

The Mangiferin (*Mangifera Indica* Linn) Effect Against the Calcium Degradation, Bones Resorption and Ossification of *Rattus novergicus* of Post-orthodontic treatment

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Abstract

To evaluate the effect of mangiferin on the calcium degradation, resorption and bone ossification of *Rattus novergicus* to prevent the relapse of post-orthodontic treatment. An in-vivo study using 15 Wistar mice for the orthodontic treatment model. The rate of calcium degradation was analysing using Energy Dispersive X-Ray Spectroscopy (EDS) while resorption and ossification of the maxillary bone were characterised by Scanning electron microscope (SEM) and analysed by ImageJ 1.46r. On the third day, mangiferin 12.5% and 6.5% were able to reduce calcium degradation better than the fifth and seventh days ($P < 0.05$). While there was no significant difference between the concentrations ($P > 0.05$). While the resorption level is in line with the level of bone ossification ($P < 0.05$), but based on concentrations of 12.5% and 6.25% there is no significant difference ($P > 0.05$).

While the SEM descriptive analysis showed that the process of bone resorption and ossification as a condition of post-orthodontic treatment remodelling. Mangiferin is able to control the degradation and resorption of calcium. It is also able to enhance the ossification of maxilla bones of the *Rattus novergicus* post-orthodontic treatment. Mangiferin with a concentration of 6.25% and 12.5% can be utilized as a standardised reference in bone remodelling research.

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Introduction

Orthodontic treatment aims to restore the ideal and balanced position of the teeth in the basal bone while improving the orofacial function, which consists of the alveolar dental system, skeletal tissue, soft tissue including muscles and bone around the mouth.¹ The basis of orthodontic treatment is pressure applying to the teeth without damaging the teeth or attachment to the bones. Post-orthodontic treatment, a retainer device is required to maintain the results of treatment and prevents the return of the tooth to its original position after being moved (relapse). This process also requires a relatively long time to maintain the moved position.²

Relapse has been reported as a major problem in post-orthodontic treatment.³ Relapse is generally caused by both intrinsic factors in the periodontal ligament and alveolar bone and extrinsic factors such as facial structure growth, soft tissue pressure and interdigation.⁴ Olive (2003) reported that stability and Relapse post-orthodontic treatment are unpredictable, with a tendency for Relapse of 33-90%, which is estimated to be ten years post-treatment.⁵ The response of periodontal tissue remodelling to orthodontic tooth movement is a key factor to prevent Relapse so that the tooth can move on the periodontal ligament to produce osteoclasts that function to absorb bone adjacent to the periodontal ligament that is under pressure. In contrast, on the strengthened side of the bone, re-formation occurs by osteoblast. Post-orthodontic treatment it is crucial to consider bone stability to prevent bone relapse.⁶ Long-Time use of retainer tool would result in a negative effect on the excessive movement of the alveolar bone, so that reduce the aesthetics

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as well as encourage malocclusion.⁷ Natural products such as polyphenols and xanthone glucosyl contained in mangiferin could be functioned as antioxidants and anti-inflammatories to prevent osteonecrosis, and osteonecrosis.⁸ Bai (2018) reported that mangiferin has also been using in bone remodelling research.⁹ Duivenvoorden (1999) also confirmed that mangiferin could immunomodulator bone growth by inducing bone growth proteins such as beta-growth factor (TGF- β), matrix metalloproteinase (MMP), and osteocalcin protein (OCN), the proteins play a role in maintaining calcium degradation in maintaining bone development.¹⁰ The increase in these three proteins is in line with bone resorption and ossification during bone development.¹¹ Both of these concepts are part of bone remodelling activities to maintain bone integrity during bone growth adaptation.¹² This research aims to evaluate the effect of mangiferin in controlling calcium degradation, resorption and bone ossification of *Rattus novergicus* on the prevention of post-orthodontic treatment relapse.

