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EFFECTIVENESS OF ROBUSTA SEMENDO COFFEE EXTRACT IN THE TREATMENT OF CHRONIC PERIODONTITIS ON REDUCING THE COUNT OF PMN CELLS IN WISTAR RATS

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Abstract

Background: Periodontitis is an oral disease with a very high prevalence in Indonesia. Periodontitis can cause tooth loss. Semendo robusta coffee extract contains active compounds as anti-inflammatory and antibacterial which can reduce the number of PMN cells in chronic periodontitis treatment. ***Objective:*** This study aimed to determine the effectiveness of Semendo Robusta coffee bean extract in reducing PMN cell counts in the treatment of chronic periodontitis in Wistar rats**.** ***Methods:*** his study used a post test only control group design. A total of 21 male Wistar rats were divided into 3 treatment groups. Making periodontitis rats is done by inducing Porphyromonas gingivalis bacteria into the gingival sulcus of rats for 14 days. Periodontitis rats were applied gel according to the treatment group twice a day for 14 days. Semendo robusta coffee extract gel applied to periodontitis rats with various concentrations of 40%, 50%, and placebo gel (negative control). All samples were euthanised on the 15th day after gel application and histological sections were made. The number of PMN cells was counted and statistically analysed. ***Results:*** Semendo robusta coffee extract gel group with 50% concentration was the group with the lowest PMN cell value. Statistically, the 40% Semendo coffee extract gel group had a significant difference ($p<0.05$) compared to the placebo gel group. ***Conclusion:*** Semendo robusta coffee bean extract gel with 50% concentration significantly decreased the number of PMN cells against chronic periodontitis treatment in Wistar rats compared to 40% semendo robusta coffee extract gel and placebo gel.

Keywords: Semendo Robusta coffee, chronic periodontitis, PMN cells, anti-inflammatory, Wistar rats

INTRODUCTIONS

Periodontitis is one of the most prevalent oral diseases in Indonesia, with a prevalence rate of 74.1% according to the 2018 Basic Health Research (RISKESDAS)[1]. Periodontitis generally develops from gingivitis, a condition initiated by the body's inflammatory response to bacterial colonization on the

surfaces of the teeth and gingiva. However, not all cases of gingivitis progress to periodontitis. Oral hygiene and poor habits in individuals with gingivitis significantly influence the progression of the disease.[2] Severe periodontitis can result in progressive damage to the periodontal ligament and alveolar bone loss, leading to loose and easily lost teeth.[3]

The inflammatory response caused by periodontitis can lead to an increase in immune cells such as PMNs (polymorphonuclear cells) or MNs (mononuclear cells), which trigger the release of various inflammatory mediators to phagocytose the bacteria causing periodontitis. PMNs are the frontline cells in the body's defense system and will react when inflammation occurs. The increased presence of immune cells like PMNs or MNs can lead to damage to the periodontal tissue, including destruction of connective tissue, damage to the periodontal ligament, and alveolar bone resorption.[2,4]

Indonesia is a tropical archipelagic country rich in natural resources and herbal materials, with great potential as an alternative source of natural medicine. There are three famous types of coffee in Indonesia: Liberica coffee *(Coffea liberica*), Arabica coffee (*Coffea arabica*), and Robusta coffee (*Coffea canephora*). The dominant coffee plantation in Indonesia is Robusta coffee.[6] The dominant coffee plantation in Indonesia is Robusta coffee. Semendo coffee is a type of Robusta coffee from Muara Enim, South Sumatra. Semendo Robusta coffee has several distinctive characteristics, such as being less bitter, thick, and having a strong aroma.[7,8] Many studies have proven the health benefits of Robusta coffee. Some research by Anwari et al. states that consuming Robusta coffee can lower the risk of type 2 diabetes and reduce blood sugar levels in individuals with type 2 diabetes. Robusta coffee can also help prevent Alzheimer's disease, Parkinson's disease, liver cirrhosis, and reduce uric acid levels.[9–12] Robusta coffee beans contain several active chemical compounds, such as caffeine, caffeic acid, and chlorogenic acid, which have antioxidant, antibacterial, and anti-inflammatory properties.[5,6]

Research by Tantin et al., conducted on animals to test the anti-inflammatory properties of Robusta coffee, showed that applying a gel of Robusta coffee bean extract could reduce the number of macrophages and lymphocytes in

rats with periodontitis.[13] Another study by Nektara et al. tested the antibacterial properties of Robusta coffee against the growth of *Porphyromonas gingivalis* bacteria and found that Robusta coffee had a bacteriostatic effect on *Porphyromonas gingivalis*, causing bacterial lysis.[14]

