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RESEARCH ARTICLE
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Arabica Coffee (*Coffea Arabica L.*) Peel Powder Increases the Number of Osteoblasts After Tooth Extraction

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ABSTRACT

The loss of structure and volume of alveolar bones due to the process of extracting teeth will result in the resorption of about 25% of the alveolar bone volume. Therefore, an effort is needed to maintain alveolar bone volume after extraction. Osteoblasts are bone-forming cells that are responsible for the formation and process of bone mineralization. therefore, it is necessary to provide alternative materials that can assist in the bone formation process, namely the arabica coffee (*Coffea arabica L.*) peel which contains active compounds such as polyphenols, tannins, chlorogenic acid, and caffeine. This studi aimed to evaluate the effect of arabica coffee peel powder on increasing the number of osteoblasts in the tooth socket after extraction. Extraction of the left mandibular first molar was performed on 24 male Wistar rats. Then divided into two groups, namely the control group by intragastric administration of sterile aquadest and the treatment group by intragastric administration of Arabica coffee peel powder for 3, 5, and 7 days. Then it was sacrificed to make tissue preparations with HE staining and counting osteoblasts in the socket after tooth extraction. Data analysis used the One Way ANOVA and LSD tests. The results of the one way ANOVA parametric test showed that there were significant differences in the number of osteoblasts between groups ($p<0.05$) on each day. The LSD results showed a significant difference ($p <0.05$) on days 5 and 7 between the treatment group and the control group. As conclusion, arabica coffee peel powder tends to increase osteoblast proliferation in the tooth socket after tooth extraction.

Keywords: flavonoids; osteoblasts; wound healing

INTRODUCTION

Tooth extraction accompanied by certain local or systemic disorders can cause trauma and cause inflammation and extensive damage to the alveolar bone of the tooth socket. Major trauma during tooth extraction can damage the alveolar bone of the jaw, which can cause resorption of the jaw alveolar bone. Therefore, resorption of the alveolar bone of the teeth must be prevented during tooth extraction.[1,2] The loss of alveolar bone structure and volume from maxillary incisor extraction results in the resorption of more than 25% of alveolar bone volume in the first year, increasing to 40-60% after 3 years.[3] Another study reported that loss of alveolar bone volume due to progressive resorption is a loss of 0.34-7.7 mm in ridge width and 0.2-3.25 mm in vertical height 6-12 months after extraction.[4]

Bone healing in tooth extraction wounds involves the activity of osteoblasts, which are bone matrix that is capable of forming cells, which play an important role in bone healing, enzyme secretion, growth factors, and osteoclast regulation. Osteoblasts are cells that play a role in the remodeling phase as new bone formation.[5] Osteoblasts will synthesize collagen and glycosaminoglycans (GAGs) which are the bone matrix and play a role in the bone mineralization process. Mature osteoblasts will express several chemical compounds that can be used to identify osteoblast activity which is referred to as biochemical bone markers, namely: type I collagen, alkaline phosphatase, osteopontin, and osteocalcin for the needs of bone formation.[6] Osteoblast formation begins on day 3 of the wound healing process as seen by the differentiation of mesenchymal cells into osteoprogenitor cells. Then on day 5, newly formed osteoblasts were seen at the edges of the bone tissue. On the 7th day, osteoblast proliferation is still ongoing and woven bone has formed at the edges of the socket.[7]

In many countries, including Indonesia, the potential of natural ingredients is used as an alternative treatment that has more advantages such as low side effects, is cheaper, and is often used by all groups of people compared to modern medicines. Today the use of materials derived from nature has been developed in the health

sector, one of which is the Arabica coffee plant. World coffee consumption is still dominated by Arabica coffee with a percentage of 70%, while Robusta coffee is 30%.[8] Coffee production produces waste in the form of coffee berry skin which is usually only used as fodder and fertilizer. The content of coffee berry skin is not much different from the coffee berry itself which contains polyphenolic compounds, chlorogenic acid, tannins, and caffeine. The polyphenol group includes flavan-3-ol, flavonoids, hydroxynamic acid, flavonols, anthocyanidins, catechins, epicatechins, and ferulic acids.[9-13]

Research conducted by Masruri,[14,15] it is proved that Arabica coffee peel can increase fibroblast proliferation and increase the thickness of the tooth socket epithelium after extraction in experimental animals. Flavonoids present in coffee berry skin have antioxidant and anti-inflammatory effects which are thought to be related to bone damage by stimulating osteoblast differentiation and inhibiting osteoclast differentiation. Flavonoids also function in regulating the balance of bone mass by increasing osteoblastogenesis, suppressing osteoclastogenesis, and as osteoimmunological.

