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Original article

Post-tooth extraction induction effect of *Moringa oleifera* leaf extract and demineralized freeze-dried bovine bone xenograft treatment on alveolar bone trabecula area

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ABSTRACT

Background: *After tooth extraction, alveolar bone resorption occurs naturally, followed by alveolar bone remodeling. Alveolar bone formation is characterized by an increase in density and expansion of the trabecular bone. Socket preservation using a combination of Moringa oleifera leaf extract and demineralized freeze-dried bovine bone xenograft (DFDBBX) is expected to increase the area of the alveolar bone trabeculae and thus accelerate the process of alveolar bone formation.* ***Purpose:*** *This study aimed to determine if a combination of Moringa oleifera leaf extract and DFDBBX could increase the area of the alveolar bone trabeculae in tooth extraction sockets.* ***Methods:*** *With their lower left incisors extracted, the 56 Cavia cobayas were divided into eight treatment groups according to the material given: polyethylene glycol (PEG), DFDBBX and PEG, Moringa oleifera leaf extract and PEG, and a combination of Moringa oleifera leaf extract, DFDBBX, and PEG. On the seventh and thirtieth days, the Cavia cobayas were sacrificed and examined. Histopathological samples were stained with Hematoxylin-Eosin (HE) to evaluate the trabecula area, and data were analyzed using one-way ANOVA and Tukey HSD.* ***Results:*** *On the thirtieth day, the group that received a combination of Moringa oleifera leaf extract and DFDBBX had the greatest area of alveolar bone trabeculae.* ***Conclusion:*** *A combination of Moringa oleifera leaf extract and DFDBBX induced in the tooth extraction socket can increase the area of the alveolar bone trabeculae.*

Keywords: *alveolar bone; DFDBBX; Moringa oleifera leaf extract; socket preservation; trabecular area*
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INTRODUCTION

Tooth extraction will result in the formation of a socket. In the post-extraction socket, bleeding will occur as an early marker of the socket healing process, followed by coagulation, inflammation, proliferation, and remodeling.[1] After tooth extraction, alveolar bone resorption happens, causing changes in the morphology and dimensions of the alveolar bone. After six months, the alveolar bone will resorb by 29–63% in the horizontal plane and 11–22% in the vertical plane.[2]

The bone remodeling process will involve concurrent bone resorption and deposition. The balance of osteoclasts and osteoblasts influences this process. Preosteoclasts will differentiate into osteoclasts, causing increased bone resorption. The activation of osteoclasts stimulates the differentiation and maturation of osteoblast precursor cells, resulting in bone matrix mineralization, which is an indicator of the bone formation process and osteoclast apoptosis.[3] On the seventh day after tooth extraction, bone regeneration will begin at the periphery and extend to the socket's middle area toward the bone trabeculae. On the twelfth day, woven bone trabeculae have formed at the socket's periphery and are surrounded by preosteoblasts, osteoblasts, and osteoprogenitor cells.[4] By the fourteenth day, trabecular bone is actively forming and will cover most of the bone graft's surface. On the twenty-eighth day, the trabecular bone will fill most of the alveolar sockets.[5] An increase in trabecular bone density and expansion also indicates the formation of new alveolar bone.[6]

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Alveolar bone resorption following a tooth extraction is a physiological process, but it can be minimized. Socket preservation is the process of minimizing alveolar bone resorption by inserting regenerative material into the post-extraction socket. Also, socket preservation is expected to preserve the dimensions of the alveolar ridge after tooth extraction. Bone graft is a regenerative material commonly used in dentistry, one of which is demineralized freeze-dried bovine bone xenograft (DFDBBX). DFDBBX is a xenograft graft material derived from bovine bone, which is biocompatible, osteoinductive, and osteoconductive.[5] DFDBBX promotes the growth of new bone.[6] Several studies have reported that *Moringa oleifera* leaves can aid in bone formation and prevent bone resorption. *Moringa oleifera* in combination with xenograft is effective in generating TGF-β1 and osteocalcin expression in alveolar bone, as well as accelerating alveolar new bone formation and decreasing bone resorption.[7,8]

Based on pharmacological studies, flavonoids and tannins have an anti-inflammatory effect by reducing TNF-α, IL-1β, and IL-6, all of which play a role in bone resorption.[6] A combination of Moringa leaf extract and DFDBBX with an effective dose of 2% can increase the number of osteoblasts, thereby accelerating the process of post-extraction alveolar bone formation on the experimental subject *Cavia cobaya*.[9] This study was conducted to determine if inducing a combination of Moringa leaf extract (*Moringa oleifera*) and DFDBBX in the tooth extraction socket increased the area of the alveolar bone trabeculae.

