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Analysis of the Potency of Robusta Coffee (*Coffea canephora*) to Increase the Expressionof FGF2, Collagen 1 and ALP in the Periodontal Ligament During Orthodontic Tooth Movement

Herniyati[1,*], Leliana Sandra Devi[1] and Noengky Prameswari[2]

[1]*Department of Orthodontic, Faculty of Dentistry, Jember University, Jember, Indonesia*
[2]*Department of Orthodontic, Faculty of Dentistry, Hang Tuah University, Surabaya, Indonesia*

([*]Corresponding author's e-mail: herny_is@yahoo.com)

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Abstract

Orthodontic treatment is relatively long and various efforts have been made to speedup the movement of orthodontic teeth. Coffee contains substances called caffeine (1,3,7 trimethylxanthine), chlorogenic acid and caffeic acid, as well as flavonoids which have antioxidanteffects. Chlorogenic acid and caffeic acid are antioxidants that can reduce oxidative stress in osteoblasts and promote osteogenesis. The study aimed to analyze the expression of FGF2, Collagen 1 and ALP, on the movement of orthodontic teeth after administration of Robusta coffeeextract. 24 wistar rats divided into 4 groups, namely groups C1 and C2: Rats were givenorthodontic mechanical force (OMF) for 2 and 3 weeks and groups T1 and T2: Mice were given OMF and Robusta coffee extract for 2 and 3 weeks. OMF in mice (rats) was carried out bymeans of the right maxillary first molars teeth and on both maxillary incisors were given ligature wires. Then the right maxillary first molars moved to the mesial with a Niti closed coil spring. Observations were made on the day 15 and day 22 with immunohistochemical examinations to determine the expression of FGF2, Collagen 1 and ALP. The results indicated that administration of Robusta coffee extract for 2 and 3 weeks significantly increased the [expression of FGF2, Collagen 1 and ALP. The expression of FGF2, Collagen 1 and ALP (expression of GFß1, IGF1 and PDGF). The expression of TGFß1, IGF1 and PDGF during 3 weeks was greater than that at 2 weeks ($p < 0.05$. Robusta coffee extract can be used as an alternative ingredient in accelerating orthodontic treatment.

Keywords: ALP, FGF2, Collagen 1, Orthodontic mechanical force, Robusta coffee extract

Introduction

Malocclusion is a deviation from the normal relationship between teeth with other teethin the same jaw arch and/or with other teeth in the opposite jaw arch [1]. Malocclusion can causepsychosocial problems related to dentofacial aesthetic disorders, impaired masticatory and phonetic functions, and increase the risk of periodontal disease [2]. Malocclusion is one of the dental and oral health problems in Indonesia which has a prevalence of 80 % and ranks third aftercaries and periodontal disease [3].

Orthodontic tooth movement occurs due to mechanical stimulation followed by remodeling of the alveolar bone and periodontal ligament (PDL). Bone remodeling is the process of bone resorption in areas of stress and bone formation in areas of tension. Bone resorption is carried out by osteoclasts on the tension side and new bone formationby osteoblasts on the tensile side. In orthodontic tooth movement the forces applied to the teeth will cause changes in the microenvironment around the PDL due to changes in blood flow, leading to the secretion of inflammatory mediators such as cytokines, growth factors, neurotransmitters, colony-stimulating factors, and arachidonic acid metabolites. As a result of this secretion, bone remodeling occurs [4].

Basic fibroblast growth factor (bFGF) or fibroblast growth factor-2 (FGF2) is acytokine involved in angiogenesis, tissue remodeling and stimulation of osteoblasts and osteoclasts. FGF2 is a polypeptide of the FGF family that can be found in dentin. The role of this molecule is similar to that of VEGF and it is involved in immigration and proliferation of endothelial cells, angiogenesis under *in vivo* conditions and bone reconstruction [5]. FGF2, is a potent angiogenic factor that shows increased expression in hypoxic conditions and during wound healing [6,7]. The role of thismolecule is similar to that of VEGF and is involved in endothelial cell immigration and proliferation, *in vivo* angiogenesis and bone tissue

reconstruction [5]. FGF2 is a potent angiogenic factor that shows increased expression under hypoxic conditions and during wound healing [6,7]. This growth factor increases endothelial cell proliferation and induces endothelial cellgermination. FGF2 is also a component of the bone matrix and plays an important role in regulating bone remodeling [8].

