

Original Research

Effects of Consumption Brew Robusta Coffee on Alveolar Bone Resorption in Rats Induced Periodontitis by *Porphyromonas gingivalis*

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Background: *Porphyromonas gingivalis* is the main cause of periodontitis. The presence of virulence factors of this bacteria increases pro-inflammatory cytokines that can interfere with the remodeling of alveolar bones that will later occur alveolar bone resorption. Robusta coffee is a natural ingredient that contains various substances that are useful for inhibiting inflammation, such as chlorogenic acid, caffeic acid, and caffeine. The purpose of this study was to analyze the effect of steeped Robusta coffee consumption on alveolar bone in rats induced by *Porphyromonas gingivalis*. **Methods:** Twenty male Wistar rats divided into 5 groups, control group (K1), rats treated with *P. gingivalis* induction and was sacrificed on the 14th day (K2), rats treated with *P. gingivalis* induction with 3.6 ml of brew coffee and was sacrificed on the 14th day (K3), rats treated with *P. gingivalis* induction and sacrificed on the 28th day (K4), rats treated with *P. gingivalis* induction with 3.6 ml of brew coffee and sacrificed on the 28th day. The specimens were then processed histologically and stained with HE to determine alveolar bone resorption. **Results:** There was no significant difference between the induction *P. gingivalis* and the consumption of robusta brew coffee for 14 days with control group. It means that treated brew robusta coffee for 14 days had alveolar bone resorption similar with the control group. The induction group *P. gingivalis* with consumption of Robusta coffee brew for 28 days there was a significant difference with the control group. The induction group *P. gingivalis* with consumption of Robusta coffee brew for 14 days differed significantly from the treatment for 28 days, this could be due to the longer treatment resulting in more bacterial tissue exposure. **Conclusion:** Consumption of brew robusta coffee can inhibit the resorption of alveolar bone caused by *Porphyromonas gingivalis*.

Keywords: Periodontitis; *Porphyromonas gingivalis*; robusta coffee; alveolar bone resorption

1. INTRODUCTION

According to the results of Riskesdas survey conducted in 2018, the percentage of periodontitis cases in Indonesia was found to be 74.1%.⁽¹⁾ Periodontitis is a type of oral cavity infection that affects the periodontal tissue and the supporting tooth structures, including the periodontal ligament and alveolar bone.⁽³⁾ The primary cause of periodontitis is the presence of microorganisms like *Porphyromonas gingivalis* that reside in plaque build up on the surface of teeth.⁽²⁾

Porphyromonas gingivalis is a microorganism that plays a major role in the occurrence of periodontitis. *Porphyromonas gingivalis* can survive and resist host defenses with its virulence factors, such as lipopolysaccharide (LPS), specific outer membrane receptors, adhesions (fimbriae), and extracellular products.⁽⁴⁾ The virulence factors of *P. gingivalis* bacteria such as fimbriae and LPS can stimulate host cells to produce proinflammatory cytokines (Tumor Necrosis Factor- α (TNF- α), Interleukin-1 alpha (IL-1 α), Interleukin-1 beta (IL-1 β), and Interleukin-6 (IL6)) which can interfere with alveolar bone remodeling because these cytokines can increase osteoclastogenesis.⁽⁵⁾ Recently there has been a lot of research focusing on natural ingredients. One of the reasons is that natural ingredients can minimize the side effects of using chemical drugs and the lack of public knowledge about the benefits found in natural ingredients.⁽⁶⁾

Robusta coffee is a natural ingredient which contains various active biological ingredients such as chlorogenic acid, caffeic acid, ferulic acid and caffeine. This biologically active content has antibacterial, anti-inflammatory and antioxidant properties which prevent bacteria from surviving and promote healing.⁽⁷⁾ Previous in vitro study has shown that caffeine in coffee can block pro-inflammatory cytokines (TNF- α and IL-6), resulting in reduced alveolar bone resorption.⁽⁸⁾ In addition, the caffeine content has the ability to destroy DNA cells of bacteria not from bacteria.⁽⁹⁾ Based on the description above, it is necessary to conduct research on the role or effect of consuming steeped robusta coffee grounds on alveolar bone resorption in a rat model of periodontitis induced by *Porphyromonas gingivalis*.