Materials and methods

This research has received ethical clearance from the Medical Faculty, University of North Sumatra, Medan Indonesia, No. 18/TGL/KEPK FK USU-RSUP HAM/2017. A total of 15 male Wistar rats (*Rattus novergicus*) aged 3-4 months, average weight 200-250 grams were used as animal models for maxillary bone relapse post-orthodontic treatment. Rats were divided into five treatment groups. Each group consisted of 3 mice based on the time of treatment (days 1, 3, 5, 7, and day 14). Each treatment involved two groups (1) the treatment group (tagged by orthodontic closed coil titanium nickel and given mangiferin with concentrations of 6.25% and 12.5%), and (2) the control group (labelled by the nickel-titanium orthodontic closed coil)

Orthodontic Treatment Model

Experimental mice were acclimatized for seven days in a light / dark cycle with room temperature. Furthermore prepared in the treatment group, positive control and negative control. All groups of mice mounted titanium nickel orthodontic closed coil in the maxilla for tooth movement for ten days, after ten days the coil was removed. This phase is considered an

orthodontic treatment phase that prevents Relapse of maxillary alveolar bone. All mice in the group were applied with mangiferin extract daily using a sterile cotton swab on the upper alveolar bone. The first application is considered zero-day treatment. Bone remodelling assessment was carried out for one day, three days, five days, seven days and 14 days. The due mice were euthanised with a 0.05 cc intraperitoneal Ketamine-Xylazin mixture, and then decapitation was carried out to obtain the maxillary alveolar bone. The bone was fixed from the tissue and musculature and then stored in a 10% buffered formalin solution at the pH of 7.0.

Measurement of Calcium

The calcium composition was determined by Scanning electron microscope (SEM) dan Energy Dispersive X-Ray Spectroscopy (EDS).¹³ The longitudinal part of maxillary alveolar bone samples of the rat was cut in 1 cm, where both ends were polished with polish paper grid 3000 and stored in Phosphate Buffer Saline (PBS) pH 6.8 for 24 hours. The sample was dried at room temperature for six hours. The surface profile of the bone was determined by using SEM followed by EDS to determine the chemical composition. In the first stage, bone samples were placed in the middle of the vacuum chamber. The height of the sample, in accordance with standard calibration, was adjusted. Then the device was turned on with 20 kV power. The sample is moved slowly to get the ideal position to be photographed on the SEM screen (Hitachi TM 3000, Japan). Then Brightness, contrast and focus are adjusted until good picture obtained. The images were taken at the enlargement of 1200x, 1500x and 2000x. Then each image with a magnification of 50-100x examined was furthered examined using EDS to determine the composition of calcium.

Bones Resorption and Ossification Analysis

Analysis of resorption and ossification of bone smeared with mangiferin was carried out based on SEM images with 1500x magnification using ImageJ 1.46r.¹⁴ The evaluation begins with opening the targeted file in ImageJ tool. By using tool-line, the resorption areas of ossification (μm) were determined. The Analyze menu was then employed to measure the distance of each target. The file was then exported to Microsoft Excel format to analyzed the resorption and ossification area.

Statistical Analysis

Calcium degradation based on exposure time was analysed using Kruskal-Wallis. The effect of mangiferin concentration on calcium degradation and bone resorption and ossification activity were analysed using independent T-test. While the intensity of bone resorption and ossification based on the time of exposure using Oneway Anova. The significant assessment was limited by $p < 0.05$.

Results

Figure 1 shows that mangiferin at concentrations of 12.5% and 6.5% on the third day had an excellent response effect in inhibiting calcium degradation from preventing relapse of post-orthodontic treatment. While the results in the 5th and 7th still provide an excellent response to prevent bone calcium degradation with control as a comparison. Kruskal-Wallis analysis showed significant differences in bone calcium degradation with time (days) 1, 3, 5, 7, and 14 ($P < 0.05 = 0.05$). Whereas based on the analysis of the Independent T-test, it was shown that there was no difference in calcium degradation after preparation with Mangiferin 12.5% and 6.25% ($P > 0.05 = 0.91$).

