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Original article

The potential of toothpaste containing Robusta coffee bean extract in reducing gingival inflammation and dental plaque formation

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ABSTRACT

Background: *The prevention of gingivitis using chemicals containing antibiotics and chlorhexidine can disrupt the balance of the oral microbiota and have side effects in long-term use. A recent development in the prevention of gingivitis is the use of natural ingredients. Coffee is a natural ingredient that compounds several antibacterial and anti-inflammation properties.* ***Purpose:*** *The study aimed to determine the potential of toothpaste containing Robusta coffee bean extract in reducing gingival inflammation and inhibiting the formation of dental plaque.* ***Methods:*** *Twenty male Rattus norvegicus were divided into four groups, namely the control group and treatment groups (TG) TG25%, TG50%, and TG75%. All groups were fitted with ligature wire on the first left molar to accumulate dental plaque. After the fourth day, the ligature wire was removed, and the TG25%, TG50%, and TG75% groups were brushed once a day using toothpaste containing various concentrations of Robusta coffee extract, while the control group was brushed without using toothpaste. Plaque index, gingival index, and interleukin-1 (IL-1) expression were observed on the fifth day. The data was statistically tested using a one-way analysis of variance and post hoc least significant difference.* ***Results:*** *The statistical test showed that the TG75% group had the lowest value of plaque, gingival index, and IL-1 expression, while the control group had the highest ($p < 0.05$).* ***Conclusion:*** *Robusta coffee bean extract toothpaste has the potential to reduce gingival inflammation and dental plaque formation in a rat with gingivitis. The most effective concentration of Robusta coffee bean extract toothpaste in reducing gingival inflammation and dental plaque formation was 75%.*

Keywords: *gingivitis; herbal toothpaste; medicine; Robusta coffee bean extract*
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INTRODUCTION

Periodontal disease is an inflammatory disease of the tissue around the teeth that begins with gingival inflammation and continues to damage the structure of other tooth-supporting tissue, such as cementum, periodontal tissue, and alveolar bone.[1,2] Based on the 2018 National Basic Health Research report,[3] in Indonesia, 73.1%–75% of the population have periodontal disease. The most common periodontal disease is gingival inflammation or gingivitis, with 13.7%–14.1% of patients experiencing bleeding gums.[3]

Gingivitis is caused by the interaction between microorganisms found in dental plaque biofilms and tissues and inflammatory cells of the host.[4] Dental plaque is a biofilm that contains a lot of bacteria and is found in both hard and soft tissues. Dental plaque is a common etiologic factor for gingivitis.[5] Dental plaque accumulation is prevented by controlling plaque mechanically, namely by brushing teeth with toothpaste. Currently, the use of toothpaste in the community has become a daily necessity because using toothpaste regularly can maintain dental and oral health.[6]

A new development in the prevention of gingivitis is the use of natural ingredients. So far, the prevention of gingivitis has been with chemicals classified as antibiotics and chlorhexidine, which can disrupt the balance of the oral microbiota and have side effects in long-term administration.[7] One natural ingredient that has the potential to reduce gingivitis is coffee. Many studies have stated that coffee contains active ingredients,

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such as chlorogenic acid, flavonoids, caffeine, phenolic compounds, and trigonelline, which have antibacterial and anti-inflammatory properties.[8–10]

Robusta coffee contains several compounds that have antibacterial properties. The antibacterial content found in Robusta coffee beans includes caffeine, phenol, trigonelline, and chlorogenic acid.[11] The compounds that have the most antibacterial activity in Robusta coffee are flavonoids.[12] The flavonoids have biological activity; they interact with bacterial cells through an adsorption process involving hydrogen bonds and then damage the cytoplasmic membrane, resulting in leakage of the bacterial cell nucleus.[13,14] Flavonoid compounds destroy bacterial cells through differences in polarity between the lipids that make up bacterial cells and the alcohol groups in flavonoids.[15]