2. METHODS

2.1 Robusta Coffee Processing

To make a brew of Robusta coffee, 10 grams of coffee powder is dissolved in 200 ml of distilled water. The conversion dose of coffee in mice is 10 grams \times 0.018 = 0.18 grams/head.⁽¹⁰⁾ After that the coffee powder is dissolved in the following ratio:

$$10 \text{ grams}/200 \text{ ml} = 0.18 \text{ gr}/X$$

$$\text{Or, } X = 3.6 \text{ ml}$$

So, 0.18g of coffee powder will be dissolved in 3.6ml of boiling water.

The dosage is given based on the single dose rule of one cup of coffee per day, which is a simulation of the habit of drinking one cup of coffee (200ml) in one day.⁽¹⁰⁾

2.2 Intervention

The experimental animal treatment was carried out by dividing 20 Wistar rats into 5 groups, namely group 1 was the control group, Group 2 was the *P. gingivalis* induction group and decapitated on the 14th day. Group 3 was the *P. gingivalis* induction group which was simultaneously given steeped robusta coffee and decapitated on the 14th day. Group 4 was the *P. gingivalis* induction group and was decapitated on the 28th day. Group 5 was the *P. gingivalis* induction group which was simultaneously given steeped robusta coffee and decapitated on the 28th day. Induction of *P. gingivalis* was given in the amount of 0.05 ml at a concentration of 1.5×10^8 CFU/ml and 3.6 ml of robusta coffee brew was given to mice by sondase using a gastric probe. Next, the tissue was fixed with 10% formalin solution and then the tissue was processed with *hematoxylin-eosin* (H&E) staining.

2.3 Experiment

Observation of histological preparations was carried out using a light microscope and 40x magnification. Measurement of alveolar bone resorption after treatment is done by determining the Cementum Enamel Junction (CEJ) on the buccal of the 1st molar, then drawing a vertical line from the CEJ in an apical direction to the highest peak of the alveolar bone.

2.4 Data Analysis

This research data is in the form of ratio scale data, and will be presented in table form. The normality test is carried out using the *Shapiro-Wilk* test, and the homogeneity test is the *Levene* test with a significance value of 95% ($p > 0.05$). If the research data is normally distributed and homogeneous then a parametric test can be carried out using *One Way Analysis of Variance* (ANOVA) and continued with the test of *Least Significance Difference* (LSD).

2.5 Ethical Clearance

The research has received approval from the Health Research Ethics Committee, Faculty of Dentistry, Jember University No. 797/UN25.8/KEPK/DL/2019.

3. RESULTS

The histological image of the tissue of the left first molar on the buccal side at 40x magnification is shown in Figure 1. Alveolar bone resorption was greatest in the group of rats modeled on periodontitis on day 28, then

the group of rats modeled on periodontitis and consuming robusta coffee brews on day 28, then the group of rats modeled on periodontitis on day 14, and the smallest is the group of rats with periodontitis model and consumption of brewed robusta coffee on day 14.