MATERIALS AND METHODS

This research began once the Airlangga University Faculty of Dental Medicine Committee approved it with the number 533/HRCEE.FODM/IX/2021. This was an experimental laboratory study with randomized post-test-only control group design samples using healthy and active *Cavia cobaya* males, weighing about 300–350 grams and at 3–3.5 months in age.

Moringa leaf extract was made at the Surabaya Industrial Consultation and Research Institute. Moringa leaves were soaked in water before being heated and filtered. The filtrate was thickened and concentrated to a volume of 1:1 with water. The extract was treated with silicate to make it non-hygroscopic and then dried. This study used four different solutions as treatments for each group: (i) only polyethylene glycol (PEG), (ii) a combination of DFDBBX and PEG, (iii) a combination of Moringa leaf extract and PEG, and (iv) a combination of Moringa leaf extract, DFDBBX, and PEG, with each concentration of the active substance being 2%. To obtain a 2% concentration of Moringa leaf extract and DFDBBX, 0.5 grams of Moringa leaf extract were combined with 0.5 grams of DFDBBX and 24 grams of PEG (PEG 400 + PEG 4000, in a 1:1 ratio). PEG was used as a carrier to turn the mixture into a gel, making it easier to fill into the socket.

Cavia cobayas were anesthetized intramuscularly (IM) with ketamine at a 20mg/300 mg body weight (BW) dose. The same motion, direction, and force were used to extract the left lower incisor. The socket was then irrigated with sterile distilled water. The 56 *Cavia cobayas* with their lower left incisors extracted were divided into eight groups of seven. As a control, groups 1 and 2 were given 25 grams of PEG. On the other hand, groups 3 and 4 received 0.5 grams of DFDBBX and 24.5 grams of PEG. Groups 5 and 6 were both given 0.5 grams of Moringa leaf extract and 24.5 grams of PEG. For groups 7 and 8, 0.5 grams of Moringa leaf extract, 0.5 grams of DFDBBX, and 24 grams of PEG were administered. The combination of Moringa leaf extract, DFDBBX, and PEG was injected into the socket (± 0.1 ml) using a syringe. Then, the post-retraction wound area was sutured with polyamide monofilament, DS 12 3/8 c, 12 mm, 6/10 meters, 0.7 sterile Braun Aesculap. The *Cavia cobayas* from groups 1, 3, 5, and 7 were terminated on the seventh day, and groups 2, 4, 6, and 8 on the thirtieth day. Their mandibular bones were collected for a 30-day decalcification using ethylenediaminetetraacetic acid (EDTA).

The tissue was then prepared for histopathological examination using paraffin blocks. Moreover, histopathological samples were prepared using Hematoxylin-Eosin (HE) staining. The area of the alveolar bone trabeculae was calculated using a light microscope with 400x magnification and ten visual fields equipped with raster 4.1 image software (OptiLab). The measurement results were analyzed using the Shapiro–Wilk, Levene's, one-way Analysis of Variance (ANOVA), and Tukey Honestly Significant Difference (HSD) tests on the Statistical Package for the Social Sciences (SPSS) version 21 software.

RESULTS

The results of the mean value of the alveolar bone trabeculae area and the standard deviation in the histological observations of five visual fields during the seventh- and thirtieth-day examinations are shown in Figure 1. The highest mean value of the alveolar bone trabeculae was found in the combined leaf extract treatment group. The Moringa + DFDBBX + PEG group had the highest value on the thirtieth day, while the control group had the lowest value on the seventh day. Furthermore, the mean alveolar bone trabecular area in the treatment group was greater compared to the control group on both the seventh and thirtieth days. Histopathological views of *Cavia cobaya* tooth sockets showing the trabecular area on the seventh and thirtieth days are depicted in Figures 2 and 3, respectively.

The research samples were normally distributed and homogeneous based on the Shapiro–Wilk test and Levene's test. The one-way ANOVA test showed that on both the seventh and thirtieth day, there were significant differences

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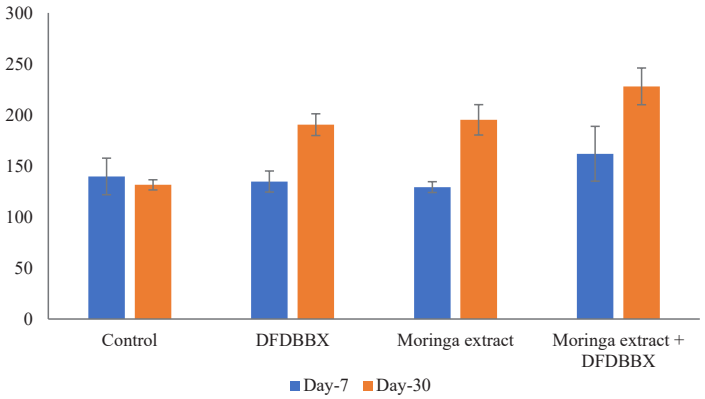

Figure 1. The mean and standard deviation of an alveolar bone trabecular area on Day-7 and -30 in the control group and treatment groups: DFDBBX, Moringa leaf extract, and combined Moringa leaf extract and DFDBBX.

