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RESEARCH ARTICLE

The Ratio of Runx-2 and Osterix in Tension Area of Orthodontic Tooth Movements on Days 7 and 14 after Giving Mangostin

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Abstract

Background: Orthodontic relapse is the return of the tooth to the first position after being treated with an orthodontic appliance. Bone density in tension area of orthodontic in new bone formation (apposition) is critical in orthodontic treatment to prevent relapse so that orthodontic treatment can be achieved optimally through runt-related transcription factor 2 (Runx-2) and Osterix evaluation. This study aims to determine the role of Runx-2 and Osterix ratio in the tension area of orthodontic tooth movement after Mangostin administration. Material and Methods: An experimental laboratory study was conducted among 30 male Wistar rats were divided into several groups. The K1 was a negative control group (K-) without treatment, K2 was a positive control group (K +) given orthodontic mechanical strength and observed for 7 days, and K3 was a positive control group, given orthodontic mechanical strength and was observed for 14 days. In addition, K4 was a treatment group (P) given orthodontic mechanical strength and were observed for 7 days, and K5 was the treatment group (P) given orthodontic mechanical strength and were observed for 14 days. Data were tabulated and analyzed using SPSS version 17 for Windows. Results: Mangostin administration is significantly increasing the expression of Runx-2 (8.3±0.4 ng/mL), Osterix (4.7 ± 1.8 ng/mL), and bone formation (1:3.23 vs. 1:1.23) in the tension area of the treatment group ($P<0.05$) after 14 days. The Osterix levels were also suggest a significantly higher among groups from K1 (0.2 ± 0.2 ng/mL), K2 (1.1 ± 0.2 ng/mL), K3 (1.4 ± 0.3 ng/mL), K4 (1.3 ± 0.6 ng/mL), and K5 (4.7 ± 1.8 ng/mL) ($P<0.05$). The ratio of Osterix (1:3.23) seemed to be higher in treatment groups, whereas Runk2 ratio was slightly lower compared with control groups (1:1.23).Conclusion: The administration of Mangostin can increase the expression of Runx-2 and Osterix in the area tension of orthodontic tooth movement on day 14.

Keywords: *Mangostin, Runx-2, Osterix, Tension area, Tooth movement.*

Introduction

Runx-2 (Runt-related transcription factor 2), also known as CBFA, is considered as the center of control of the osteoblast phenotype gene. Runx-2 is a marker that is expressed by osteoblast precursors. Physical interactions and functions between Runx-2 as osteogenic factors and HIF-1α as angiogenic factors stimulate VEGF expression in mesenchymal cells[1].Runx-2 is an important transcription factor in osteogenic differentiation, one of which is shown by stimulating the formation of transcription genes in osteoblasts, such as osteocalcin[2].The movement of orthodontic teeth activates osteoblasts, osteoblasts produce a number of molecules, one of which is the Runx-2 transcription factor, Runx-2 also regulates Osterix and helps differentiate osteoblasts, Runx-2 also induces BMPs [3,5].Malocclusion is a dental growth disorder and associated anatomical structure that can interfere with a person's psychological condition, Malocclusion can be treated using orthodontic devices to obtain normal and pleasant facial occlusion [6,8].

Orthodontic treatment always uses mechanical strength to move teeth. The mechanical strength of the teeth to be moved orthodontically will be forwarded to all supporting tissues of the teeth and cause a remodeling process to facilitate the movement of teeth through the bones [9].Giving orthodontic mechanical pressure causes the area around the teeth to be divided into two regions, namely the area of tension/pressure and pull. In the area of pressure, the mechanical force will stimulate osteoclasts to resorb alveolar bone. After the resorption process is complete, the osteoclasts will experience apoptosis so that the resorption process stops.