Collagen is the main protein that makes up the extracellular matrix and the most abundantprotein found in the human body. Collagen is a component main fiber in bones, teeth, cartilage, dermis tendons and cartilage [9,10]. Includes connective tissue composed of collagen fibers [9,10]. Bone metabolism can be seen in biochemistry using bone formation markers. Bone formation markers consist of type I collagen, alkaline phosphatase and osteocalcin [11]. Type I collagen is the most common type of collagen, distributed in almost all connective tissue except in hyaline cartilage. Type I collagen is also the main protein in bone, skin, tendons, ligaments, sclera, cornea, and blood vessels and comprises 95 % of the collagen content of whole bones and about 80 % of the total protein in bones Type I collagen is the most common type of collagen, distributed in almost all connective tissues except for hyalinecartilage. Type I collagen is also the main protein in bone, skin, tendons, ligaments, sclera, cornea,and blood vessels and this type I collagen accounts for 95 % of the collagen content of all bones and about 80 % of the total protein present in bone [12]. The periodontal ligamen mainly containstype I and type III collagen fibers and type I is the predominant collagen [13,14]. Type I collagen is the highest (90 %) collagen produced by osteoblasts, whereas osteocalcin is a non-specific collagen produced only by osteoblasts. Both collagens have an important role in matrix formationin the regeneration process [15].

Alkaline phosphatase (ALP) is a protein bound to cell membranes and synthesized by various tissue cells. The biological response to orthodontic tooth movement involves changes in the surrounding bony architecture. Bone metabolism is related to alkaline phosphatase (ALP) by osteoblasts and acid phosphatase (ACP), by osteoclasts. ALP is a glycoprotein involved in the mineral formation of bone and cementum tissue. This enzyme is thought to release phosphate ionsfrom organic phosphate esters leading to the deposition of tissue phosphate salts [16]. The ALP enzyme is contributed in the blood from the liver, placenta and intestine, ALP from the osteoblastmembrane and then enters the blood circulation plays a role in the mineralization of new bone formation known as Bone Alkaline Phosphatase (BALP) tissue [17]. Continuous orthodontic forces can exert pressure that disrupts the integrity of the vascular compartment in the PDL. Excessive pressure causes ischemia, gradual capillary reduction, presence of thrombus, impaired nutrition and cell death [18], with the almost unavoidable formation of necrotic or hyaline zones, especially on the pressure side. In contrast, dilated blood vessels were found on the side of pull [19].

The success of orthodontic treatment is influenced by a number of factors, including periodontal health, oral hygiene, and orthodontic strength. Efforts to accelerate the movement of orthodontic teeth have been made, including the use of drugs [20].

Coffee is a type of plantation crop that has long been cultivated and has high economic value. Coffee is one of the popular drinks consumed by the public. Coffee, among other things, contains a substance called caffeine (1,3,7 trimethylxanthine) [21], chlorogenic acid and caffeic acid, as well as flavonoids which have antioxidant effects (22). Robusta coffee has high antioxidant activity compared to Arabica coffee and others [23]. Flavonoids stimulate prolidase activity which catalyzes the final step of collagen and plays an important role in collagen biosynthesis through integrin-mediated signaling [24].

The results showed that chlorogenic acid promoted osteogenesis in human adipose tissue derived mesenchymal stem (hAMSCs), which wasindicated by increased mineralization [25].

The results also showed that giving 50 mg/kg caffeine to pregnant rats showed that rats had osteoblasts with high osteogenic potential which was characterized by increased expression of osteocalcin, osteopontin, sialoprotein, Runx-2, alkaline phosphatase and type 1 collagen and increased synthesis of mineralized nodules [26].

The previous study shows showed that chlorogenic acid promoted osteogenesis in human adipose tissue derived mesenchymal stem (hAMSCs), which was indicated by increased mineralization [25]. The previous study also showed that administration of 50 mg/kg of caffeine to pregnant rats showed that the rats had osteoblasts with high osteogenic potential, characterized by increased expression of osteocalcin, osteopontin, sialoprotein, Runx-2, alkaline phosphatase, collagen type 1 and synthesis of mineralized nodules. [26].