The active ingredients of coffee can reduce inflammation through the mechanism of inhibiting nuclear factor kappa B (NF-κB) activation, thereby inhibiting the synthesis of interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) and inhibiting vasodilation of blood vessels and capillary permeability. The adhesion of neutrophils to the blood vessel walls is inhibited and causes the infiltration of neutrophils into the tissue to decrease so that inflammation decreases.[8,12,13]

Flavonoid compounds in Robusta coffee beans might be used as an additive for plaque control agents, such as toothpaste. A previous study explained that green coffee bean extract showed a significant reduction in the *Streptococcus mutans* colony count before and after it was used.[16] Therefore, green coffee bean extract as a mouthwash can be a safe and effective alternative for decreasing bacterial plaque.[16] To prove the effectiveness of toothpaste containing Robusta coffee bean extract as a plaque control agent, we used it in various concentrations as an antibacterial and anti-inflammation agent in gingivitis rats. We observed the anti-bacterial and anti-inflammatory potential of Robusta coffee bean extract toothpaste using the plaque index, gingival index, and IL-1 expression.

MATERIALS AND METHODS

This research is a laboratory experimental study with a post-test-only control group design, and it has passed the ethical clearance issued by the Dentistry Research Ethics Commission of Jember University with No. 1281/UN25.8/KEPK/DL/2021. The sample of this research comprised 20 male Wistar white rats (Rattus norvegicus) aged around 12 to 14 weeks with weights of 200–250g that were divided into four groups: a control group and treatment groups (TG) TG25%, TG50%, and TG75%. All groups were fitted with ligature wire on the first left molar for three days to accumulate dental plaque and induce gingivitis. All rats were also monitored for their food and drink intake so that they consumed the same quantities.[17]

Robusta coffee extract toothpaste was made using Robusta coffee bean extract ingredients with concentrations of 25%, 50%, and 75% mixed with a placebo. The placebo paste consisted of magnesium carbonate, calcium carbonate, glycerin, propylene glycol, triethanolamine, sterile distilled water, and oleum menthae piperithae.[18]

On the fourth day, the ligature wire on the first left molar of all groups was removed, and the tooth was brushed once at 9 a.m. on the fourth and fifth days using an interdental brush with a roll technique. The TG25%, TG50%, and TG75% groups were brushed using toothpaste containing 25%, 50%, and 75% Robusta coffee extract, respectively, while the control group was brushed without using toothpaste.[18]

The plaque index and gingival index were observed on the fifth day.[18] The plaque was measured using the personal hygiene performance index, and the gingival inflammation was measured using the gingival index from Loe & Sillness.[19] After the plaque index and gingival index were obtained, all samples were decapitated on the fifth day, and the left gingival tissue was taken. Gingival tissue was put into a 10% formalin buffer solution for at least eight hours before decalcification so that the tissue to be observed was not damaged.[18] Samples were decalcified using an ethylene diamine tetra acetic acid solution. After the decalcification process, examination continued of the expression of IL-1 using immunohistochemistry (IHC). The IL-1 smear results were read by two pathologists using the Allred method.

The research data obtained was tested for normality using the Shapiro–Wilk test and homogeneity using the Levene test. Furthermore, a differences test was performed using a one-way analysis of variance (ANOVA) and a post hoc least significant difference (LSD) test with a significance level of 95% ($p = 0.05$) to see which concentration of Robusta coffee bean extract toothpaste was most effective in reducing inflammation and inhibiting dental plaque formation.

RESULTS

All groups were fitted with ligature wire on the first left molar, as shown in Figure 1A, to accumulate dental plaque. After three days of placement, the ligature wire of all groups was removed. The gingiva of the ligature area showed redness compared to another gingival area without ligature wire. The redness of the gingival indicates inflamed gingiva, as shown in Figure 1B.

The plaque index and gingival index were observed on the fifth day. The results of plaque measurements showed a decrease in plaque value in each study group, along with a large content of Robusta coffee bean extract in toothpaste. The mean and standard deviation (SD) of plaque values from each group can be seen in Table 1.