Whereas in the pull region, the osteoblasts are activated to perform new bone formation activities (apposition).If the orthodontic mechanical pressure is adequate, the resorption process and the apposition of the alveolar bone are in balance [10, 11].Mechanical pressure in orthodontic treatment gives rise to various responses to the periodontal ligament tissue, dental pulp, alveolar bone in the form of an inflammatory reaction. The initial phase of orthodontic tooth movement always involves an acute inflammatory response characterized by capillary vasodilation and leukocyte migration to capillaries.

These migrating cells produce various cytokines. These cytokines stimulate the synthesis and secretion of different target substances such as prostaglandin, growth factors and various cytokines [12].The bone remodeling process caused by the pressure or tension of an orthodontic device will lead to tooth movement [13].Bone remodeling is beneficial in orthodontic treatment, specially to prevent relapse of treatment results.

Several studies have shown that if tooth movement is not followed by remodeling of supporting tissue, the teeth will relapse [14].The development of treatment using natural ingredients where one that is proven effective as an anti-inflammatory and antioxidant is the extract of mangosteen peel (Garcinia Mangostana) which contains Mangostin. The results show that the mangosteen is rich in amazing nutrients called xanthones, which are widely found on the skin. Extracted mangosteen peel found xanthone 95%, isoflavones, tannins, flavonoids, Vitamin C, phenols, and anthocyanins, which are high antioxidant activities [15].Xanthone of mangosteen peel in the form of α-Mangostin and γ-Mangostin has been studied to have potent anti-inflammatory and antitumor effects [16].The substance of α-Mangostin is the main component (78%) which is used throughout the world as a traditional treatment for anti-inflammatory, anti-bacterial, and anti-cancer [17].

Both types of xanthone in the form of α-Mangostin and γ-Mangostin in the mangosteen peel play a role in stopping inflammation or inflammation by inhibiting prostaglandin synthesis through inhibition of the cyclooxygenase enzyme that causes inflammation [18]. Based on the aforementioned, this study aims to analyze the effect of Mangostin on orthodontic pull regions in the expression of Runx-2 and Osterix in osteoblasts in the process of bone remodeling to prevent relapse through bone apposition.

Material and Methods

An experimental study was conducted among Wistar rats aged 2-3 months with a bodyweight of about 250-350 grams. Furthermore, randomly allocated 30 rats were divided into 3 research groups, namely. The negative control group (K1) as the initial treatment that was not treated was only given standard food and sterile distilled water which was decapitated on day 8.

The positive control (K+) group was divided into 2: K2 group as given mechanical stressors and standard food and CMC without administration of Mangostin and decapitated on day 8 and K3 as the group given mechanical stressors without the providing Mangostin and standard food or CMC, sacrificed on day 15. The treatment groups were divided into K4 as given a mechanical stressor and Mangostin as well as CMC by oral sondage, decapitated on the day 8.

K5 group was assigned mechanical stressors and oral Mangostin as well as CMC and were sacrificed on day 15. The administration of Mangostin (*Garcinia mangostana*) was standardized containing 90% mangostin produced by Xi'an Biof Bio-Technology Co., Ltd (Room 1-1111, High-tech Venture Park, No.69 Jinye Road Gaoxin District of Xi'an, People Republic of China) Ni coil spring is

placed between the maxillary central incisor and the upper right first molar to move the molar toward the mesial with a constant force of 10 g / cm2. Spring is attached to the first molar and right upper jaw incisor using stainless steel wire. Ligh-curing bonding was applied to the perforation made with a round bur along the line angle on the mesio-lingual and disto-lingual side of the maxillary first molar and distal side of the incisor to increase retention of the coil spring .Anesthetic injection of intraperitoneal Ketamine hydrochloride and acepromazine for the installation of closed coil spring as a mechanical stressor. After that, three groups of male Wistar rats were sacrificed for immunohistochemical observations by anesthetizing using Ketamine and acepromazine then decapitation, maxillary bone tissue was then taken in a buffer formalin solution. The number of Runx-2 and Osterix expressions was observed and calculated using a light microscope on a number of 10 view fields with 400x magnifications. Based on the results of the calculation, One Way Anova test was carried out, followed by the Post Hoc test to analyze differences between groups. Data were analyzed using SPSS version 17 for Windows.

