and 28 days (b)

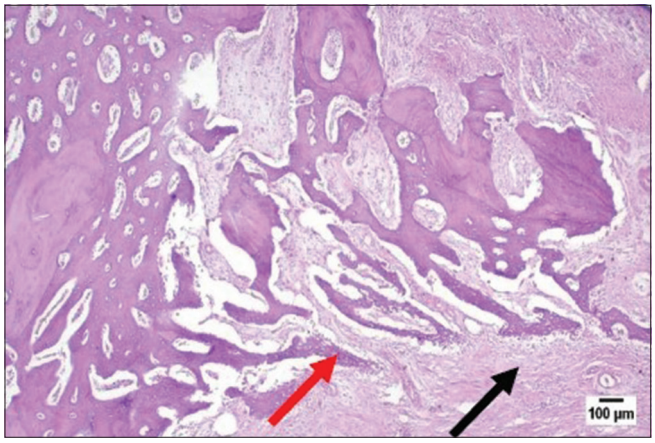

Figure 6: Photomicrograph of bone, after 14 days, Moringa + PRF Group 1 showing filling of the defect area by fibrous tissue (black arrow) with the presence of newly formed bone (red arrow) (H&E)

Table 6: The healing score of bone defect

	Rt. side (PRF + Moringa group)	Lt. side (PRF group)
14 days	6.5±0.47 b	4.9±0.52 a
28 days	8.5±0.34 c	6.6±0.33 b

Data were presented as mean±SE. a, b and c indicate statistically significant differences at $P< 0.05$

Chandradas et al.[33] demonstrated that PRF improves clinical and radiological parameters compared to open flap debridement (OFD) alone in intrabony defects. The addition of demineralized bone matrix (DBM) enhances the effects of PRF in relative attachment level (RAL) gain and radiographic defect fill. This goes with our study, which

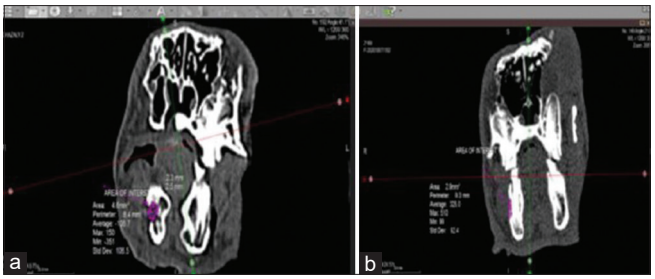

Figure 3: Showed coronal cut in right side group (1) PRF + Moringa at baseline (a) and 28 days (b)

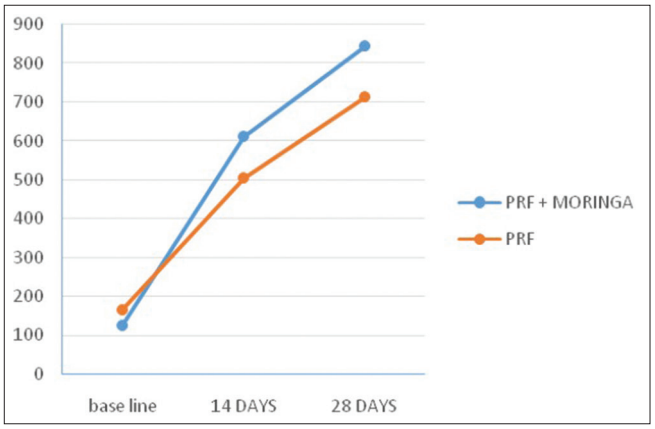

Figure 5: Comparison over time in each group and between groups

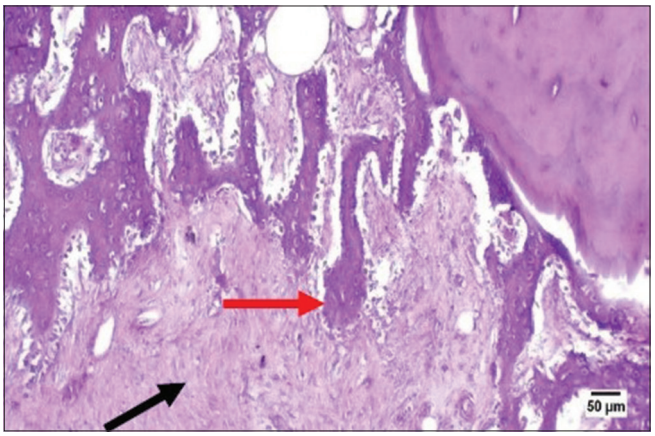

Figure 7: Photomicrograph of bone, after 14 days, PRF Group 2 showing newly formed bone (red arrow) filling the defect area with a moderate amount of organized tissue (black arrow) (H&E)

revealed that new bone tissue is formed within the defect area treated with PRF after 30 days of the beginning of the experiment.