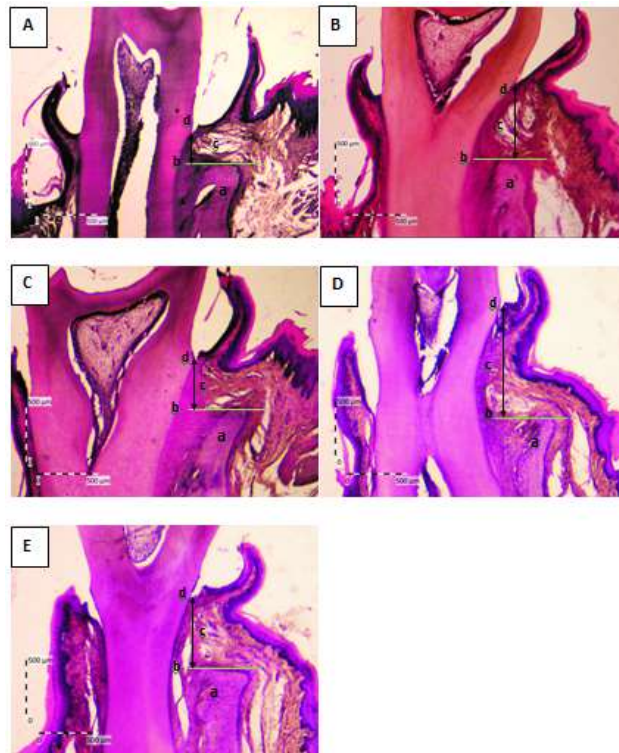


Figure 1. Histological image of alveolar bone resorption with HE staining and 40x magnification. (A. Control group; B. Group of rats modeled on periodontitis on day 14; C. Group of rats with periodontitis model and consumption of brewed robusta coffee on day 14; D. Group of rats modeled on periodontitis on day 28; E. Group of rats modeled on periodontitis and consuming robusta coffee brews on day 28; a. Alveolar bone; b. Alveolar bone crest; c. Line connecting the CEJ with the crest of the alveolar bone; d. CEJ)

The results of the study showed inhibition of alveolar bone resorption in the group given steeped robusta coffee (Figure 2). In the figure it can be seen that there is a difference in alveolar bone resorption between the groups treated with robusta coffee brewing and those not treated. The group treated with Robusta coffee brewing was able to suppress alveolar bone resorption compared to those not treated with Robusta coffee brewing.

Data from measurements of alveolar bone resorption carried out by the One-Way Anova test showed significant differences with a value of $\alpha: 0.00$ ($\alpha < 0.05$). Furthermore, the results of the LSD test analysis showed significant differences ($\alpha < 0.05$) between groups 2, 4, and 5 against group 1 (Control); group 4 against group 2; groups 4 and 5 against group 3, showed in Table 1.

Table 1. Summary of Least Significance Difference (LSD) Test Results

Group	I	II	III	IV	V
I	-	0.016*	0.092	0.000*	0.001*
II	0.016*	-	0.377	0.005*	0.198
III	0.092	0.377	-	0.001*	0.039*
IV	0.000*	0.005*	0.001*	-	0.073
V	0.001*	0.198	0.039*	0.073	-

Note: (*) = mean difference at the significance level $\alpha < 0.05$

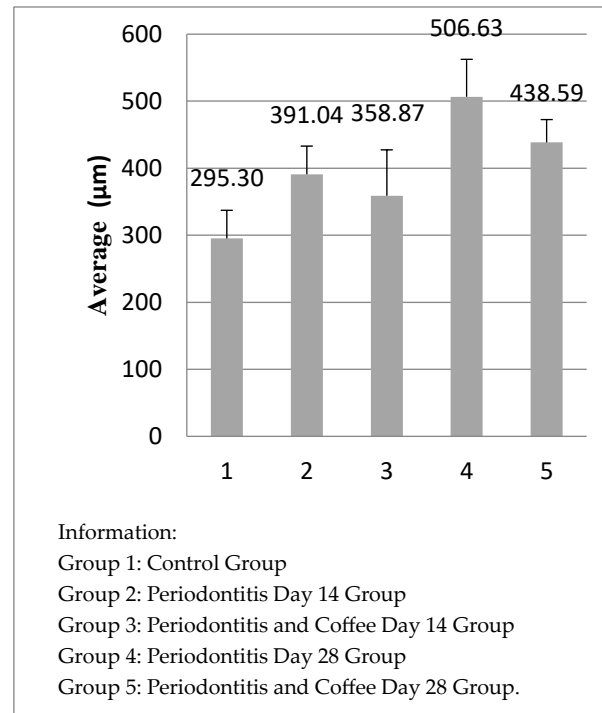


Figure 2. Bar graph showing the average amount of alveolar bone resorption in Wistar rats calculated from the CEJ in the apical direction to the highest peak of the alveolar bone.

