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(RESEARCH ARTICLE)

The potential of cocoa bean extract (*Theobroma cacao* L.) Lindak variety on increasing the number of osteocyte cells in tension area in orthodontic tooth movement

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Abstract

Nowadays, many people experience malocclusion. The incidence of malocclusion can be reduced by providing orthodontic treatment to sufferers. During orthodontic treatment, various inflammatory mediators, which are one of the primary sources of increased Reactive Oxygen Species, which can damage the structure and function of osteoblasts so that they cannot differentiate into osteocytes. Giving cocoa bean extract can play a role in increasing the number of osteocytes in tension area. This study aims to analyze the potential of cocoa bean extract in increasing the number of osteocytes in the tension area during orthodontic tooth movement on 7 and 14 days. This research is a laboratory experimental type with the research subjects being 36 male which divided into three main groups, namely C-: without any treatment, C+: with only NiTi Closed Coil Spring application, CX: with cocoa bean extract and NiTi Closed Coil Spring application. NiTi Closed Coil Spring installation was carried out on the RA and M-1 RA incisors to move the M-1 RA mesially. The rats were euthanized after 7 and 14 days of treatment; then preparations were made using HE staining, and the number of osteocytes was counted. The results of the study showed that there was an increase in the number of osteocytes in the treatment group, as indicated by the results of calculating the average number of osteocytes in each group. It can be concluded that cocoa bean extract has the potential to increase the number of osteocytes in the tension area during orthodontic tooth movement.

Keywords: Osteocytes; Tension Areas; Cocoa Bean Extract; Orthodontic Tooth Movement

1. Introduction

Nowadays, many people experience malocclusion. Based on the results of the 2018 National Basic Health Research, malocclusion ranks third in dental and oral health problems in Indonesia, with a prevalence of around 80%. The incidence of malocclusion can be reduced by providing orthodontic treatment to sufferers [1]. However, orthodontic treatment requires quite a long duration, so efforts are needed to speed up it. Many efforts have been made to speed up orthodontic treatment because it not only shortens the duration of treatment but also correlates with reducing the risk of excessive resorption and the risk of root resorption [2]. Providing orthodontic treatment to correct malocclusion involves a process of remodeling the alveolar bone which will create two areas of tension and an area of tension [3]. In tension area of orthodontic tooth movement, the formation and activation of osteoblasts will occur to carry out the bone formation process [4]. At the end of bone formation, some mature osteoblasts will settle in the lacuna and differentiate into osteocytes [5]. Osteocytes have a role as a trigger for the formation of osteoblasts to carry out the process of bone

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apposition in the pulling area which can increase bone mass and accelerate the formation of new bone in the pulling area of the tooth orthodontics [6].

During orthodontic treatment, various inflammatory mediators are released after the application of mechanical force to the teeth, which is one of the primary sources of excess ROS (Reactive Oxygen Species) in the body, thereby triggering oxidative stress [7]. Oxidative stress produced by orthodontic mechanical force can damage the structure and function of osteoblasts so that they cannot differentiate into osteocytes [8]. This will hinder the bone apposition process from running optimally. The increase in ROS is also increasingly significant due to factors outside the body that can cause excess free radicals in the body [9]. Other facts show that during orthodontic treatment, the process of bone resorption, which causes tooth movement to occur, is greater than bone apposition, so it is necessary to balance bone remodeling by increasing the number of osteocytes to increase bone formation activity [10,11].

Oxidative stress can be overcome by supplying antioxidants from outside the body, which can be obtained through synthetic and natural antioxidants. However, the use of synthetic antioxidants has several side effects, such as damage to the lungs and liver, which is carcinogenic. Therefore, safe natural antioxidants are needed to overcome oxidative stress [3]. One ingredient that has natural antioxidants is non-fermented cocoa beans. Cocoa beans contain quite high polyphenols when compared to other parts of the cocoa plant [12]. The types of polyphenols contained in cocoa beans include catechin, epicatechin, procyanidin, and flavonoids, which are the types of polyphenols most commonly found in cocoa beans [13]. Polyphenol is a substance that has an antioxidant effect to ward off free radicals, which reduces oxidative stress in cells, thereby preventing cell damage during orthodontic treatment [14]. Apart from that, flavonoid polyphenol compounds also have anti-inflammatory properties to increase the cell proliferation process. The various ingredients contained in cocoa beans will cause increased maturation and differentiation of osteoblasts into osteocytes so that it can increase the number of osteocytes, which will increase the bone formation process in the tension area during the orthodontic treatment process and will speed up the bone remodeling process in orthodontic teeth [15].