This finding is consistent with another study by Sulistiawati et al., which showed that Semendo Robusta coffee extract had an antibacterial effect in killing *Streptococcus sanguinis*, a bacterium that can initiate plaque formation leading to periodontitis.[5] Another scientific study by Sulistiawati et al. demonstrated that Semendo Robusta coffee gel extract significantly increased the number of fibroblast cells during the gingival wound healing phase in Wistar rats, possibly due to the presence of active compounds in Semendo Robusta coffee with antioxidant properties that can protect tissues from oxidative damage.[6]

To date, no studies have been conducted on the effects of Semendo Robusta coffee bean extract on PMN cells in the treatment of chronic periodontitis. Based on this, the author is interested in conducting research on the effectiveness of Semendo Robusta coffee bean extract in the treatment of chronic periodontitis, specifically focusing on the reduction of PMN cell count in Wistar rats.

METHODS

This type of research is an in vivo laboratory experiment with a post test only control group design and ethical feasibility test has been approved by the Medical and Health Research Ethics Committee (KEPKK) Faculty of Medicine, Sriwijaya University with ethical certificate number 117-2023.

This study used 21 male rats (*Rattus norvegicus*) aged 2-3 months, weighing 150-200 grams with a healthy state randomly divided into 3 groups, each of 4 heads, namely: group 1 is a chronic periodontitis rat treatment group given 40% robusta coffee extract gel for 14 days, group 2 is a chronic periodontitis rat treatment group given 50% robusta coffee extract gel for 14 days, and group 3 is a chronic periodontitis rat treatment group given placebo gel for 14 days. The concentration of robusta semendo coffee extract gel in this study was 40% and 50%. Induction of *Porphyromonas gingivalis* bacteria (strain ATCC

33277) in rats using a 1 ml syringe and 30 gauge needle into the gingival sulcus of the right central incisor of the mandible of Wistar rats as much as 0.5 ml every 3 days for 14 days, the rats were still fed and drank as usual during the induction process.

On the 15th day, the process of making periodontitis rats was successful when the clinical appearance of the wistar rat gingiva contained erythema, edema, gingival recession, especially in the anterior region of the mandible, and the formation of periodontal pockets with a depth of> 0.4 mm, especially on the gingiva of the right central incisor of the mandible of wistar rats. The depth of the pockets was measured from the bottom of the pockets using a calibrated periodontal probe instrument (UNC 15). If on day 15 there are rats that have not shown a clinical picture of periodontitis, the rats will be induced again *Porphyromonas gingivalis* bacteria (strain ATCC 33277) once a day for 3 days, and if the rats have not also shown a clinical picture of periodontitis, the rats will be replaced with spare samples. Semendo robusta coffee extract was applied to each treatment group twice a day as much as 1 ml which was applied topically from the interdental papillae area to the gingival sulcus, especially on the right central incisor of the mandible of Wistar rats. The gel was applied to the buccal and lingual anterior gingival areas and allowed to stand for 2 minutes by retracting the rat's oral cavity so that the gel was not swallowed by the rat during application.

All groups were euthanized on the 15th day after the administration of Semendo robusta coffee gel extract and decapitation for the collection of rat mandibular gingival tissue. The tissues were sent to the anatomical pathology laboratory for processing, tissue observation, PMN cell count (using Olympus BX51 microscope), and data analysis. Calculated data were analyzed using SPSS. Normality test using Shapiro-Wilk test, homogeneity test using Levene test, parametric statistical test with One Way ANOVA method, and Post Hoc test.

RESULTS

The results of the PMN cell count data obtained were tested for normality using Shapiro-wilk with the results ($p>0.05$) meaning that all data for each group

were normally distributed. Homogeneity test using Levene test with the results of data variance is not homogeneous ($p<0.05$) because the average value of placebo gel group data is very high and far compared with the treatment group of robusta coffee extract gel semendo concentration 40% and 50%.