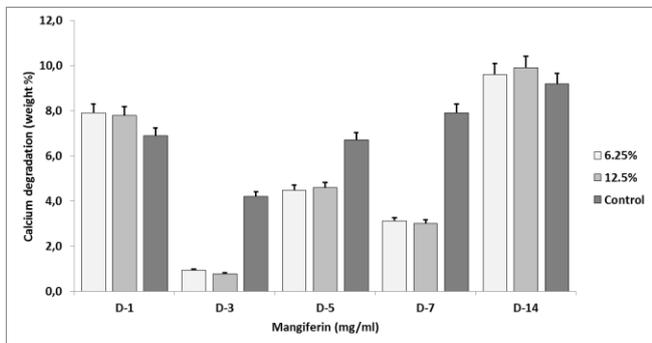


Figure 1. EDS Profile of Wistar rat maxillary bone calcium composition post-orthodontic treatment. The best reduction of calcium degradation occurred at 3, 5, and 7: bar (calcium degradation) and bar error (standard deviation).

Figure 2 depicted that the level of resorption is in line with the degree of bone ossification as a necessary condition for bone remodelling. It indicates that mangiferin has an effect of preventing Relapse post-orthodontic treatment. Based on these results, a concentration of 6.25% shows better performance compared to 12.5% in inhibiting

bone resorption even though they both demonstrate the ability to reduce relapse and increase ossification.

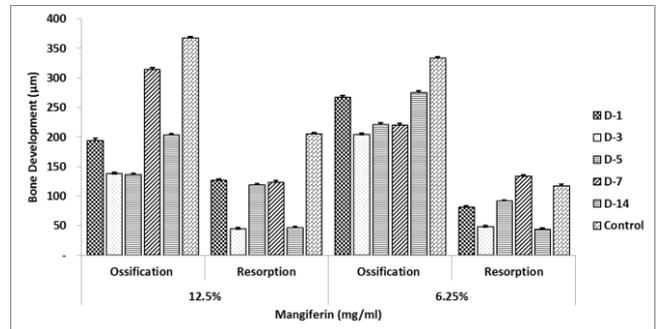


Figure 2. ImageJ analysis of Wistar rat maxillary bone Post-orthodontic treatment based on bone resorption and ossification from SEM image (1500 x). Generally, the concentration of 6.25% has a better effect on bone resorption compared to a concentration of 12.5% (D:day). Bar (resorption and ossification / bone development). Bar error (standard deviation).

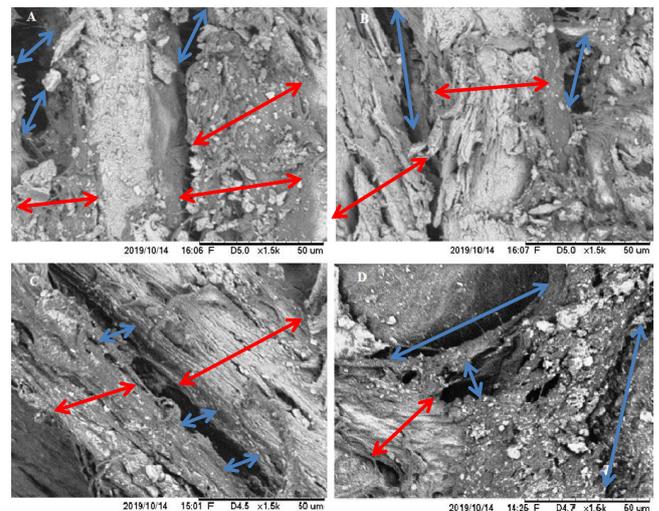


Figure 3. SEM image of Wistar rat maxillary resorption and ossification for seven days of post-orthodontic treatment. A (Mangiferin 12.5%), B (Mangiferin 6.25%), C (Control), and D (no treatment). Blue arrow (resorption) and red arrow (ossification).