This study aims to investigate the potential of cocoa bean extract (*Theobroma cacao* L.) in increasing the number of osteocytes in the tension area of the alveolar bone of Wistar rats during orthodontic tooth movement. The benefit of this research is to provide information regarding the benefits of administering cocoa bean extract in increasing the number of osteocytes in the tension area during orthodontic tooth movement, thereby increasing the bone remodeling process.

2. Material and Methods

This is a laboratory experimental type of research with a posttest-only control group design. The total sample of male Wistar rats was 36, and they were divided into six groups. The negative control groups are the groups without cocoa bean extract and NiTi Closed Coil Spring application for 7 and 14 days (C-7, C-14); the positive control groups are the groups with only NiTi Closed Coil Spring application for 7 and 14 days (C+7, C+14); and the treatment groups are the groups with cocoa bean extract and NiTi Closed Coil Spring application for 7 and 14 days (CX-7, CX-14). The treatment series process has received permission in the form of Ethical Clearance No. 1310/UN25.8/KEPK/DL/2021.

The process of making cocoa bean extract comes from non-fermented Lindak cocoa varieties taken from the PTPN X Kertosari plantation, which is then extracted using the maceration method. A total of 6 kg of cocoa beans were extracted using 96% ethanol solvent with a ratio of 1:4 material to solvent for three days and covered using aluminum foil with occasional stirring. The resulting macerate was concentrated using a rotary evaporator for 2 hours at a temperature of 40-50℃. The final result was 62 grams of cocoa bean extract. The dose of cocoa bean extract given to each rat was 50 mg diluted in 2 ml of distilled water.

The NiTi Closed Coil Spring was installed between the right M-1 RA tooth and the RA central incisor with a force of 10grF, which was measured using a tension gauge to move the molar teeth mesially. The treatment group was given cocoa bean extract once a day every evening for 7-days and 14-days. Next, the experimental animals were euthanized after 7-days and 14-days of treatment, then the tissue was fixed using 10% BNF followed by 10% formic acid decalcification before making histology preparations. The stage of making histological preparations begins with dehydration using alcohol, then the clearing stage using xylol, followed by impregnation and embedding, namely, the tissue embedded in paraffin. After the paraffin was frozen, cuts were made using a microtome with a thickness of 5 mm in the mesiodistal direction, then continued with staining using hematoxylin-eosin (HE).

Observation and calculation of the number of osteocytes using a binocular light microscope with 40x magnification followed by 400x magnification to view osteocytes. Osteocyte counting was carried out by looking at three selected fields of view, namely the top of the alveolar bone to the apical direction of the alveolar bone in the area of attension of

the right maxillary 1st molar or in the upper third of the alveolar bone, the middle third and the lower third of the bone by three observers which produced three results osteocyte counting. In this study, to see the differences between the control group and the treatment group, the LSD post hoc test was used, and all data obtained was processed using the SPSS program.

3. Results

The average value was obtained from the calculation of the number of osteocytes in the tension area of the alveolar bone of the male Wistar rats (*Rattus norvegicus*) with the standard deviation of the three groups: The group with cocoa bean extract and NiTi Closed Coil Spring application (CX/Treatment Group), the group with only NiTi Closed Coil Spring application (C+/Positive Control Group) and the group without cocoa bean extract and NiTi Closed Coil Spring application (C-/Negative Control Group). The research results showed that the average number of osteocytes in the 7-days and 14-days CX/treatment groups was greater than C+/the positive and C-/the negative control groups (Table 1).

Table 1 The mean and standard deviation (SD) of the number of osteocytes in the tension area of the C-, C+, and CX groups.