Tooth movement in orthodontic treatment which takes a long time, if efforts to accelerate treatment are not carried out, malocclusion can negatively impact oral hygiene, making it more susceptible to periodontal disease and caries. In addition, it creates a big psychological burden because the sufferer feels embarrassed by his irregular teeth. The relatively expensive cost of orthodontic treatment will also add to the patient's economic burden, so efforts to accelerate orthodontic treatment need to be continued.

The movement of teeth in orthodontic treatment that takes a long time, if no efforts are made to speed up treatment, can have a negative impact on oral hygiene, making it more susceptible to periodontal disease and caries. Besides, it causes a great psychological burden because the sufferer feels ashamed of the condition of his teeth that are not good. The cost of orthodontic treatment which is relatively expensive will also increase the economic burden of thepatient, so efforts to accelerate orthodontic treatment need to be carried out continuously.

Orthodontic treatment to correct dental malocclusion requires a long time. Dental malocclusion can have an adverse impact on oral hygiene, making it more susceptible to periodontal disease and caries. Besides that, dental malocclusion causes a large psychological burden because sufferers feel embarrassed by the condition of their teeth that are not good. The cost of orthodontic treatment which is relatively expensive will also add to the economic burden of the patient, so efforts to speed up orthodontic treatment need to be carried out continuously.

This study aimed to analyze the molecular aspects of Robusta coffee extract in the remodeling process of periodontal tissue during orthodontic tooth movement through the expression of FGF2, Collagen 1 and ALP using immunohistochemical techniques.

Materials and method

Research design

This laboratory experimental study was conducted using 24 male Wistar rats with an agerange of 3 - 4 months and a body weight of 250 - 300 g. The rats were in good health and wereselected who had complete dental structures, healthy oral cavity conditions and periodontal tissue.

Settings & samples

Rats were randomly divided into 4 groups: Control group (C1 and C2): Rat mice were given orthodontic mechanical force (OMF) and 2 mL of distilled water for observation time for 2 and 3 weeks and treatment group (T1 and T2): Given OMF and freeze-dried extract 0.050 g of Robusta coffee (equivalent to 1 cup of adult coffee) dissolved in 2 mL of distilled water, given twicea day, morning and evening for 2 and 3 weeks of observation time. The orthodontic mechanical force on rats was performed by using ketamine anesthesia for rats. The right maxillary first molarand both maxillary incisors were given a ligature wire with a diameter of 0.20 mm.

Then the right maxillary the rightright maxillary first molar was moved mesial using Nickel Titanium Orthodontic closed coil spring with a length of 6 mm. The orthodontic strength used was 3.5 oz measured using an orthodontic force gauge [26].

Data collection procedures

Observations of expression of FGF2, Collagen 1 and ALP were made by sacrificing rats on the 15^{th} and 22^{nd} days, then taking the right M1-1 and M-2 RA teeth and their periodontal tissue. Subsequently, histological preparations were made and immunohistochemical examinations were carried out to determine the expression of FGF2, Collagen 1 and ALP. Counting of the expression of FGF2, Collagen 1 and ALP was carried out in alveolar bone osteoblasts in the tension area using a microscope with a magnification of 400x.

Statistical analysis

The expression data of FGF2, Kolagen 1 and ALP were analyzed using the independentt test and the Mann Whitney test, with a confidence level of 95 % ($\alpha = 0.05$). This research was approved by the research ethics commission of the Faculty of Dentistry, University of Jember, Number: No. 1294/UN25.8/KEPK/DL/2021

Results and discussion

FGF2 expression

The results of the research on the effects of Robusta coffee extract on FGF2 expression are shown in **Table 1**. The results of immunohistochemical examination of expression are shown in **Figure 1**.

Table 1 Mean and standard deviations of FGF2 expressions on day 15 and day 22 and different tests of TGFß1 expressions between day 15 and day 22 in each research group.

