Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2018 June; 51(2): 86–90

Research Report

Effects of *Moringa oleifera* leaf extract combined with DFBBX on type-1 collagen expressed by osteoblasts in the tooth extraction sockets of *Cavia cobaya*

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ABSTRACT

Background: *Tooth extraction is a common procedure in dentistry after which the residual ridge will no longer receive stimulus leading to volume, height and width loss. These anatomical changes can then result in difficulties with future denture fabrication and implant placement. Preservation of the alveolar ridge, therefore, assumes considerable importance after tooth extraction. Moringa oleifera, on the other hand, can enhance bone formation. Type-1 collagen is a marker of osteoblast formation.* ***Purpose:*** *This research aimed to analyze the effects of Moringa oleifera leaf extract combined with DFBBX on type-1 collagen expressions in tooth extraction sockets.* ***Methods:*** *56 Cavia cobaya subjects were divided into eight groups. Their lower left incisors were then extracted prior to the sockets of the first and fifth groups being filled with PEG, those of the second and sixth groups with DFBBX, those of the third and seventh groups with Moringa oleifera leaf extract and a combination of DFBBX and Moringa oleifera leaf extract in those of the fourth and eighth groups. The sockets were then examined on days 7 and 30 by means of an immunohistochemical technique. The data collected was subsequently subjected to analysis by One Way Anova and Tukey HSD tests.* ***Results:*** *There were significant differences between the control group and the treatment group administrated with Moringa oleifera leaf extract combined with DFBBX. On days 7 and 30, the groups treated with the combination of DFBBX and Moringa oleifera leaf extract had the highest number of type-1 collagen expressions.* ***Conclusion:*** *A combination of DFBBX and Moringa oleifera leaf extract is effective in increasing type-1 collagen expressions in tooth extraction sockets.*

Keywords: *alveolar bone; DFBBX; Moringa oleifera leaf extract; type-1 collagen; socket preservation.*

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INTRODUCTION

Tooth extraction, one of the most common procedures performed in dentistry, can unfortunately cause alveolar ridge resorption. The largest bone loss usually occurs in the horizontal dimension, especially on the facial side of the ridge and the buccal side of the vertical dimension. Post-extraction alveolar bone resorption is, thus, an unavoidable physiological process.[1] In the manufacture of dentures and dental implants, bone resorption is one of the complications potentially impeding the success of the therapy.[2] As a result, it is important to maintain the height of the alveolar ridge after the tooth extraction process has been completed. In order to maintain the alveolar ridge post-extraction, graft material with or without a membrane is usually applied.[3]

Graft material is a natural or synthetic material that can be used to repair defects.[4] Xenograft is a graft material transferred from one species to another. One of the most commonly used xenograft materials is demineralized freeze bovine bone xenograft (DFBBX). derived from cow bone whose particles are of a specific size. The removal of minerals contained in the bone was effected by soaking it in an acidic solution.[5]

More recently, however, treatments have been developed and improved by the use of drugs derived from plants or herbs whose medicinal properties have been

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.
 Available at http://e-journal.unair.ac.id/index.php/MKG
DOI: 10.20473/j.djmkg.v51.i2.p86–90

known for many years. One such medicinal herb is Moringa oliefera which can be used to accelerate the process of bone formation and prevent that of bone resorption.[6] A study conducted by Chirag Patel argues that flavonoid compounds contained in *Moringa oliefera* leaf extract can generate alkaline phosphatase (ALP) and hydroxyproline when applied to $SaOS^{-2}$ cell line culture.[7] Another piece of research indicated that *Moringa oliefera* leaf extract is capable of increasing cell proliferation and survival, as well as cell migration of human dermal fibroblasts (HDF).[8]

Moreover, research conducted by Pudianto reveals that a combination of *Moringa oliefera* leaf and demineralized freeze bovine bone xenograft (DFBBX) at an effective dose of 2% can generate osteoblasts, leading to the acceleration of alveolar bone formation after tooth extraction in *Cavia cobaya* subjects.[9] Similarly, research conducted by Wirawan also found that a combination of *Moringa oliefera* leaf extract and DFBBX can significantly increase the number of osteoblasts in their tooth extraction sockets.[10] During the bone formation process, many markers can be observed, one of which is type 1 collagen expression. Type 1 collagen is the most dominant extracellular matrix protein present in bone and plays an important role in the bone formation process.[11] Nevertheless, the effects of *Moringa oliefera* leaf extract combined with DFBBX on type-1 collagen expressions are still unclear. As a result, this research aimed to analyze the effects of *Moringa oleifera* leaf extract combined with DFBBX on type-1 collagen expressions in post-extraction sockets by observing osteoblast cells.