Results

The recent findings found that there were a significant differences in the mean level of Runx-2 expressions in the group with or without Mangostin administration as well as orthodontic mechanical strength of the K1 on day 7, K2 on days 7 and 14, treatment group (P) on days 7 and 14 (P<0.05) (Figure 1).

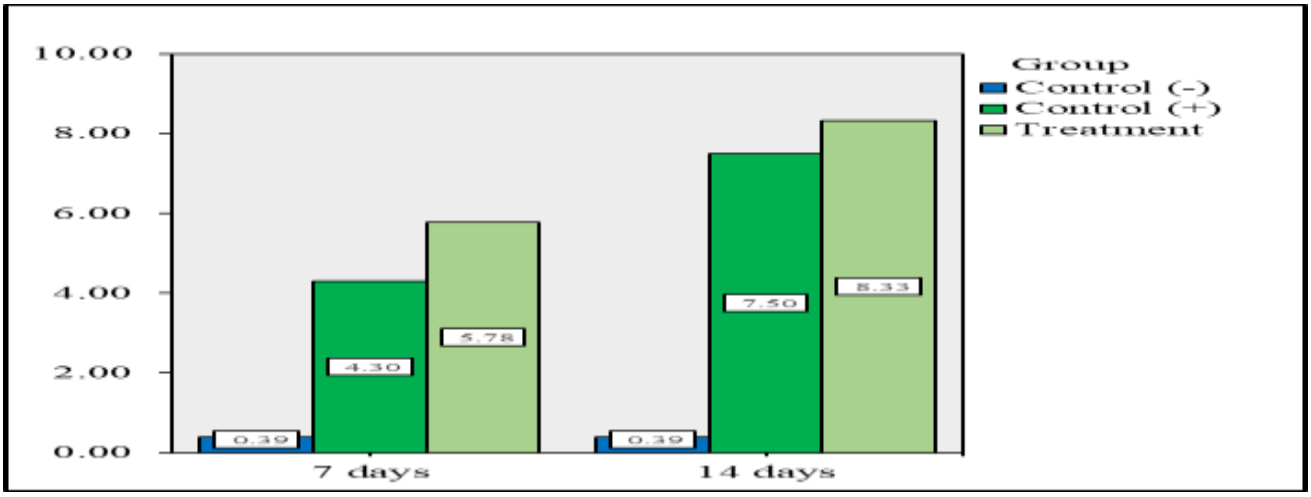

Figure 1: The number of Runx-2 expressions on days 7 and 14 between groups

The Runx-2 expression was predominant in K5 group (8.3 ± 0.4 ng/mL), followed by K4 (5.8 ± 0.8 ng/mL), K3 (7.5 ± 0.9 ng/mL), K2 (4.3 ± 0.4 ng/mL), and K1 (0.4 ± 0.1 ng/mL). The Post Hoc analysis also suggested a statistically significant difference of Runx-2 levels between groups (P<0.05) (Table 1). The immunohistochemical assessment of Runx-2 expression is depicted in Table 2.

Table 1: The Runx-2 expressions in the negative control (K1), positive control (K2 and K3), and treatment (K4 and K5) groups

Groups		Runx-2 (Mean ± SD) (ng/mL)	p
K1		0.4 ± 0.1	
	K2	4.3 ± 0.4	0.000*
	K3	7.5 ± 0.9	0.000*
	K4	5.8 ± 0.8	0.000*
	K5	8.3 ± 0.4	0.000*
K2		4.3 ± 0.4	
	K3	7.5 ± 0.9	0.000*
	K4	5.8 ± 0.8	0.000*
	K5	8.3 ± 0.4	0.000*
K3		7.5 ± 0.9	
	K4	5.8 ± 0.8	0.000*
	K5	8.3 ± 0.4	0.018*