4. DISCUSSION

Based on the results of this study, the periodontitis group that was given robusta coffee steeping for 14 days (Group 3) had a resorption rate that was not significantly different from the control group, which means that the robusta coffee brewing group for 14 days had a resorption rate that was close to the control group, so the steeping was given Robusta coffee on day 14 can inhibit alveolar bone resorption in mice induced by *P. gingivalis* bacteria. Similar results were also seen when giving coffee for 14 days and 28 days (Groups 3 and 5) which resulted in smaller resorption rates compared to the group that was not given coffee (Groups 2 and 4). This is because robusta coffee contains biologically active components such as caffeine, Chlorogenic acids (CGAs), Phenol, Ferulic acid, Caffeic acid which have anti-inflammatory, antioxidant and antibacterial activity. In research, Hall et al., 2015 explains that caffeine has anti-inflammatory properties which can inhibit the production of TNF- α stimulated by LPS, so it can suppress inflammation.⁽⁷⁾ Chlorogenic acids (CGAs) also have anti-inflammatory properties which can inhibit production. a number of pro-inflammatory mediators, including TNF- α , IL-1 β , IL-6

and interferon- γ (IFN- γ) in macrophage cells which can suppress osteoclast activity.^{(11),(12)} Apart from that, there is also Ferulic acid which can trigger a decrease in IL-1 β and TNF- α , so that they can improve osteoblast activity which can help restore resorbed alveolar bone.⁽¹³⁾ Caffeine, Caffeic acid and CGAs also have antioxidant properties to neutralize ROS produced by PMN, so they can suppress oxidative stress which can damage tissue.⁽¹⁴⁾ Caffeine, CGAs, trigonelline, and phenol are coffee compounds that have antibacterial activity. These contents can damage bacterial cell walls through differences in polarity between the lipids that make up DNA and are able to destroy DNA cells, so that bacteria experience damage and cannot survive in tissue.⁽⁹⁾

The *P. gingivalis* bacterial induction group that was given robusta coffee powder for 14 days (Group 3) showed a significant difference from the *P. gingivalis* bacterial induction group that was given robusta coffee powder for 28 days (Group 5). This can happen because the condition of the periodontitis mice on day 14 was at a moderate inflammatory stage where alveolar bone resorption was not as severe as the periodontitis mice on day 28 which were at the severe inflammatory stage, therefore robusta coffee brewing was given to the mice at the same time as bacterial induction for 14 days. days

can suppress alveolar bone resorption, so the robusta coffee steeping group for 14 days had a smaller value.⁽¹⁵⁾

The *P. gingivalis* bacterial induction group on days 14 and 28 (Groups 3 and 5) had a mean that was significantly different from the control group. This was caused by the treatment given, namely the induction of *P. gingivalis* bacteria in the buccal gingival sulcus of the lower left first molar. Newman et al., 2018 stated that periodontitis is an inflammatory disease caused by bacteria in dental plaque. This process begins when dental plaque bacteria, one of which is *P. gingivalis*, have virulence factors that diffuse into the gingival epithelial layer and stimulate epithelial cells to produce mediators that can cause alveolar bone resorption.⁽³⁾ *P. gingivalis* bacteria that invade periodontal tissue can trigger the body's response. by stimulating cellular components such as fibroblasts and monocytes to stimulate cytokines such as TNF- α , Interleukin-1 (IL-1), IL-6, IL-8 and prostaglandin E2 (PGE2). These pro-inflammatory products can stimulate the release of matrix metalloproteinases (MMPs) which will degrade extracellular matrix proteins, thereby triggering bone resorption and collagen tissue damage.⁽¹⁶⁾

5. CONCLUSION

The effect of consuming steeped robusta coffee can reduce alveolar bone resorption caused by the induction of *Porphyromonas gingivalis* bacteria in Wistar rats for 14 days, but with a longer treatment of 28 days, the effect of consuming robusta coffee becomes insignificant. Further research is needed regarding the method of steeping robusta coffee simultaneously with *P. gingivalis* bacterial induction treatment but using larger doses to prove that this method can be effective in inhibiting alveolar bone resorption and can be used as a basis for further research.

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This research received no fund.

Conflict of Interest

The authors declare no conflict of interest.

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