The values in Table 1 show a decrease in average plaque value in the control group, TG25%, TG50%, and TG75%. The TG75% group had the smallest average plaque value of 0.8 ± 0.84, while the control group had the largest plaque

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value of 2.2 ± 0.84. The plaque value data was analyzed using statistical tests, which showed the data was normal and homogenous. The results of the one-way ANOVA test showed a significant difference between groups ($p < 0.05$), as seen in Table 2.

The gingival index was also measured for the presence of inflammation in the rat gingiva. The results in Table 3 show a decrease in the value of the gingival index in the control group, TG25%, TG50%, and TG75%. The lowest gingival index value in the TG75% group was 0.75 ± 0.04, including the criteria for mild inflammation, while the highest gingival index value in the control group was 2.02 ± 0.23, including the criteria for severe inflammation. The data of the gingival index was analyzed using statistical tests, which showed the data was normal and homogenous. The results of the one-way ANOVA test showed a significant difference between groups ($p < 0.05$), as shown in Table 4.

The image of the results of the IL-1 examination using IHC can be seen in Figure 2. From the results of the calculation of IL-1 expression, it was found that the lowest average of IL-1 expression was in the TG75% group, and the highest average of IL-1 expression was in TG25%. The results of the examination of IL-1 expression can be seen in Table 5.

The data on IL-1 expression were analyzed using statistical tests. The statistical test showed the data was normal and homogenous. The results of the one-way ANOVA test showed a significant difference between groups ($p < 0.05$), as shown in Table 6. The statistical test was then continued using the LSD post hoc test to see the differences between each group. The results of the LSD post hoc test in the four groups can be seen in Figure 3.

The results of the LSD post hoc test of IL-1 expression showed the differences between each group. The TG75% group was significantly lower than the control group ($p < 0.05$). However, the TG25% and TG50% groups did not significantly differ from the control group. The TG75% group is also significantly lower than the TG25% and TG50% groups.

Figure 1. (A) Ligature wire placement on the first left molar (red arrows). (B) The ligature wire was removed after three days of placement, and the gingiva in the ligature area was red (blue arrows).

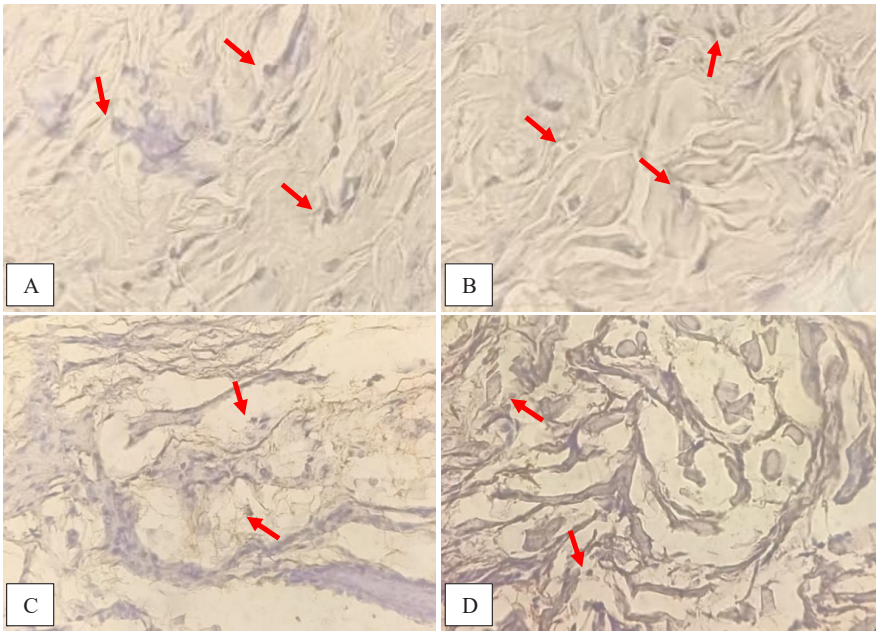

Figure 2. Immunohistochemistry examination results. (A) Control group, (B) TG25% group, (C) TG50% group, (D) TG75% group. IL-1 expression is shown with red arrows. 400x magnification.