Its indicates that mangiferin from the two concentrations gives a response to changes in the tensile force by the nickel-titanium orthodontic closed coil on orthodontic treatment. Independent T-test analysis showed that the level of resorption with bone ossification was significantly different ($P < 0.05 = 0.00$). Base on the concentration of Mangiferin did not show a

significant difference in resorption and ossification ($P > 0.05 = 0.97$). Besides, based on the analysis of One-way ANOVA, there are significant differences in the level of resorption and ossification based on time (days) 1, 3, 5, 7, and 14.

Figure 3 shows the SEM profile of the maxillary bone post-orthodontic treatment of Wistar rats. Image analysis of bone resorption and ossification areas showed that mangiferin had a good effect of preventing bone relapse in the post-orthodontic treatment as a process of bone remodeling. Although the mangiferin's concentration has significantly affected the resorption and ossification of maxilla bone, the descriptive analysis of these images indicates that mangiferin has a good effect on bone remodeling activity post-orthodontic treatment.

Discussion

This study discusses the effect of mangiferin in preventing maxillary bone relapse post-orthodontic treatment. In this study, two characteristics were measured namely decreased calcium degradation and bone resorption, as two key factors involved in bone development and integrity, because more than 99% of the calcium content plays an essential role in the bone-forming elements.¹⁵ Whereas resorption is associated with the degradation of calcium as a process of bone resorption in the form of minerals facilitated by osteoclasts.¹⁶ Therefore, the ability of mangiferin to reduce the degradation of calcium and bone resorption is the focus of this study.

Figure 1 shows a decrease in calcium degradation, especially on the third, fifth and seventh days of treatment. These data indicate that mangiferin can play a role in bone remodelling by controlling bone calcium degradation as the main requirement to maintain bone compactness and prevent post-orthodontic treatment of maxillary bone relapse because mangiferin can act as an immunomodulator by regulating calcium and phosphorus formation. Also, mangiferin can control growth while repairing bone damage.¹⁷

Changes in post-orthodontic bone calcium degradation are affected by the mechanical response of the closed coil spring, which produces a constant force of 0.5 N to move the maxillary first molar to the mesial. So

that the maxillary bone adapts lead to the impact of calcium degradation. Qin (2013) states that bone growth and response to mechanical strength are highly dependent on several systemic hormones that could respond to calcium and phosphorus. In vivo modeling in mice using ortho wire resulted in movement until the specified time. This movement is the basis for bone remodeling to adapt to the surrounding environment. Calcium loss at the time of withdrawal by the treatment of ortho wire is replaced by hormonal mechanisms, in which systemic hormones regulate and excrete it from the bone to facilitate vital functions in other body systems, but excessive withdrawal can cause bone weakness.¹⁸ So it can be assumed that mangiferin can play a role in inducing the secretion of systemic hormones such as parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D to regulate bone remodelling activities.¹⁹

Bone remodeling is characterised by resorption and ossification in response to new bone formation.²⁰ The regulation of bone remodeling involves signals specifically in osteoclast function. Calcium is involved in the formation and activation of bone-taken osteoclasts. Parathyroid hormone and vitamin D are reported to be part of a systemic mechanism that regulates the availability, storage and disposal of calcium.²¹ Fischer et al. (2018) report that calcium and vitamin D is essential for maintaining bone health. Therefore, lack of calcium and vitamin D can be a significant risk factor for osteoporosis.²² Indirectly, the mangiferin investigated in this study can prevent maxillary osteoporosis by preventing calcium degradation. This ability is related to the chemical configuration of mangiferin influenced by the antioxidant activity of phenolic compounds.