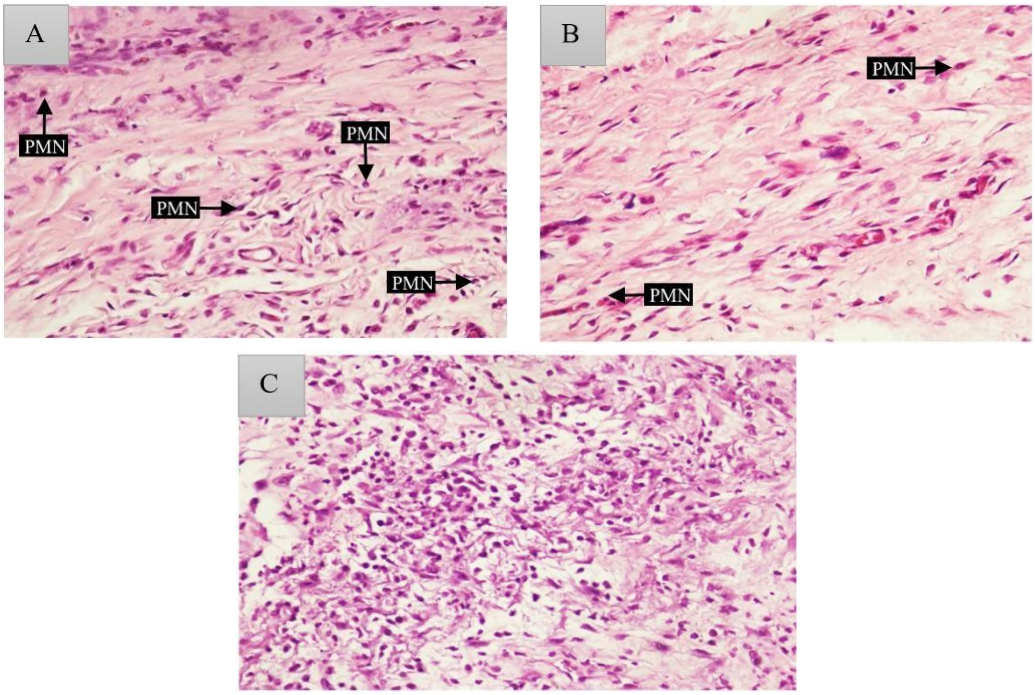

Figure 1. Histological Images of Gingival Tissue in Treatment Groups: (A) Group with 40% Robusta Semendo Coffee Extract Gel, (B) Group with 50% Robusta Semendo Coffee Extract Gel, and (C) Group with Placebo Gel.

Tabel 1. Average PMN Cell Count of Wistar Rats after Treatment with Semendo Robusta Coffee Extract Gel

Treatment Group	Mean ± SD PMN count
40% Semendo Robusta Coffee Extract Gel	11,17 ± 2,12
50% Semendo Robusta Coffee Extract Gel	4,5 ± 1,07
Plasebo Gel	123,33 ± 45,76

Parametric test using One Way ANOVA test with the results (p <0.05) which shows that there is a difference in the average number of PMN cells in each treatment group of semendo robusta coffee bean extract gel on reducing the number of PMN cells in chronic periodontitis treatment of Wistar rats.

Table 2. Parametric Test Results with One Way ANOVA test of Robusta Semendo Coffee Extract on PMN Cell Decrease in Chronic Periodontitis Treatment of Wistar Rats

	Sum Of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	44175,926	2	22087,963	31,556	0,000
Within Groups	8399,497	12	699,958		
Total	52575,423	14			

* Shows a significant difference in (p<0,05)

The analysis was continued using the Games Howell Post Hoc test with a value of (p <0.05) for all treatment groups which stated that the 50% robusta semendo coffee bean extract gel group had experienced an excellent reduction in PMN cells compared to the 40% robusta semendo coffee bean extract gel group and the placebo gel group.

Tabel 3. Results of Post Hoc Games Howell Parametric Test of Average PMN Cell Counts Between Each Group

Group Treatment	**40% Semendo Robusta Coffee Extract Gel**	**50% Semendo Robusta Coffee Extract Gel**	**Plasebo Gel**
40% Semendo Robusta Coffee Extract Gel		0,003	0,012
50% Semendo Robusta Coffee Extract Gel	0,003		0,010
Plasebo Gel	0,012	0,010	

*****Shows a significant difference in (p<0,05)

DISCUSSION

Inflammation that occurs in chronic periodontitis is caused by an increase in PMN cells or MN cells as the body's immune response and is also caused by toxin products produced by the pathogenic bacteria *Porphyromonas gingivalis* that cause periodontitis. An increase in the number of PMN cells or MN cells in the body triggers the release of proinflammatory cytokines for phagocytosis, but this also results in the destruction of periodontal tissue and can cause tooth loss. This study shows that Semendo robusta coffee bean extract gel is effective in reducing the number of PMN cells in periodontitis rats. The 40% and 50% concentration groups had lower PMN cell counts compared to the control group.