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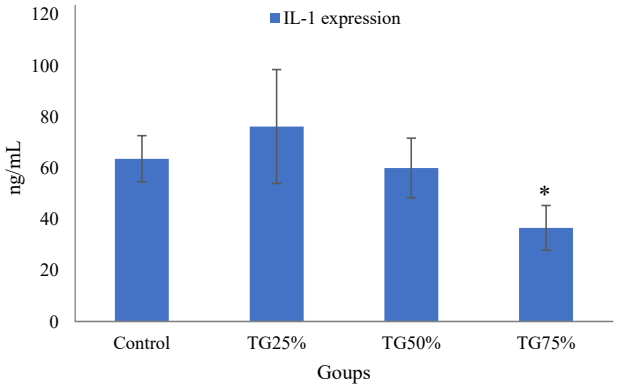

Figure 3. The results of the LSD post hoc test of IL-1 expression. The TG75% group was the only group that was significantly lower than the control group. (*) significant $p < 0.05$.

Table 1. The mean and SD of plaque values on the first left molar tooth

Groups	N	Mean ± SD (mm)
Control	5	2.2 ± 0.84
TG25%	5	2 ± 0.71
TG50%	5	1.2 ± 0.84
TG75%	5	0.8 ± 0.84

Table 2. The results of statistical tests of plaque value

Groups	Normality (Shapiro–Wilk test)	Homogeneity (Levene test)	Difference test (One-way ANOVA)
Control	0.314**	0.801**	0.045*
TG25%	0.325**		
TG50%	0.314**		
TG75%	0.314**		

(*) significant $p < 0.05$; (**) significant $p > 0.05$

Table 3. The mean and SD of the gingival index on the first left molar tooth

Groups	N	Mean ± SD	Criteria
Control	5	2.02 ± 0.23	Severe
TG25%	5	1.75 ± 0.07	Moderate
TG50%	5	1.5 ± 0.32	Moderate
TG75%	5	0.75 ± 0.04	Mild

Table 4. The results of statistical tests of the gingival index

Groups	Normality (Shapiro–Wilk test)	Homogeneity (Levene test)	Difference test (One-way ANOVA)
Control	0.368**	0.056**	0*
TG25%	0.623**		
TG50%	0.484**		
TG75%	0.325**		

(*) significant $p < 0.05$; (**) significant $p > 0.05$

Table 5. The mean and SD of IL-1 expression

Groups	N	Mean ± SD
Control	5	63.6 ± 9.02
TG25%	5	76.2 ± 22.26
TG50%	5	60 ± 11.64
TG75%	5	36.6 ± 8.74

Table 6. The results of statistical tests of IL-1 expression

Groups	Normality (Shapiro–Wilk test)	Homogeneity (Levene test)	Difference test (One-way ANOVA)
Control	0.979**	0.061**	0.003*
TG25%	0.894**		
TG50%	0.942**		
TG75%	0.999**		

(*) significant $p < 0.05$; (**) significant $p > 0.05$

DISCUSSION

In this study, the first left molar was ligated using a wire so that plaque accumulation occurred. Ligation aims to cause plaque accumulation that will induce gingivitis. After five days of ligation, the gingival margin was clinically reddish. This was following the second stage of gingivitis. In the second stage of gingivitis (early lesion), we found red gingiva appearing and bleeding on probing that occurred 4–7 days after plaque accumulation.[20]

In Table 1, the results of the measurement of plaque values in the TG25%, TG50%, and TG75% groups decreased, along with the large concentration of Robusta coffee bean extract in toothpaste. The results of this study are in line with Prasasti et al.,[18] which explains that the 75% concentration of Robusta coffee bean extract found in toothpaste is effective in inhibiting the formation of dental plaque. The higher the concentration of Robusta coffee bean extract, the more active chemical compounds are contained therein. The decrease in plaque value may be