Mangiferin shows a higher ability of chelating iron due to its sizeable antioxidant potential. The properties of antioxidants can prevent the formation of free radicals, thus preventing the release of bone calcium ions.²³ Besides, mangiferin contributes to increasing the role of pro-hypoglycemic by modulating glucose metabolism, improving insulin resistance, reducing cholesterol synthesis, so that it does not interfere with excessive calcium intake in both blood and bone when bone resorption occurs.²⁴ The results of the study in Figure 1 also showed that on the 14th day there was an increase in

calcium degradation, meaning that the concentration of Mangiferin 6.25% and 12.5% was only adaptive to work until the 7th day while on the 14th day had a weak protective power in preventing calcium degradation because several active compounds contained in mangiferin have a working period of only up to the seventh day, but the use of 25% mangiferin concentration needs to be considered to achieve optimum time to reduce bone degradation to prevent post-orthodontic treatment of bone relapse.

Figure 3 shows the post-orthodontic treatment of maxillary bone resorption and ossification based on the results of the analysis using the ImageJ device, where resorption is in line with the level of bone adoption as a condition for remodelling. It thus indicated that mangiferin is capable of preventing Relapse post-orthodontic treatment. Based on these results, the concentration of 6.25% showed better activity than 12.5%, but both have the same capability of decreasing bone resorption and increasing bone ossification. Therefore, 6.25% of mangiferin is the minimum concentration that shows a better ability to prevent calcium degradation in modelling in-vivo orthodontic treatment. In this study, it can be assumed that mangiferin can respond to changes in tensile forces by nickel-titanium orthodontic closed coil during orthodontic treatment to prevent maxillary bone relapse in the process of bone remodelling.

Jimi (2017) reported that the main cells involved in bone remodelling are osteoclasts and osteoblasts, where bone resorption and ossification are closely related during bone remodelling, the imbalance of these two processes lead to an increase or decrease in bone mass. Osteoclasts are multinucleated cells responsible for physiological and pathological bone absorption, which play a role in maintaining bone volume and homeostasis. Osteoclastic bone resorption is regulated by several cytokines, calcium signalling, and transcription factors.²⁵ Boabaid et al. (2001) reported that mangiferin is a polyphenolic compound capable of reducing bone damage and inhibiting osteoclastic by inducing WST-1 activity for cell proliferation.²⁶ In addition, mangiferin significantly reduces the formation of multinuclear acid phosphatase cells that are resistant to tartaric acid, thereby inhibiting bone resorption.²⁷ Sekiguchi (2017) confirms that mangiferin can inhibit osteoclastic bone resorption by suppressing osteoclast

differentiation and promoting ER β mRNA expression in mouse bone marrow of macrophage cells that benefit osteoblastic processes by promoting cell proliferation as well as promoting cell differentiation.²⁸ These results are consistent with previous reports that, bone remodelling is always followed by the intensity of the production of proteins involved in the bone formation such as MMP-8 which is involved in bone matrix formation and TGF- β protein which is involved in the development and growth of bone elements based on responding conditions required by the bone.²⁹

Tanaka et al. (2005) reported that osteoblasts and osteoclasts are actively involved during bone remodeling and inflammation. Osteoblasts not only play a central role in bone formation by synthesising several bone matrix proteins but also regulates osteoclast maturation to produce bone resorption.³⁰ Its means that bone resorption and ossification are normal processes of bone remodelling, but the use of mangiferin can increase control of bone remodelling based on bone resorption and ossification intensity compared to controls without mangiferin administration as shown in Figure 3, where the concentration of responses (6.25 and 12.5%) of mangiferin to resorption and ossification differed between both concentrations but based on the descriptive analysis of the image, there was a post-orthodontic treatment of bone remodelling activity characterised by resorption and ossification processes.

Conclusions

Mangiferin is able to control calcium degradation as well as bone resorption. It is also able to enhance the ossification of maxilla bones of the *Rattus novergicus* post-orthodontic treatment. Mangiferin with a concentration of 6.25% and 12.5% can be utilized as a standardised reference in bone remodelling research.

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Declaration of Interest

The authors report no conflict of interest.

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