Group	n	Expression of FGF2 (Mean ± Standard deviation)		P
		Day - 15	Day - 22	
Control	6	31.83 ± 1.169	48.33 ± 1.033	0.000*
Treatment	6	49.33 ± 1.211	55.50 ± 1.049	0.000*
P		0.000*	0.000*	

*$p < 0.05$ = significant
**$p < 0.05$ = not significant

Table 2 illustrates mean and standard deviations of FGF2 expression in the control group and treatment group on day 15 and day 22.

Testing based on the independent t-test of FGF2 expression between the study groups on day 15 and day 22 showed that the expression of FGF2 in the treatment group was greater than that in the control group and there was a significant difference ($p > 0.05$).

Tests based on the independent t-test of FGF2 expression on day 15 and day 22 showed that FGF2 expression in the treatment groups was significantly greater than that in the control groups ($p < 0.05$).

Tests based on the independent t-test showed that the expression of FGF2 in the control and treatment groups on day 22 was significantly greater than day 15 ($p < 0.05$).

The histological image of FGF2 expression in the control group and the treatment groupis shown in **Figure 1**.

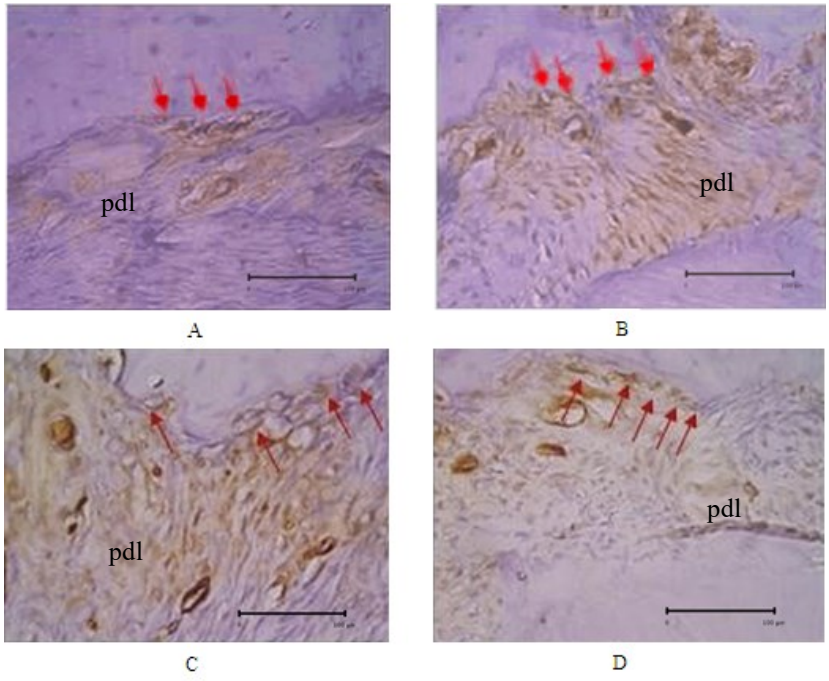

Figure 1 Expression of FGF2 alveolar bone observations on day 15 and day 22, indicated by arrows: In the control group day 15 (A), the treatment group on day 15 (B), the control group on day 22 (C), and Treatment group day 22 (D), bone (b), periodontal ligament (pdl), dentine (d). (Immunohistochemistry, 400x magnification).

Basic fibroblast growth factor (bFGF) or fibroblast growth factor-2 (FGF2) is a cytokine involved in angiogenesis, tissue remodeling and stimulation of osteoblasts and oteoclasts. FGF2 is a polypeptide from the FGF family that can be found in dentine. The role of this molecule is similar to that of VEGF and is involved in endothelial cell immigration and proliferation, *in vivo* angiogenesis and bone tissue reconstruction [5].

Coffee is one of the main sources of antioxidants in people's daily diets. The beneficial health effects of coffee are usually associated with high antioxidant activity (ability to inhibit oxidation processes). Robusta coffee beans contain a lot of polyphenolic antioxidants, such as chlorogenic acid, caffeic acid, ferulic acid and n coumaric acid. The main polyphenolic compounds in robusta coffee are chlorogenic acid and caffeic acid. The amount of chlorogenic acid reaches 90 % of the total phenols found in coffee [22].