This is due to the content of active chemical compounds in robusta semendo coffee bean extract such as chlorogenic acid which is high in robusta semendo green coffee beans has an anti-inflammatory effect on chronic periodontitis treatment by neutralizing and reducing the release of proinflammatory cytokines triggered by PMN cells. The high caffeine content in robusta semendo coffee beans is an anti-inflammatory chemical compound that works by reducing the proliferation and phagocytic activity of macrophages and lymphocytes during the inflammatory phase. In addition, caffeine can also reduce the release of proinflammatory cytokines (such as TNF-α and IL-6) by PMN cells induced by bacterial toxin products. Caffeine is also antibacterial because it can cause pathogenic bacteria to lyse by damaging the bacterial cell wall and DNA.

Robusta coffee beans, especially green coffee, as used in this study, contain high levels of the active compound trigonelin. Trigonelin is antibacterial in the treatment of chronic periodontitis because it can inhibit the growth of pathogenic bacteria which is one of the causes of periodontitis. Trigonelin works by disrupting the stability of the bacterial cytoplasmic membrane, resulting in unbalanced bacterial metabolic functions. Other active compounds contained in robusta coffee beans and can provide anti-inflammatory effects in the treatment of chronic periodontitis, namely flavonoids and phenols which are included in the polyphenol chemical group. Flavonoids and phenols work as anti-inflammatory by inhibiting the accumulation of PMN cells in inflammatory areas.[4-6,26,44]

The value of significant differences in the average number of PMN cells in the Semendo robusta coffee bean extract gel treatment group with the control group. The control group with placebo gel showed the highest average number of PMN cells, which was 122.6. The control group was only given placebo gel, placebo gel only contains gel base material and has no active ingredients to reduce the number of PMN cells in periodontitis rats. Semendo robusta coffee bean extract gel group with a concentration of 40% can already reduce the number of PMN cells in periodontitis rats and has an average value of PMN cell count of 10.67. In the Semendo robusta coffee bean extract gel group with a concentration of 50%, the average value of PMN cell count was 4.53 and had a significant difference compared to all groups. The average number of PMN cells was less in the treatment group compared to the control group.

The application of robusta coffee bean extract gel in the treatment group was carried out for 14 days to be effective in reducing the degree of inflammation in the healing process of periodontitis rats against chronic periodontitis treatment.[44] The results of the research in this study indicate that the administration of robusta coffee bean extract with a concentration of 50% is the most optimal concentration in reducing the number of PMN cells. Where the higher the concentration of the extract, the higher the content of active ingredients contained therein. This is supported by previous research conducted by Wulandari et al. in testing robusta coffee bean extract with several concentration variations, namely 6.25%, 12.5%, 25%, and 50% in inhibiting the growth of *Porphyromonas gingivalis* bacteria and getting the results of robusta coffee bean extract with a concentration of 50% is the most optimal concentration in inhibiting the growth of *Porphyromonas gingivalis* bacteria.[50]

This study has several obstacles such as pain and stress experienced by mice after induction of *Porphyromonas gingivalis* bacteria. Prolonged pain in rats can prolong the inflammatory phase and inhibit the decrease in the number of PMN cells in Wistar rats. Another obstacle in this study, namely when applying Semendo robusta coffee bean extract to Wistar rats made in gel dosage form requires time to apply the gel right to the gingival sulcus of rats due to the

movement of the oral cavity of Wistar rats and the active reaction of Wistar rats at the time of application of the gel.

CONCLUSION

This study can be concluded that the administration of semendo robusta coffee bean extract gel has an effective effect on reducing the number of PMN cells, especially at a concentration of 50% which can significantly reduce the number of PMN cells in chronic periodontitis treatment of Wistar rats.

SUGGESTIONS

1. Further research needs to be done on the cytotoxic test at the concentration of robusta semendo coffee bean extract against chronic periodontitis treatment in wistar rats.
2. Further research needs to be done on the minimum concentration of robusta semendo coffee bean extract to get the maximum effect in reducing the number of PMN cells in chronic periodontitis treatment of Wistar rats.
3. Further research needs to be done on more effective preparations such as liquids for the application of robusta semendo coffee bean extract to the treatment of chronic periodontitis.

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