The purpose of this study is to evaluate the effects of the Coffee arabica **(*Coffea arabica L.*)** peel on increasing the number of osteoblasts as an indicator of bone healing in wound healing after tooth extraction in Wistar rats.

METHODS

This study was a laboratory experiment with a post test only control group design. Ethical Clearance No. 1557/UN25.8/KEPK/DL/2022 had been done. The sample size was 24 male Wistar rats aged 2-3 months and weighing about 200-225 grams which were separated into 2 groups, namely the control and treatment groups. All experimental animals were subjected to extraction of the left lower first molar. The control group was given intragastrically 2 ml distilled water for 3, 5, and 7 days. The treatment group was given the powdered skin of Coffee arabica's berries intragastrically and a dose of 0.117 g/day for 3, 5, and 7 days. The male Wistar rats were then sacrificed on days 3, 5, and 7, then their mandibles were taken and made into tissue preparations with HE staining. After that, observations and counting of osteoblasts in the tooth socket after extraction were carried out using an optilab connected to a binocular microscope with 400 times magnification.

Data analysis used the One Way ANOVA test with a 95% confidence level. And then tested with the post hoc Least Significant Difference test to see the differences between each group

RESULTS

The histological figure of osteoblasts in the treatment group that was given the coffee arabica (*Coffea arabica L*) peel powder and the control group on days 3, 5, and 7 can be seen in the Figure 1.

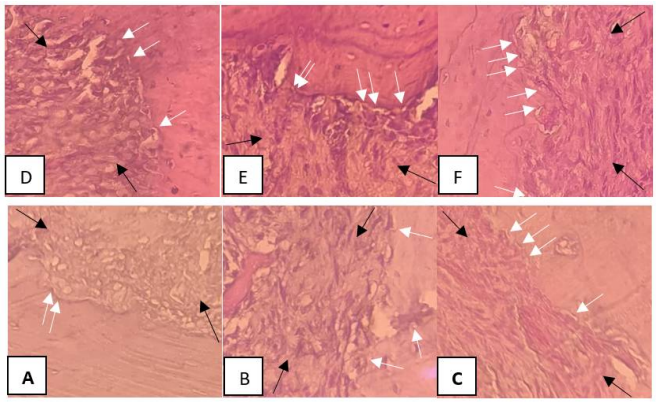

Figure 1. Histological picture of osteoblasts in the tooth socket after tooth extraction (magnification 400X).(A) control group on day 3, (B) control group on day 5, (C) control group on day 7, (D) treatment group on day 3, (E) treatment group on day 5, (F) treatment group on day 7, (G) The white arrows indicate osteoblasts in the tooth socket, and (H) The black arrows indicate the tooth socket after-extraction area

Based on figure 1, it can be seen that the tooth extraction socket with osteoblasts is located on the edge of the bone tissue. short cuboidal or cylindrical in shape, the nucleus of the osteoblasts is at the top of the cell, and the Golgi complex is located in the basal part of the cell with cytoplasm from osteoblasts that appear basophilic. Osteoblasts were observed using an optilab connected to a binocular microscope with 400X magnification. in three fields of view that was previously stained with HE (Hematoxylin Eosin).

Based on Table 1, it can be seen that there was an increase in the number of osteoblasts both in the control group and the treatment group. However, in the treatment group, it was higher when compared to the control group. Based on the One-way ANOVA test, a significance value was obtained ($p < 0.05$). This shows that there is a significant difference between the control and treatment groups.