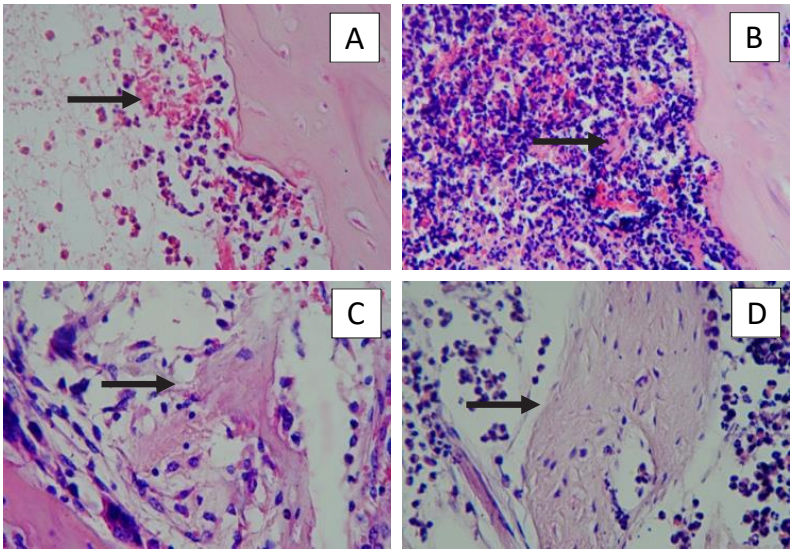

Figure 2. Histopathological view of Cavia cobaya tooth sockets in each group on Day-7. (A) Control group (PEG); (B) DFDBBX treatment group; (C) Moringa leaf extract treatment group; (D) Combined DFDBBX + Moringa leaf extract treatment group.

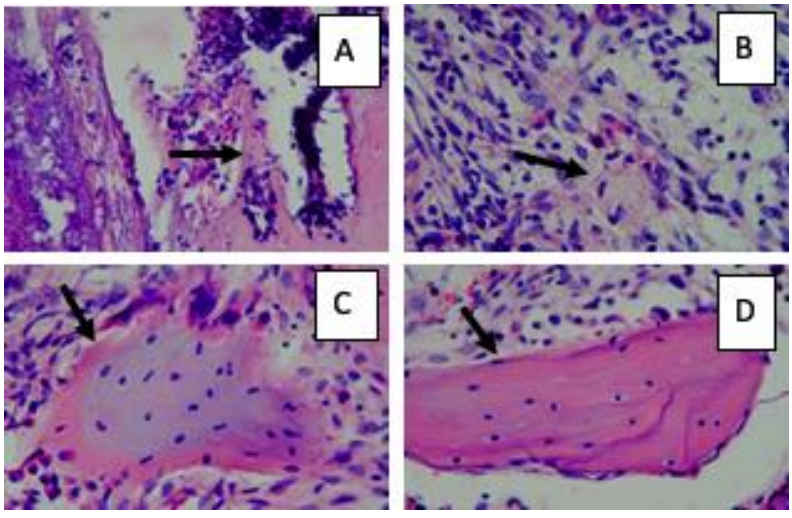

Figure 3. Histopathological view of Cavia cobaya tooth sockets in each group on Day-30. (A) Control group (PEG); (B) DFDBBX treatment group; (C) Moringa leaf extract treatment group; (D) Combined DFDBBX + Moringa leaf extract treatment group.

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between the treatment groups ($p < 0.001$). The Tukey HSD test also revealed a significant difference on the seventh day between the control group and the Moringa leaf extract group ($p = 0.026$), between the control group and the Moringa leaf extract and DFDBBX combination group ($p = 0.001$), and between the DFDBBX group and the Moringa leaf extract and DFDBBX combination group ($p = 0.034$). On the thirtieth day, however, a significant difference was noted between all groups ($p = 0.001$) except for the Moringa leaf extract group and the DFDBBX group ($p = 0.913$).