Among all PCs, PRFs have been shown to possess fibrin, leucocytes and a variety of growth factors that could promote wound healing. PRF alone in ridge preservation did not provide significant additional benefit when compared to natural healing sockets with regard to bone volume, bone density and osteoblastic activity.[24]

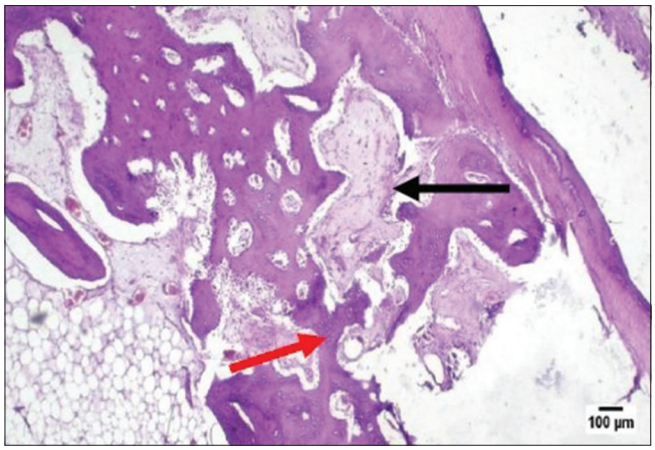

Figure 8: Photomicrograph of bone, after 28 days, PRF + Moringa Group 1 showing an increased amount of newly formed bone filling the defect area (red arrow) with few areas of uncalcified tissue (black arrow) (H&E)

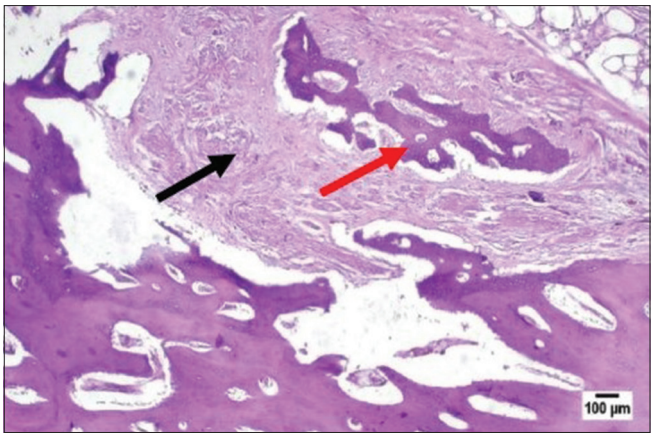

Figure 9: Photomicrograph of bone, after 28 days, PRF Group 2 showing filling of the defect area by fibrous tissue (black arrow) with the presence of newly formed bone (red arrow) (H&E)

The same observations were clearly obvious in our study, as the left side (PRF group) defect area showed retarded healing by being partially filled with non-ossifying fibrous tissue with the presence of only small fragments of newly formed bone and increased numbers of inflammatory cells. While a significant elevation of bone defect healing score was observed on the right side (Moringa + PRF group) when compared to the left side (PRF group) at both times of evaluation.

Wound healing necessitates a complicated interaction between diverse cell types, a three-dimensional extracellular matrix as well as soluble growth factors that aid regeneration. Since then, four principles of wound healing have been identified as crucial to the effective regeneration of human tissues. Haemostasis, inflammation, proliferation and maturation and the overlaps between each of the stages and the population of cells found in each category. Whereas lymphocytes normally appear after 7 days, the ability for PRF to introduce a large number at day 0 helps to accelerate the regenerative stages during this process. Each phase includes a variety of cell types.[33]