Continuous orthodontic force can apply pressure that disrupts the integrity of the vascular compartment in the PDL. Excessive pressure causes ischemia, gradual reduction of capillaries, presence of thrombus, impaired nutrition and cell death 18]. with the almost unavoidable formation of necrotic or hyaline zones, especially on the pressure side. In contrast, dilated vessels are found on the traction side [19]. These vascular changes may be mediated by different growth factors, such as fibroblast growth factor-2 (FGF2) and vascular endothelial growth factor (VEGF). FGF2, also known as basic FGF, is a potent angiogenic factor that exhibits increased expression under hypoxic conditions and during wound healing [28,29]. This growth factor promotes endothelial cell proliferation and induces endothelial cell germination [26]. Likewise, FGF2 is also a component of the bone matrix and plays an important role in regulating bone remodeling [30].

The increased expression of FGF2 in osteoblasts when given Robusta coffee extract was caused by the flavonoid content in coffee which is an antioxidant. Flavonoids in coffee can act as primary antioxidants, namely antioxidants that prevent the formation of free radicals in cells. These antioxidants play an important role in neutralizing free radicals that are formed by donating an electron to an unpaired electron in a free radical, so that it becomes a relatively stable free radical [31].

Stable free radicals can protect cells and tissues from oxidative damage and can cause uninhibited macrophage stimulation. Macrophages will secrete growth factors such as fibroblast growth factor (FGF), platelet derived growth factor (PDGF), TGFβ, and vascular endothelial growth factor (VEGF) which are able to attract more fibroblasts to the wound area and synthesize collagen so as to accelerate the regeneration of the periodontal ligament [32].

In addition, flavonoids also act as chelating agents for metal ions forming Reactive Oxygen Species (ROS) such as Fe2+ and Cu+ ions. Binding of metal ions by flavonoids can reduce the catalytic activity of Fe and Cu metals thereby reducing the formation of free radicals [28]. Flavonoids have anti-inflammatory activity by inhibiting pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) and lipoxygenase which causes decreased stimulation of phospholipid cell membranes so that arachidonic acid cannot be released from phospholipid cell membranes through phospholipase activation. Cyclooxygenase and lipoxygenase cycles that are inhibited will suppress prostaglandins, endoperoxidase, thromboxane, acid hydroperoxidase and leukotrienes so that the inflammatory phase can be reduced and accelerate the process of fibroblast proliferation [33].

Anti-inflammatory in flavonoids can also stimulate macrophages to secrete growth factors (TGF-ß, EGF, PDGF, bFGF, VEGF) and cytokines (Interleukin-1 (IL-1), Interleukin-4 (IL-4) and Interleukin-8 (IL) -8)) which functions to induce migration and proliferation of fibroblast cells, as well as induce the production of extracellular matrix such as collagen and proteoglycans [34].

Collagen1 expression

The results of the research on the effects of Robusta coffee extract on Collagen 1 expression are shown in **Table 2**. The results of immunohistochemical examination of Collagen 1 expression are shown in **Figure 2**.

Table 2 Mean and standard deviation of Collagen 1 expression on day 15 and day 22 and differenttest of IGF1 expression between day 15 and day 22 in each research group.

Group	n	Expression of Collagen 1 (Mean ± Standard deviation)		p
		Day - 15	Day - 22	
Control	6	25.17 ± 1.169	31.83 ± 1.169	0.000*
Treatment	6	30.83 ± 1.169	40.00 ± 0.894	0.000*
P		0.000*	0.000*	

*$p < 0.05$ = significant
**$p < 0.05$ = not significant

Table 2 illustrates mean and standard deviations of Collagen 1 expression in the controlgroup and treatment group on day 15 and day 22.

Testing based on the independent t-test of Collagen 1 expression between the study groups on day 15 and day 22 showed that the expression of Collagen 1 in the treatment group was greater than that in the control group and there was a significant difference ($p < 0.05$) ($p > 0.05$).

Tests based on the independent t-test showed that the expression of collagen 1 in the control and treatment groups on day 22 was significantly greater than day 15 ($p < 0.05$).