Groups		Runx-2 (Mean ± SD) (ng/mL)	p
K4		5.8 ± 0.8	
	K5	8.3 ± 0.4	0.000*
*Statistically significant if less than 0.05; ng: nanogram; mL: milliliter; Runx-2: Runt-related transcription factor 2; SD: Standard Deviation			

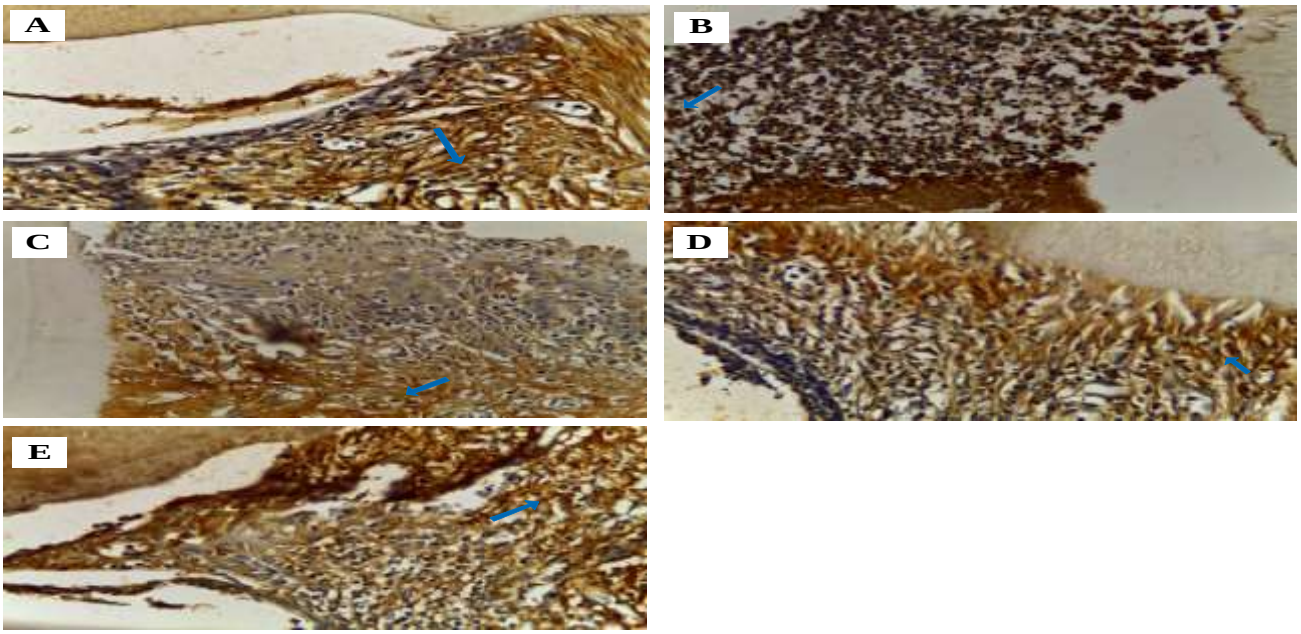

Figure 2: The immunohistochemical evaluation of Runx-2 in alveolar bone osteoblasts (brownish) marked with a blue arrow. (A) Negative control group (K-); (B & D) Positive control group (K +); (C) Treatment group (P) with Mangostin administration on day 7; (E) Treatment group (P) with the provision of Mangostin on day 14 with 400x magnification

In Figure 3, it illustrates that there is a significant differences in the average number of Osterix expressions in the group without Mangostin administration and without giving orthodontic mechanical strength with the Mangostin administration group, as well as the orthodontic mechanical strength of K1 on day 7, K2 on days 7 and 14, treatment group (P) on days 7 and 14 (P<0.05). The highest mean level of Osterix expression was K5 group (4.7 ± 1.8 ng/mL) (Table 2). The immunohistochemical approach also found that the Osterix expression was higher in the K5 group (Figure 4).