MATERIALS AND METHODS

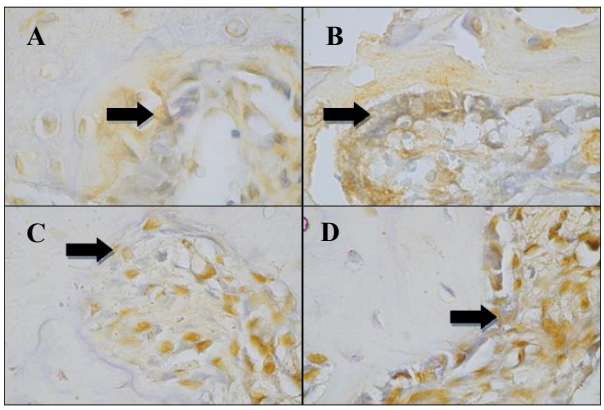
This research was a laboratory experiment involving a sample size of 56 *Cavia cobaya* subjects that filled certain inclusion criteria, namely: male, body weight of 300-350 grams, aged 3-3.5 months, healthy and active, having a normal appetite, free of limb injuries or skin complaints and able to run freely. Ethical approval was granted by the Ethics Commission, Faculty of Dental Medicine, Universitas Airlangga with No. 0009/HRECCFODM/I/2017.

The 56 *Cavia cobaya* subjects were subsequently divided into eight groups, each of which contained seven members. Their left mandibular incisor was then extracted. Following extraction of their incisors, the resulting sockets of Groups I and V as the control groups were filled with polyethylene glycol (PEG). The animals in Group I were then terminated on day 7, while those in Group V were terminated on day 30. Meanwhile, the empty sockets in Groups II and VI were filled with *Moringa oliefera* leaf extract and PEG. The subjects in Group II were executed on day 7, while those in Group VI were executed on day 30. Furthermore, the tooth extraction sockets in Groups III and VII were filled with DFBBX and PEG. The animals in Group III were terminated on day 7, with those in Group VII being terminated on day 30. Meanwhile, the tooth extraction sockets of Groups IV and VIII were filled with a combination of *Moringa oliefera* leaf extract, DFBBX and PEG. The members of Group IV were executed on day 7, while those in Group VIII were executed on day 30.

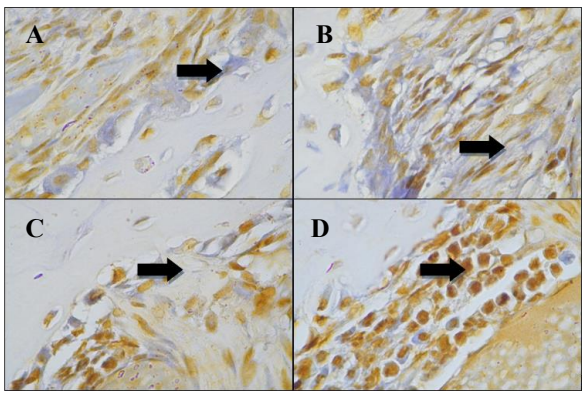
All of these animals were executed using a cervical dislocation method. Their tooth extraction sockets were then subjected to immunohistochemical analysis. Immunohistochemical staining was conducted using polyclonal anti-collagen type I antibody (D-Bio System USA®) with the number of type 1 collagen expressions being calculated by observing osteoblasts with a light microscope at a magnification of 1000x. The data collected was subsequently subjected to a normality test (a Saphiro-Wilk statistical test). A one-way ANOVA was carried out to calculate the difference in the number of type 1 collagen expressions between the groups.