Testing based on the independent t-test in the control group and in the treatment group showed that the expression of Collagen 1 in group K and group P on day 22 was significantly greater thanday 15 ($p < 0.05$), which means that there was an increase in Collagen 1 expression. on day 22 significantly.

The histological image of Collagen 1 expression in the control group and the treatment group is shown in **Figure 2**.

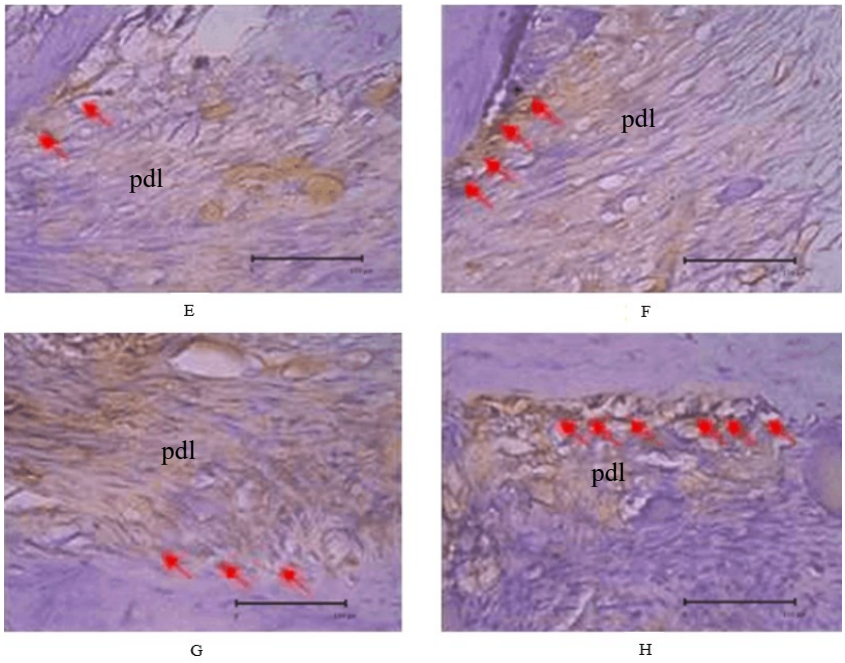

Figure 2 Expression of collagen 1 bone observations on day 15 and day 22, indicated by arrows: In the control group day 15 (E), the treatment group on day 15 (F), the control group on day 22 (G), and Treatment group day 22 (H), bone (b), periodontal ligament (pdl). (Immunohistochemistry, 400x magnification).

Collagen is the main protein that makes up the extracellular matrix and is the most abundant protein found in the human body [9,10]. Bone metabolism can be seen in biochemistry by using bone formation markers. Bone formation markers consist of collagen type I, alkaline phosphatase and osteocalcin [11].

One of the markers of bone formation is collagen type 1. Collagen type I is the most common type of collagen, spread in almost all connective tissues except in hyaline cartilage. Type I collagen is the main protein in bones, skin, tendons, ligaments, sclera, cornea, and blood vessels and this type I collagen includes 95 % of the collagen content of all bones and about 80 % of the total protein found in bones [12].

Fibroblasts are the most abundant connective tissue cells in the periodontal ligament which play a role in synthesizing extracellular matrix proteins such as elastic, reticular and collagen fibers which are the structural proteins of the periodontal connective tissue [35]. The formation of fibroblast cells is related to the inflammatory response due to orthodontic tooth movement.

The initial phase of orthodontic tooth movement always involves an acute inflammatory reaction in the periodontal tissues that lasts 1 - 2 days. Furthermore, a chronic inflammatory process occurs which is characterized by the migration of monocytes to the wound area to differentiate into macrophages. Macrophages are able to secrete anti-inflammatory cytokines such as IL-4, IL-10, IL-13, as well as growth factors that stimulate fibroblast proliferation and collagen production. The next phase, namely the proliferative phase, takes place from the 3rd to the 20th post-traumatic day, marked by the replacement of the provisional matrix which was initially dominated by platelets and macrophages gradually being replaced by migration of fibroblast cells and deposition of extracellular matrix synthesis [36]. In this phase,

M2 macrophages produce growth factors (eg PDGF, FGF and TGF-β) which induce fibroblast proliferation and migration, and form the extracellular matrix [37].