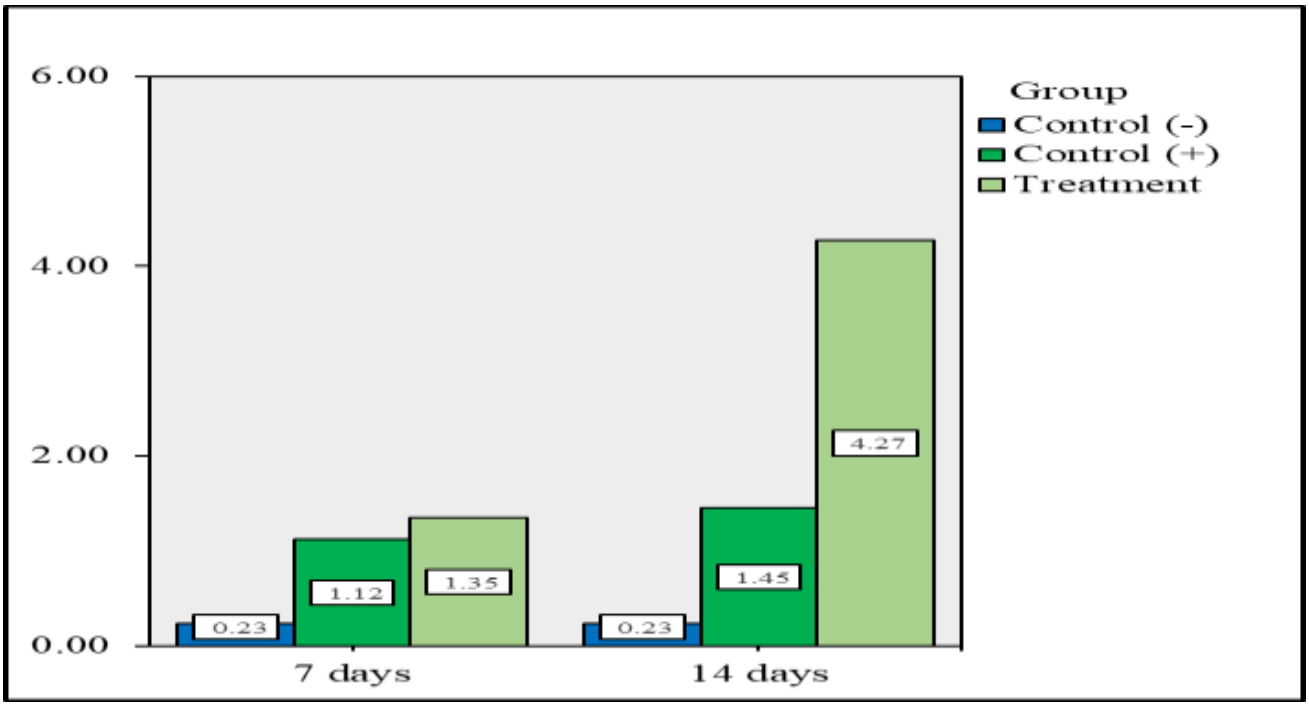

Figure 3: The expression of Osterix between groups on days 7 and 14

Table 2: The mean level of Osterix expressions between groups of Mangostin administration

Groups	Osterix (Mean ± SD) (ng/mL)	P
K1	0.2 ± 0.2	
K3	1.4 ± 0.3	0.014*
K4	1.3 ± 0.6	0.024*
K5	4.7 ± 1.8	0.000*
K2	1.1 ± 0.2	
K5	4.7 ± 1.8	0.000*
K3	1.4 ± 0.3	
K5	4.7 ± 1.8	0.000*
K4	1.3 ± 0.6	
K5	4.7 ± 1.8	0.000*

*Statistically significant if less than 0.05; ng: nanogram; mL: milliliter; SD: Standard Deviation