Days	C- (*mean*±SD)	C+ (*mean*±SD)	CX (*mean*±SD)
7-days	50.00±3.89	60.77±2.96	78.10±2.38
14-days	51.77±3.23	69.87±2.86	86.47±3.58

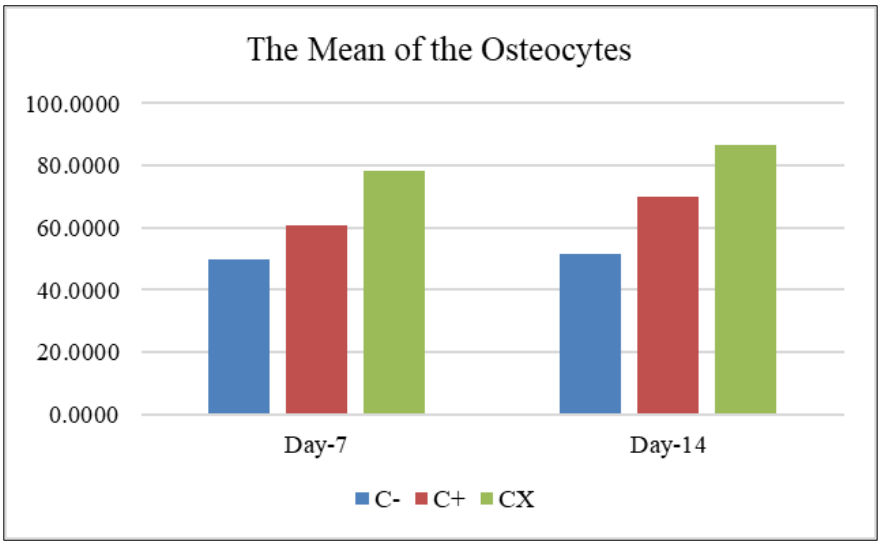

Figure 1 Bar chart of osteocytes number in the tension area between groups. Data are expressed as mean±SD.

Table 2 The summary of *post hoc LSD test* results for the number of osteocytes in each sample group.

Groups	C-7	C-14	C+7	C+14	CX7	CX14
C-7						
C-14	0.442					
C+7	0.000*	0.001*				
C+14	0.000*	0.000*	0.001*			
CX7	0.000*	0.000*	0.000*	0.002*		
CX14	0.000*	0.000*	0.000*	0.000*	0.002*	

(* : There are significant differences (P≤0.05)

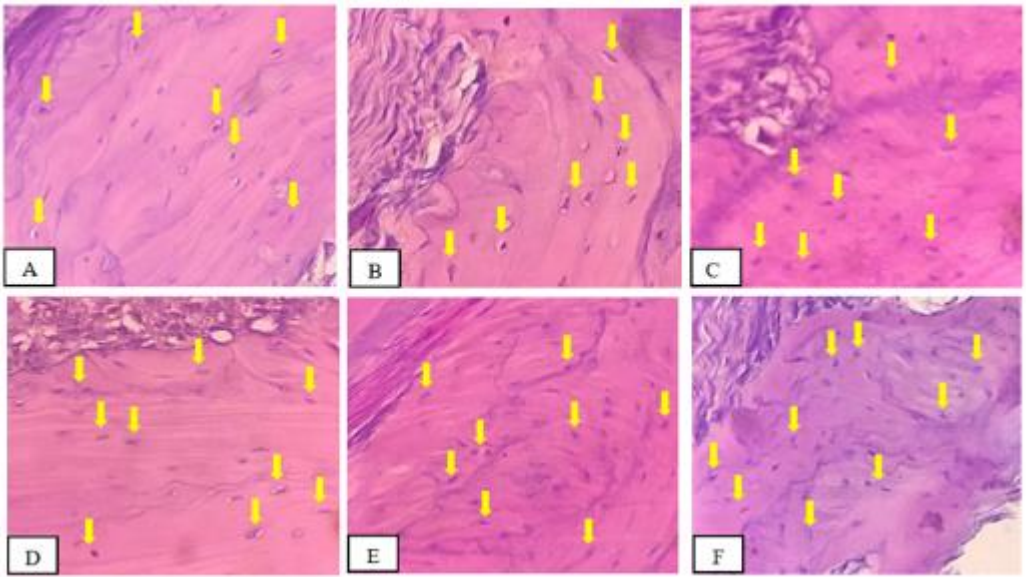

Figure 1 Histological examinations for osteocytes in the tension area of the alveolar bone of the male Wistar rats with Hematoxylin Eosin staining using a light microscope at 400x magnification. Yellow arrows indicate osteocytes. A) C-7 group, B) C+7 group, C) CX7 group, D) C-14 group, E) C+14 group, F) CX14 group.