Coffee is one of the popular drinks consumed by the community. Coffee, among other things, contains a substance called caffeine (1,3,7 trimethylxanthin) [7], chlorogenic acid and caffeic acid, as well as flavonoids which have an antioxidant effect [8]. Robusta coffee has high antioxidant activity compared to Arabica coffee and others [9].

The increased expression of collagen1 in fibroblasts when given Robusta coffee extract was due to the coffee content which has an antioxidant effect which can increase the number of fibroblasts. The high antioxidant content can convert the ROS that is released during the application of orthodontic appliances into a stable product resulting in repair of the periodontal ligament by fibroblast cells [38].

The increased expression of collagen1 in fibroblasts after administration of Robusta coffee extract was due to the coffee content which has an antioxidant effect which can increase the number of fibroblasts. High antioxidants can convert the ROS released during the application of orthodontic devices into stable products, resulting in the repair of the periodontal ligament by fibroblasts.

ALP expression

The results of the research on the effects of Robusta coffee extract on ALP expression are shown in **Table 3**.

Table 3 Mean and standard deviation of ALP expression on day 15 and day 22 and different testof IGF1 expression between day 15 and day 22 in each research group.

Group	n	Expression of ALP (Mean ± Standard deviation)		P
		Day - 15	Day - 22	
Control	6	21.17 ± 1.169	25.00 ± 0.894	0.000*
Treatment	6	27.00 ± 0.894	33.00 ± 0.894	0.000*
P		0.000*	0.000*	

*$p < 0.05$ = significant
**$p < 0.05$ = not significant

Table 3 illustrates. Mean and standard deviations of ALP expression in the control group and treatment group on day 15 and day 22.

Testing based on the independent t-test of ALP expression between the study groups onday 15 and day 22 showed that the expression of ALP in the treatment group was greater than thatI in the control group and there was a significant difference ($p > 0.05$).

Testing based on the independent t-test in the control group and in the treatment group showedthat the expression of ALP in group K and group P on day 22 was significantly greater than day15 ($p < 0.05$), which means that there was an increase in ALP expression. on day 22 significantly.

Tests based on the independent t-test showed that the expression of ALP in the control and treatment groups on day 22 was significantly greater than day 15 ($p < 0.05$)

The histological image of ALP expression in the control group and the treatment groupis shown in **Figure 3**.

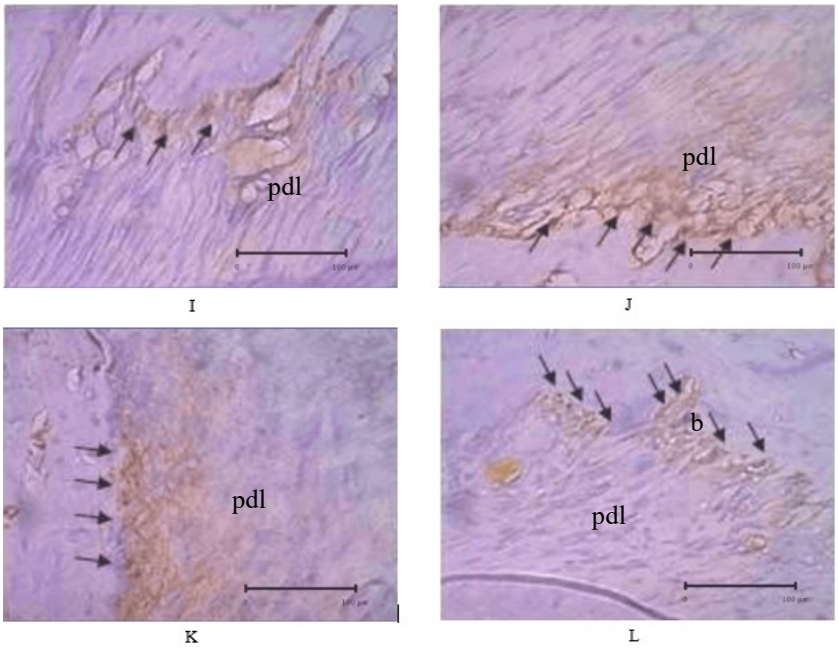

Figure 3 Expression of ALP on bone observations on day 15 and day 22, indicated by arrows: Inthe control group day 15 (I), the treatment group on day 15 (J), the control group on day 22 (K), and Treatment group day 22 (L), bone (b), periodontal ligament (pdl). (Immunohistochemistry, 400x magnification).