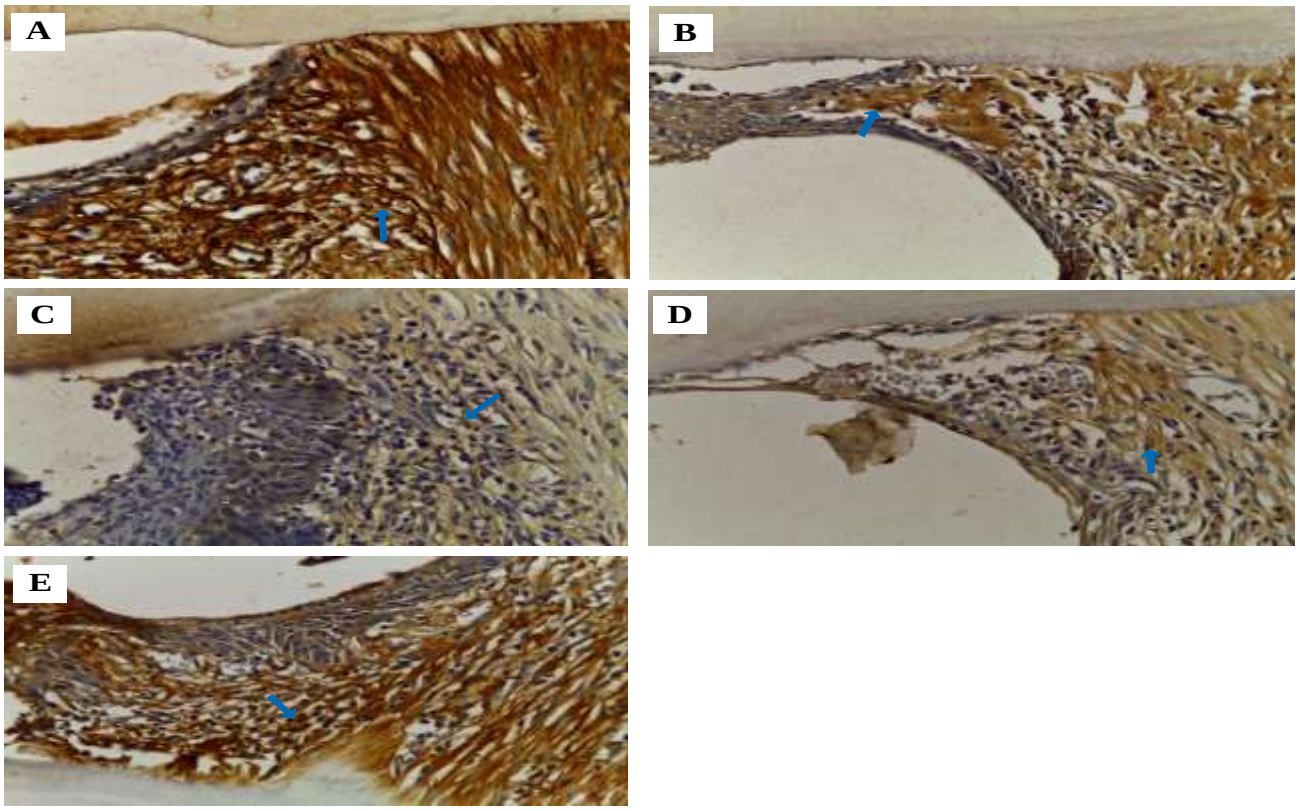

Figure 4: The Osterix expression in alveolar bone osteoblasts (brownish) marked with blue arrows on immunohistochemical evaluation. (A) Negative control group (K-); (B&D) Positive control group (K+); (C) Treatment group (P) with Mangostin administration on day 7; (E) Treatment control group (P) with the administration of Mangostin on day 14 with 400x magnification

The comparison ratio of Runx-2 and Osterix expression between days was evaluated. The findings exhibited that the Osterix ratio was higher in the treatment group between days (Day 7 vs. Day 14) compared with controls (1:3.23) (Table 3). However, in vice versa, the Runx-2 expression ratio was slightly lower in the treatment group compared with controls (Table 3). The schematic evaluation of those ratios is depicted in Figure 5.