MO is crucial in stimulating osteoblastic cells.[34,35] It is high in flavonoids (kaempferol and quercetin), saponins, alkaloids and tannins. Flavonoids inhibit the cyclooxygenase enzyme, which reduces prostaglandin synthesis (PGE_2) as well as the release of histamine and pro-inflammatory cytokines (tumour necrosis factor-α, interleukin [IL]-1 and IL-6), therefore have anti-inflammatory properties.[36] Flavonoids can increase osteoblast proliferation and differentiation, according to research by *Zhang et al*.[36] This is further supported by Patel's research, which found that flavonoids obtained from Moringa leaf extract can enhance osteoblast differentiation, resulting in bone formation.[35]

Quercetin can reduce the number of osteoclasts and increase the number of osteoblast cells by inhibiting differentiation and activation as well induces apoptosis of osteoclast cells.[37,38] Saponin has an influence on osteogenic activity which encourages the proliferation and differentiation of osteoblasts.[39] Tannin inhibits receptor activator of nuclear factor κB (RANK) activation, making it an effective inhibitor of osteoclast differentiation.[40] As a consequence, the number of osteoclasts will decrease, with the result that bone resorption decreases.

In accordance with our results, *Soekobagiono et al*.[41] measured receptor activator for nuclear factor κB ligand (RANKL) expressions in tooth extraction socket treated with Moringa leaf extract combined with demineralized freeze-dried bone bovine xenograft (DFDBBX). They concluded that the combination of Moringa leaf extract and DFDBBX can decrease the number of RANKL expressions as indicators of bone formation in the tooth extraction sockets of the Cavia cobaya rats on days 7 and 30.

Our result is consistent with research conducted by *Kresnoadi, et al*.[42] who studied the combination of Moringa leaf extract and DFDBBX during the preservation of tooth extraction sockets in the alveolar bone of Cavia cobaya and they concluded that the combination of Moringa leaf extract and DFDBBX leads can effectively generate TGF-β1 and osteocalcin expressions which leads to a greater chance of successful post-extraction socket preservation by accelerating alveolar bone regeneration.

In agreement with our results, *Pachimalla et al*.[14] formulated a hydrophilic gel from MO to be used along with the dental

implant placement, to create a hydrophilic surface for the dental implant, to enhance implant to blood contact and also bone to implant contact (BIC). They concluded that the hydrophilic implant surface showed new bone formation with increased BIC. There was an absence of degenerative changes, necrotic changes, fibrosis and inflammation at the new BIC.

Areej Salim Al-Azzawi, et al.[43] studied the differences between the healing process in Albino rats. MO, marine collagen (MC) and their combination (MM) were utilized to treat bone deformities. The combination group (MO&MC) had an accelerated rate of bone repair than the other groups, according to histological findings. Almost all histomorphometric measures employed in this study showed highly significant differences in duration between the MO with MC and control groups.

Regarding our study, the defect area from Rt. side (Moringa + PRF group) after 14 days showed better healing signs, the defect area was filled by organized non-inflammatory fibrous tissue with a higher percentage of defect bridging by newly formed bone.

Moreover, after 28 days, the defect area was almost filled completely by newly formed bone and the surface of the bone facing the defect area was filled by an increased number of active osteoblasts. A healing score was observed in Rt. side (Moringa + PRF group 1) significant elevation of bone defect healing score when compared to Lt. side (PRF group 2) at both times of evaluation.

MO's mechanism of action includes stimulating osteoblastic cells and possessing anti-inflammatory properties such as inhibiting the release of histamine and pro-inflammatory cytokines, whereas PRF's mechanism of action includes a rapid proliferation of various cell types, as well as being an essential regulator for the migration, proliferation and survival of mesenchymal cells. The synergistic impact of Moringa hydrogel and PRF may be superior to their individual usage.

CONCLUSION

Radiographical examination, histological and healing scores confirmed the superiority of Moringa + PRF mix results in an increase in bone fill and density in induced periodontal intrabony defects regeneration in rabbits.

Author contributions

- Kareman Sayed El Soudany: Conceptualization, Data curation, Formal analysis, Validation, and original draft preparation.
- Huda Ahmed Amin EL Gendi: Investigation, Methodology, Resources, Supervision, Writing- review and editing.
- Heba Abdul Fattah El said: Data curation, Formal analysis, Validation, and original draft preparation.

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Conflicts of interest

There are no conflicts of interest.

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