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due to several ingredients in Robusta coffee beans, namely caffeine, flavonoids, trigonelline, and chlorogenic acid, which have antibacterial activity. Each component has a different antibacterial mechanism.[14,21]

Caffeine is one of the most important alkaloid compounds found in coffee beans. The caffeine content in Robusta coffee beans is between 1.5%–2.25%.[22] The ability of alkaloid compounds to be antibacterial is influenced by the active compounds of alkaloids, which are basic groups containing nitrogen. When this base group is in contact with bacteria, it will react with amino acid compounds that make up the bacterial cell wall and bacterial DNA, which is the main constituent of the cell nucleus. With DNA damage, bacteria will become inactive and lyse.[8,23]

Phenolic compounds are flavonoids found in coffee beans. The flavonoid content in Robusta coffee ranges from 7.3–7.5 mg/g. Flavonoids damage the bacterial cell wall due to the difference in polarity between the lipids that make up bacterial DNA and the alcohol groups in flavonoid compounds; these compounds can enter the bacterial cell nucleus.[24]

Robusta coffee contains 1.18% trigonelline. Trigonelline works by disrupting the stability of the bacterial cytoplasmic membrane. Disturbances in the membrane will cause an imbalance in the metabolic function of bacteria, which causes bacterial growth to be inhibited.[25]

Chlorogenic acid is the most abundant component in coffee that can neutralize free radicals in the body by maintaining normal cell structure and function. Chlorogenic acid works by entering the nucleus of bacterial cells and destroying the structure of the cell wall.[26]

In Table 3, the results of the measurement of the gingival index value in the TG25%, TG50%, and TG75% groups have a decreasing gingival index value. In the TG75% group, the lowest gingival index value is 0.75, including the category of mild inflammation. Mild inflammation in the TG75% group was probably caused by the content of Robusta coffee bean extract, namely flavonoids. The flavonoid content in coffee also has an anti-inflammatory effect by binding to proteins and reducing hydrophobicity in host cell membranes and bacteria.[25] Flavonoids can inhibit inflammation in two ways: by inhibiting arachidonic acid and the secretion of lysosomal and endothelial enzymes so that proliferation and exudation of the inflammatory process occur. The inhibition of the release of arachidonic acid from inflammatory cells leads to less availability of arachidonic substrates for the cyclooxygenase pathway and the lipoxygenase pathway.[12,13,26]

The results of the one-way ANOVA test showed a significant difference in IL-1 expression between groups. The statistical test was then continued using the LSD post hoc test to see the differences between each group. The LSD post hoc test result showed that the TG75% group was significantly lower than the control group ($p < 0.05$). However, the IL-1 expression of the TG25% and TG50% groups did not significantly differ from the control group. These results indicate that toothpaste containing 75% of Robusta coffee bean extract can reduce inflammation more than the other groups. Pro-inflammatory IL-1 is associated with gingival inflammation. According to Gao et al.,[27] flavonoids have the potential to inhibit the cyclooxygenase enzyme so that the formation of prostaglandins is inhibited. Inhibition of prostaglandin formation can reduce inflammation. With reduced inflammation, proinflammatory cytokines, such as IL-1α, IL-1β, IL-6, and IL-8, are also reduced.[27] The results of our study are also consistent with Martin et al.,[28] who show that the substances contained in Robusta coffee bean extract play a role in inhibiting the production of TNF-α, IL-1β, IL-6, and COX-2 through inhibition of the NF-κB pathway. Further research also explains that Robusta coffee bean extract enhances osteocalcin and alkaline phosphatase expression that leads to bone regeneration in periodontal rat models.[28] In conclusion, Robusta coffee bean extract-containing toothpaste has the potential to reduce gingival inflammation and dental plaque formation in a gingivitis rat. The most effective concentration of Robusta coffee bean extract toothpaste in reducing gingival inflammation and dental plaque formation was 75%.

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