In orthodontic tooth movement the forces applied to the teeth will cause changes in the microenvironment surrounding the PDL due to changes in blood flow, leading to the secretion of inflammatory mediators such as cytokines, growth factors, neurotransmitters, colony-stimulating-factors, and arachidonic acid metabolites. As a result of this secretion, bone remodeling occurs [4].

Alkaline phosphatase (ALP) is a protein bound to cell membranes and synthesized by various tissue cells. The biological response to orthodontic tooth movement involves changes in the surrounding bony architecture. Bone metabolism is related to alkaline phosphatase (ALP) by osteoblasts and acid phosphatase (ACP), by osteoclasts. ALP is a glycoprotein involved in the mineral formation of bone and cementum tissue. This enzyme is thought to release phosphate ions from organic phosphate esters causing the precipitation of tissue phosphate salts [5]. Osteoblast activity can be determined from alkaline phosphatase (ALP). In orthodontic tooth movement ALP will be released which can increase the number of osteoblasts. [39].

The results showed that administration of robusta coffee extract increased the number of osteoblasts and bone formation due to the content of chlorogenic, ferulic, caffeic and n-coumaric acids which have antioxidant properties [36]. This is because the content of chlorogenic acid and caffeic acid in robusta coffee beans will act as antioxidants that can convert ROS released during the inflammatory process into stable products by means of the process of transferring electrons through hydrogen atoms, namely H2O2 will be converted to H2O and O2 [37-39].

Coffee is one of the main sources of antioxidants in people's daily diets. The beneficial health effects of coffee are usually associated with high antioxidant activity (ability to inhibit oxidation processes). Many publications provide comparisons of the antioxidant activity in popular beverages such as coffee, tea and cocoa. Robusta coffee beans contain many polyphenolic antioxidants, such as chlorogenic acid, caffeic acid, ferulic acid and cuminic acid. The main polyphenolic compounds in robusta coffee are chlorogenic acid and caffeic acid. The amount of chlorogenic acid reaches 90 % of the total phenols found in coffee [22].

The increase in ALP expression in osteoblasts when given Robusta coffee extract was due to the caffeine content in coffee increasing ALP expression. Caffeine binds to adenosine receptors and modulates several other receptors including glucocorticoid receptors, insulin, estrogen, androgen, vitamin D, cannabonoid, glutamate, and adrenergic receptors, all of which are expressed in osteoblasts or progenitor cells and have important functions during osteoblast differentiation [40]. Therefore, this study is supported by the results of previous studies which showed that giving 50 mg/kg of caffeine to pregnant rats had osteoblasts with high osteogenic potential characterized by increased expression of osteocalcin,

osteopointin, sialoprotein, RUNX-2, ALP, and type 1 collagen as well as increased synthesis of mineralized nodules [26].

Caffeic acid in coffee, which is phenolic acid, has an antioxidant effect that can reduce oxidative stress on osteoblasts [41]. Antioxidant activity is important in stimulating osteoblastic activity through specific receptors [42], so that ALP expression in osteoblasts also increases.

The results showed that administration of Robusta coffee extract on day 15 and day 22 showed a significant increase in ALP expression compared to controls ($p < 0.05$). This shows that Robusta coffee extract was effective in increasing ALP expression on day 15 and day 22. ALP expression in the treatment group on day 22 increased significantly compared to day 15 ($p < 0.05$). ALP is expressed in osteoblasts which play a role in increasing bone formation in the alveolar bone remodeling process, so that the administration of Robusta coffee extract can be used as an alternative ingredient in accelerating orthodontic treatment.

Conclusions

The administration of Robusta coffee extract increased FGF2, Collagen 1, and ALP expression in the periodontal ligament during orthodontic tooth movement.

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