RESULTS

Figures 1 and 2 show type-1 collagen expressed by osteoblasts in the tooth extraction sockets on days 7 and 30, subjected to immunohistochemical examination by light



Figures 1. The arrows indicate type-1 collagen expressions by osteoblasts in the tooth extraction sockets on day 7. A: Group I; B: Group II; C: group III; D: Group IV.



Figures 2. The arrows indicate type-1 collagen expressions by osteoblasts in the tooth extraction sockets on day 30. A: Group I; B: Group II; C: group III; D: Group IV

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.

DOI: 10.20473/j.djmkg.v51.i2.p86–90

Table 1. The mean and and standard deviation values of type-1 collagen

Group	n	Mean ± Standard Deviation
Group I	7	3.00 ± 0.816[a]
Group II	7	11.42 ± 1.511[b]
Group III	7	15.14 ± 1.672[c]
Group IV	7	15.85 ± 1.772[c]
Group V	7	10.57 ± 1.902[dew]
Group VI	7	13.28 ± 1.380[d]
Group VII	7	15.71 ± 2.690[e]
Group VIII	7	19.57 ± 1.902[f]

Note: Different superscripts showed a statistically significant difference ($p<0.05$)

microscope at a magnification of 1000x. Table 1 contains the mean number of osteoblast cells expressing type 1 collagen in the tooth extraction sockets in each treatment group. On day 7, the highest number of type 1 collagen expressions was found in Group IV, while the lowest was in Group I. Meanwhile, on day 30, the highest number of type 1 collagen expressions was found in Group VIII, with the lowest in Group V.

The results of the normality test in the form of a Saphiro-Wilk test showed that the data on days 7 and 30 was normally distributed with respective p values of 0.383 and 0.340 ($p>0.05$). The results of the Levene's test showed that the data on the 7th and 30th days were homogeneous with p values of 0.056 and 0.089 ($p>0.05$). The results of the one-way ANOVA test conducted on days 7 and 30 indicated that there were significant differences in the treatment groups with p values of 0.000 and 0.000 ($p<0.05$). The results of the Tukey HSD test carried out also indicated that there were significant differences between the treatment groups as illustrated in Table 1.

As Figure 3 shows, the highest level of type 1 collagen expression was found in Group VIII, while the lowest occurred in Group I. The number of type 1 collagen expressions in the tooth extraction sockets on day 7 also showed statistically significant differences between the treatment groups, except between Group III and Group IV. Similarly, the number of type 1 collagen expressions in the tooth extraction sockets on day 30 indicated statistically significant differences between the treatment groups, except between Group V and Group VI and between Group V and Group VII. The number of type 1 collagen expressions on day 30 was higher than that on day 7. The number of type 1 collagen expressions in the groups with the combination of *Moringa oleifera* leaf extract and DFBBX on both days 7 and 30 was significantly higher than in the other groups.

DISCUSSION

In this research, the lower left mandibular incisors of the Cavia cobaya subjects were extracted. The resulting extraction sockets were then filled with PEG, DFBBX, *Moringa oleifera* leaf extract or a combination of *Moringa oleifera* leaf extract and DFBBX. Xenograft is known to have osteoconductive properties with porous internal surfaces allowing for revascularization and osteoblast migration from the socket base which will support osteogenesis. The inorganic bone matrix structure and content of xenograft also renders it more osteoconductive which facilitates bone formation.[12]

Consequently, there were no significant differences in the amount of type-1 collagen between several groups treated with DFBBX on days 7 and 30. This may be because the xenograft inserted into the tooth extraction sockets serves as a scaffold for new bone growth, derived from the osteoblasts at the base of the sockets.[13]