DISCUSSION

Alveolar bone consists of trabecular and cortical bone but is mainly composed of the former.[10] Cortical bone has a mechanical function, while trabecular bone plays a role in metabolic processes and physiological responses.[11] Alveolar bone has biological properties in the form of plasticity, which allows it to adapt to physiological or pathological changes in the oral cavity.[12] The resorption process is characterized by a decrease in alveolar bone, which is influenced by osteoclasts.[13] Alveolar bone deposition is characterized by an ossification process that begins with the mineralization of the bone matrix and continues with osteoblast proliferation and differentiation into osteocytes. Ossification changes osteocytes, which leads to the formation of bone trabeculae. Therefore, increased trabecular bone expansion is a marker for the formation and mineralization of new alveolar bone.[5]

The results of this study prove that inducting a combination of Moringa leaf extract and DFDBBX can increase the area of the alveolar bone trabeculae. DFDBBX is a bovine xenograft with a structure and inorganic content similar to humans, which gives it osteoconductive properties. Also, DFDBBX will act as a scaffold to support cell adhesion and proliferation, as well as stabilize blood clotting to prevent tissue formation from being disturbed.[6] Its osteoconductive properties will stimulate osteoblast migration from the socket base, thereby supporting osteogenesis. Moreover, DFDBBX will decrease Receptor Activator of Nuclear Factor Kappa β-Ligand (RANKL) expression while increasing osteoprotegerin (OPG) expression, which leads to a decrease in osteoclasts, the main factor in bone resorption.[7]

Moringa oleifera is rich in flavonoids, tannins, terpenoids, alkaloids, saponins, ascorbic acid, phenolics, carotenoids, potassium, calcium, β carotene, protein, and vitamin C.[9] The flavonoid compounds in Moringa leaves are phytochemical compounds with high osteoinductive and anti-inflammatory properties. Flavonoids stimulate osteoblast proliferation and differentiation, as well as prevent bone resorption by inhibiting the cyclooxygenase-2 (COX-2) enzyme, which hinders prostaglandin synthesis and decreases PEG-2 and macrophage infiltration.[14] Reduced macrophage infiltration results in fewer inflammatory mediators and proinflammatory cytokines (TNF-α, IL-1β, and IL-6). Furthermore, RANKL production is disrupted, which inhibits osteoclast formation and stimulates osteoclast growth for apoptosis, reducing bone resorption and activating osteoblastogenesis, which plays a role in the formation of new bone.[8] Flavonoids can also regulate cell function by producing TGF-β, which induces osteoblast proliferation and migration while inhibiting osteoblast apoptosis.[15] Flavonoids also increase the enzyme alkaline phosphatase (ALP), which is an indicator of osteoblast proliferation. ALP participates in the mineralization process that facilitates calcium storage in tissues and reflects the activation of osteoblast cells during bone formation.[7,16] Phytochemical compounds of tannins play a role in inhibiting osteoclast differentiation by preventing Receptor Activator of Nuclear Factor Kappa-β (RANK) activity.[15] In comparison, phytoestrogens stimulate increased osteoblast activity, because they have bioactivity similar to estrogen produced by the body. Estrogen is involved in bone growth and bone homeostasis. Estrogen also increases osteoblast activity by inhibiting osteoclast activity, preventing osteoblast and osteocyte apoptosis, and stimulating osteoblast activity. Phytoestrogens stimulate new bone formation by binding to estrogen on target cells and increasing osteoblast activity. Hence, estrogen mediates the activity of phytoestrogens in the bone formation process.[17] Flavonoids have the potential to stimulate osteoblasts, while saponins in Moringa leaf have an osteogenic effect, aiding in osteoblast proliferation and differentiation.[18] Flavonoids can also regulate cell function by stimulating TGFβ production, which induces osteoblast proliferation and migration.[19]

Kaempferol and quercetin in Moringa can also directly inhibit RANKL and TNFα activity by inhibiting the inflammatory pathway. It is expected that there will be a reduction in macrophage infiltration, followed by a decrease in proinflammatory cytokines (TNFα, IL1β, and IL6), which will then reduce RANKL production and decrease alveolar bone resorption.[20]

The Moringa leaf extract and DFDBBX treatment group having the highest mean area of alveolar bone trabeculae is a result that is in line with a study conducted by Rostiny et al. (2016), which found that when given a Moringa leaf extract and DFDBBX combination, the number of osteoblasts increased, accelerating the formation of alveolar bone post-extraction.[9] This is because the combination of osteoconductive and osteoinductive properties found in DFDBBX and Moringa leaf extract can significantly increase the area of alveolar bone trabeculae. DFDBBX, which acts as a scaffold, will facilitate new bone formation and stabilize blood clots, as well as be a source of minerals.[6] Moreover, the Moringa leaf extract's flavonoids, tannins, and phytoestrogens have anti-inflammatory osteoinductive properties that will reduce bone resorption and stimulate osteoblast proliferation and differentiation. Increased migration, proliferation, and differentiation of osteoblasts will accelerate new bone growth along the socket and trabecular space in the extraction area, resulting in woven

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bone trabeculae and an increase in the area of the alveolar bone trabeculae.[7] This study's results established that a combination of Moringa leaf extract and DFDBBX induced in the tooth extraction socket can increase the alveolar bone trabeculae's area on the seventh and thirtieth day.

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