4. Discussions

Orthodontic treatment is a long-term procedure and requires a long time to achieve harmonious and optimal occlusion. The length of orthodontic treatment encourages many patients to avoid orthodontic treatment. Therefore, innovation and various research are needed in the field of orthodontics to reduce treatment time without sacrificing results. Many efforts have been made to speed up orthodontic treatment because it not only shortens the duration of treatment but also correlates with reducing the risk of excessive resorption and the risk of root resorption [16]. One effort to speed up orthodontic treatment is by providing drug therapy derived from herbal plants such as Lindak varieties of cocoa beans [3,12]. This research was conducted to investigate the potential of cocoa bean extract (*Theobroma cacao L.*) Lindak variety to increase the number of osteocytes in the tension area of the alveolar bone of the male Wistar rats on the 7th and 14th days during the application of orthodontic force. The way to administer diluted cocoa bean extract is to use 2 ml of gastric probe.

In the results of the *LSD post hoc test* (Table 2), the treatment and control groups had significant differences with a significance value of 0.000 ($p \leq 0.05$). From the results of this study, it was found that the number of osteocytes on the treatment group day 7 and day 14 (CX7 & CX14) is greater than the control group on day 7 and day 14 (C+7 & C+14). These data results prove that Lindak cocoa bean extract is effective in increasing the number of osteocytes on the 7th and 14th days. Osteocytes in the treatment group increased due to the presence of antioxidant compounds in the Lindak variety cocoa beans. Lindak cocoa beans contain large amounts of polyphenolic compounds in the form of flavonoids; where various studies have shown that flavonoid polyphenolic compounds can increase the number of mature osteoblast cells, which will differentiate into osteocytes so that the number of osteocyte cells increases [17].

The increasing number of osteocytes that occurred in the treatment group was caused by the content of the Lindak cocoa beans given to the experimental animals. Lindak variety cocoa beans contain high levels of antioxidants in the form of polyphenols. The types of polyphenols that are most abundant in cocoa beans are flavonoids, which contain large amounts, where the flavonoids contained in cocoa beans are useful for reducing oxidative stress (an imbalance between the number of free radicals and antioxidants) in cells by inhibiting redox transcription factors so that cells and The tissue will be protected from oxidative damage, thereby preventing cell damage during orthodontic treatment [18].

Flavonoids contain quercetin as an antioxidant, which can prevent osteoblast damage due to the increased release of various inflammatory mediators that cause inflammation in the periodontal ligament after the application of mechanical force to the teeth, which is one of the main sources of excess ROS (Reactive Oxygen Species) [19,20]. Flavonoids will directly prevent the formation of highly reactive oxygen species and inhibit the continuation of oxidative processes so that mature osteoblast cells can differentiate into osteocytes and increase the number of osteocyte cells. In the bone remodeling process, flavonoids play a role in increasing the expression of osterix and RUNX-2. Flavonoids have an

important role in the bone formation process by stimulating the differentiation and maturation of osteoblast cells. Flavonoids will induce the expression of osterix and RUNX-2 mRNA, which will then stimulate the maturation of osteoblast cells so that it can increase the number of osteoblast cells that will differentiate into osteocyte cells [21,22].

Flavonoids also play a role in increasing lymphocyte proliferation. Lymphocytes will produce transforming growth factor-β (TGF-β), which plays a role in the maturation and activation of osteoblasts. In the process of bone formation, some mature osteoblasts will turn into osteocytes. It can be concluded that the polyphenols in cocoa beans are able to stimulate the maturation and differentiation of osteoblasts into osteocytes; this will increase the number of osteocyte cells [3,12,14]. An increase in the number of osteocyte cells will cause an increase in bone apposition so that the bone remodeling process runs optimally; this will speed up the process of forming new bone in orthodontic teeth [23].

5. Conclusions

Based on this study, it can be concluded that the administration of cocoa bean extract (*Theobroma cacao* L.) Lindak variety is effective in increasing the number of osteocytes in the tension area of the alveolar bone of the male Wistar rats on the 7th and 14th days during the application of orthodontic force.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors hereby declare that there is no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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