Table 3: Osterix and Runx-2 ratio on 7 and 14 days of Mangostin administration

Group	Parameters	Days	Value	Ratio
Control (-)	Osterix	7 days (I)	0.6	1: 1
	Runx-2	7 days (I)	0.6	
Control (+)	Osterix	7 days (I)	1.5	1:1.32
		14 days (II)	1.98	
	Runx-2	7 days (I)	5.2	1:1.76
		14 days (II)	9.14	
Treatment	Osterix	7 days (I)	2.59	1:3.23
		14 days (II)	8.36	
	Runx-2	7 days (I)	7.38	1:1.23
		14 days (II)	9.07	

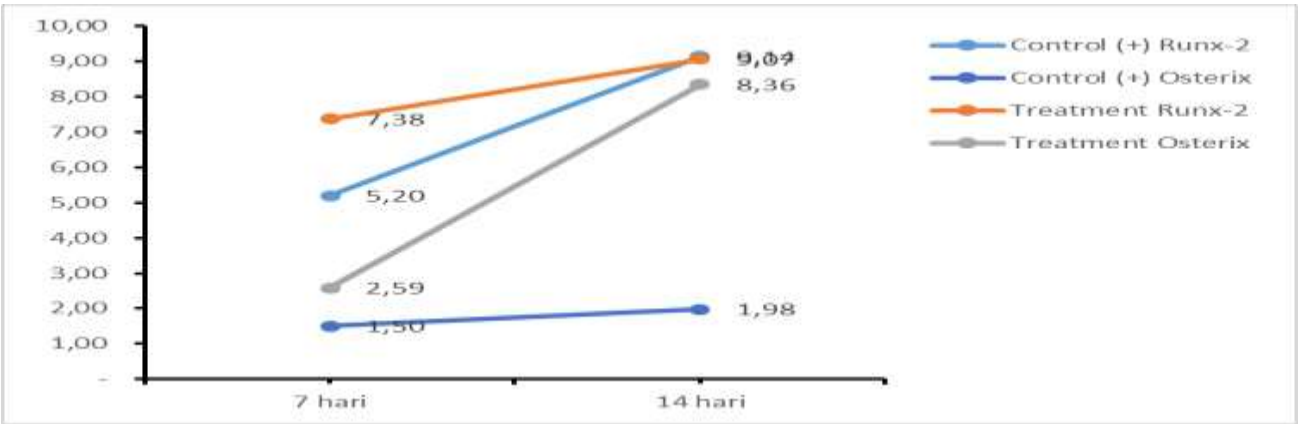

Figure 4: The schematic plots of Osterix and Runx-2 ratio on 7 and 14 days of Mangostin administration

Discussion

Mangosteen peels exude yellow resin which is rich in Xanthone [19].Xanthone is included in the class of flavonoid compounds. Xanthone is a polyphenolic compound with a chemical structure containing an aromatic tricyclic ring. This structure has biological activities such as antioxidants, anti-inflammatory, antibacterial, anticancer [20]. Extracted mangosteen peel found xanthone 95%, isoflavones, tannins, flavonoids, Vitamin C, phenols, and anthocyanins which are high antioxidant activities [15].Mangostin is the main xanthone found in the mangosteen peel (78% level) which is used worldwide for traditional medicine as an anti-inflammatory, anti-bacterial and anti-cancer [17].

In this study, the group given Mangostin had a significant difference where the highest Runx-2 expression was seen on day 14 compared to the control group (negative control) and the group not given Mangostin (positive control group). This could be caused that mangosteen skin extract (*Garcinia mangostana*) is rich in xanthones, especially α-Mangostin and γ-Mangostin as an anti-inflammatory which plays a role in inhibiting the production of cyclooxygenase (COX) enzymes which are inflammatory causes [21].