Table 1. Mean and standard deviation of the number of osteoblast

Group	Observation day	N	Mean ± Standard Deviasi	p
Control	Hari 3	4	5.083±0.875	0.000*
	Hari 5	4	10.333±1.362	
	Hari 7	4	13.750±0.568	
Treatment	Hari 3	4	5.918±0.571	0.000*
	Hari 5	4	12.833±0.429	
	Hari 7	4	17.250±1.752	

*One-Way Anova test; p<0,05; significant

Table 2. LSD posthoc test on the number of osteoblast on day 3,5,7 after tooth extraction

Group	n	3 days	5 days	7 days	P
Control	4	5.0825 ± 0.87^{aA}	10.3325 ± 1.36^{bA}	13.75 ± 0.56^{cA}	0,000*
Treatment	4	5.9175 ± 0.57^{aA}	12.8325 ± 0.42^{bB}	17.25 ± 1.75^{cB}	0,000*
P		0,272	0,003*	0,000*	

Explanation:
(*): There was a significant difference (p<0,05);
(abc): Superscript in a column shows significant difference
(AB): Superscript in a row shows significant difference between each group.

The results of the LSD (Least Significant Difference) post hoc test showed significant results for both the control group and treatment group. However, for the treatment group on day 3 to the control group on day 3 it was not significant (p> 0.05).

DISCUSSION

The results of the study shown in Tables 1 and 2 show that the number of osteoblasts on day 3 in the control and treatment groups did not show a significant difference. It was because it was on day 3 which is still in the inflammatory phase. This is consistent with the theory that osteoprogenitor cell proliferation occurs on days 3-4. Osteoblasts found on day 3 originate from old bone remains.[16] on days 5 and 7 there was a significant increase in the number of osteoblasts in the control group and the treatment group, with the treatment group having a higher average number than the control group.

Arabica coffee *(Coffea arabica L.*) peel contains many compounds such as caffeine, chlorogenic acid, tannins, and polyphenols. These compounds work together to increase the proliferation of osteoblast cells, which helps the healing process in the tooth socket after extraction. Arabica coffee peel, namely flavonoids and chlorogenic acid, have antioxidant and anti-inflammatory effects due to their ability to reduce several pro-inflammatory neurotransmitters.[17] TNF-α cytokines stimulate inflammation by stimulating mononuclear phagocytes to release IL-1β cytokines. The cytokine IL-1β together with TNF-α drives neutrophils and monocytes to sites of inflammation to degrade antigens or stimulate inflammation. A decrease in the number of pro-inflammatory mediators inhibits the formation and activity of osteoclasts, causing osteoblasts to proliferate and differentiate into mature osteoblasts, increasing the number of osteoblasts, and also an increase in bone density.[18]

Flavonoids are also classified as phytoestrogens because they have the same activity as estrogen. Phytoestrogens also act as effective alternative substances to prevent bone damage due to estrogen deficiency. Phytoestrogens can induce osteoblasts via estrogen receptors. Estrogen receptors are present in osteogenic cells and act directly on the process of osteogenesis. Estrogen receptor activity increases in the process of bone mineralization and phytoestrogens can bind to estrogen receptors and stimulate the proliferation process.[19]

Another mechanism of increasing the number of osteoblasts olesth flavonoids which have anti-inflammatory and antioxidant effects is anti-inflammatory activity related to inhibition of pro-inflammatory enzymes such as (COX-2, LOX, and iNOS), inhibition of NF-kB, activating protein-1 (AP- 1) and activation of antioxidant detoxification enzymes II, where these inflammatory factors play a role in osteoclast activation. Flavonoids also function in regulating the balance of bone mass by increasing osteoblastogenesis, suppressing osteoclastogenesis, and also experiencing osteoimmunological action. Another mechanism of action of flavonoids is to inhibit the Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL), which is also called tumor necrosis factor. Osteoprotegerin (OPG) can bind to RANKL to block the bond between RANKL and RANK thereby reducing osteoclast activity and increasing osteoblast formation activity.[18,20]

CONCLUSION

Based on the results of the research, it can be concluded that Coffee arabica (*Coffea arabica L.*) peel powder which contains chemical compounds of polyphenol, tannin, chlorogenate acid, and caffeine tends to increase the proliferation of osteoblasts on teeth socket after tooth extraction.

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