On the other hand, *Moringa oleifera* leaf extract contains flavonoid compounds, especially kaempferol and quercetin, which inhibit prostaglandin synthesis, especially PGE-2 which decreases macrophage infiltration.[14,15] The decrease in macrophage cells will be subsequently followed by a decrease in inflammatory mediators, such as histamine, serotonin, and all three proinflammatory cytokines (TNF a, IL-1, IL-6).[14] The decrease in proinflammatory cytokines then induces a decrease in bone resorption.[16] Prostaglandins (PGE-2) is known to play a role in stimulating osteoclast formation directly or indirectly through RANKL, resulting in differentiation and fusion of osteoclast precursors into osteoclasts. Hence, the presence of barriers to PGE-2 and cytokine synthesis can serve as an inhibitor of osteoclast formation so that the number of those osteoclast cells and proinflammatory cytokines are also capable of inhibiting osteoprotegerin (OPG).[17] In other words, the decrease in PGE-2 synthesis indirectly induces new bone formation through biological cascade activation of osteoblastogenesis by deactivating RANKL.[16]

Certain researchers have already indicated that *Moringa oleifera* leaf has many phytochemical variants, especially phytoestrogen that produces a positive effect on bone formation.[18] Phytoestrogen is a flavonoid compound belonging to the isoflavonoid group. Phytoestrogen demonstrates a similar bioactivity to estrogen because of structural similarities between phytooestrogens and estradiol (17-beta-estradiol), which is a naturally-produced estrogen in the body.[19] The presence of estrogen activity can then lead to increased osteoblast activity.[20,21]

Osteoblast is one of the cells that can synthesize type-1 collagen.[22] Thus, in the research reported here, osteoblasts were also expected to express type 1 collagen. *Moringa oleifera* leaf, on the other hand, is assumed to

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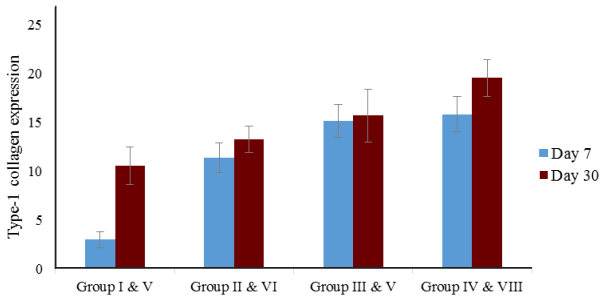

Figures 3. The diagram of type-1 collagen expressions on days 7 and 30.

increase the number and activity of osteoblasts indicated by an increase in the concentration of anti-collagen type I antibody observed using immunohistochemical techniques. Therefore, the combination of *Moringa oleifera* leaf extract and DFBBX (in Groups IV and VIII) generated type-1 collagen expressed by osteoblasts on days 7 and 30.

In this research, the mean number of type-1 collagen expressions in those treatment groups on day 30 tended to increase compared to those on day 7. This indicates that the number of osteoblasts on day 30 was higher than on day 7. The results above are consistent with the findings of research conducted by Guskuma *et al*.[23] showing that bone defect on day 7 is still in the inflammation stage and starts to experience the early stage of resorption. On day 30, the bone defect then starts to experience the early stage of bone formation process. The bone deposition process in the tooth extraction sockets is known to occur on day 28. At that time, osteoblasts and other osteogenic tissues begin to form significantly.[24] Similarly, research conducted by Kresnoadi et al. also revealed that the number of osteoblasts on day 30 increase significantly compared to the previous day.[25]

In conclusion, the post-extraction preservation of sockets using a combination of *Moringa oleifera* leaf extract and DFBBX may increase the activity of alveolar bone formation as indicated by an increase in type 1 collagen expressions. The selection of materials used in the preservation of tooth extraction sockets, nevertheless, plays an important role in the process of bone formation. DFBBX, according to research,[26] has osteoconductive properties that function as scaffolds for new bone growth, derived from the osteoblasts at the base of the sockets. On the other hand, *Moringa oleifera* leaf extract possesses osteoinductive properties since it can increase the proliferation and differentiation of osteoblasts.[27] Therefore, the combination of *Moringa oleifera* leaf extract and DFBBX in this research can significantly increase type-1 collagen expressions. The increased type 1 collagen expression indicates the occurrence of osteoconduction and osteoinduction activities in the tooth extraction sockets. This is likely to further enhance the success of socket preservation, enabling bone dimensions and volume after the tooth extraction to be maintained. However, further research is needed to improve the efficacy of the combination of *Moringa oleifera* leaf extract and DFBBX.

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DOI: 10.20473/j.djmkg.v51.i2.p86–90

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