Research on mouse glioma cells against in vitro anti-inflammatory activity of Mangostin against prostaglandin-estradiol 2 (PGE2) and cyclooxygenase (COX) synthesis which is a mediator of inflammatory occurrence In vitro enzymatic experiments, these compounds inhibit COX-1 and COX-2 activity where γ-Mangostin inhibits COX-2 protein and mRNA expression induced by lipopolysaccharide [18].Increasing COX-2 will increase PGE2 synthesis. Increased synthesis of PGE2 will increase vasodilation and endothelium permeability, which increases inflammatory cell infiltration. In the group given Mangostin, it will reduce COX-2, thereby decreasing the inflammatory process because γ-Mangostin can inhibit the expression of MAPK, NF-K, and AP-1 in macrophages. Inhibition of COX-2 can reduce pro-inflammatory cytokines (IL-1, TNF-). With decreased levels of IL-1 and TNF-α, the inflammatory process and cellular responses involved in inflammation and immune regulation can be inhibited.

The process of endothelial dysfunction can be prevented where IL-1 and TNF-α are NF-KB activation stimuli which further inhibit osteoclast formation through RANKL, which leads to inhibition of osteoclast formation through RANKL, so that osteoclast differentiation and formation do not occur and reduce the osteocalcin process which results in increased osteoblast formation. In addition, the content of γ-Mangostin decreases lipopolysaccharide induction (LPS) in the synthesis of TNF-and IL-4 by inhibiting the oncostatin M gene expression in the MAPK pathway [22].

Mangostin contained in mangosteen peel extract will enable osteoprogenitor cells from osteoblasts to become osteochondral progenitors that convert Runx-2 and Osterix and catenin to preosteoblasts which then form ALP and Collagen type 1.Runx-2 here as an important transcription factor in bone formation. Mangostin administration on the 14th day in the treatment group increased Osterix expression in osteoblasts in the pull area. By giving orthodontic mechanical movements in the area of attraction will increase Osteoprotegerin (OPG). Mangostin content found in mangosteen peel extract, especially γ-Mangostin, has anti-inflammatory properties in cyclooxygenase

activity which can inhibit the convention of arachidonic acid to PGE2, thus inhibiting the activity of IKB Kinase inhibitors that activate NF-K thereby reducing the activity of inflammatory cells [23].Mangostin can increase type 1 collagen as an osteoblast formation so that it can accelerate bone formation. Various studies indicate Osterix (Osx) triggers the precursor Runx-2 to become osteoblasts by expressing osteoblast genes [24].

Bone formation in orthodontic tooth movement is the result of osteoblast differentiation controlled by the Cbfa 1 gene (core-binding factor alpha-1) and Osterix. Cbfa-1, known as Runx-2, is the earliest expressed marker by osteoblasts and is associated with bone formation. Osterix is an advanced transcription factor that induces osteoblasts to produce osteocalcin genes that control osteoblast differentiation through inhibitory effects [24].

It can be concluded that the administration of Mangostin to orthodontic tooth movement in the pull area can increase osteoblast formation, thereby reducing osteoclast formation. In this study shows Runx-2 and Osterix are the main transcription factors in the osteogenesis process. The results showed that Runx-2 and Osterix ratios in the area of attraction of orthodontic tooth movement on the 7th day with the administration of Mangostin would increase in Runx-2 while on the 14th day there was no increase, and there were no significant differences while Osterix would occur raised on day 7 and day 14. On day 14, Runx-2 will stop differentiating while Osterix will increase to osteocytes.

The decrease in Runx-2 on day 14 was due to Runx-2 being the earliest transcription factor which was associated with the bone formation so that on day 14 there was no increase [24].In this study, on the 14th day, Osterix showed improvement but there were no significant differences. It could be caused by the administration of Mangostin can increase the formation of type 1 collagen as forming osteoblasts, so Osterix also increases and accelerates bone formation.

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