EFFECT OF ROBUSTA COFFEE BEANS AND ARABICA EXTRACT GEL AS INFLAMATION PULP MATERIAL

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ABSTRACT

Background: Reversible pulpitis is a mild to moderate inflammatory pulp condition often treated with pulp capping treatments. Current study states that calcium hydroxide ($CaOH_2$) has drawbacks for a long-time use. One of the alternative pulp capping materials is Robusta and Arabica coffee bean extract gel which contains flavonoid compounds and chlorogenic acid which have immunomodulatory properties that play role in the pulp inflammatory process by increasing the count of lymphocyte. **Objective**: The goal of this study is to determine and analyse the effect of Robusta coffee bean and Arabica coffee bean extract gel 95% to the of lymphocyte cells count on the 3rd and 5th day of pulp inflammation compared to placebo gel. **Materials and Methods**: This research is a true experimental with a post-test only control group design. This study used three treatment groups; Robusta coffee bean extract gel 95%, Arabica coffee bean extract gel 95%, and control group placebo gel. **Results**: Two Way Anova test results obtained $p<0.05$, showed significant difference. Data analysis was continued with the Post Hoc Bonferroni test which showed that there was a significant difference between the 95% Robusta coffee bean extract gel group and the 95% Arabica coffee bean extract gel group with $p<0.05$. **Conclusion**: The administration of Robusta coffee bean extract gel with a concentration of 95% can increase the of lymphocyte cells count in pulp inflammation on the 3rd and 5th days more effectively than the 95% Arabica coffee bean extract gel.

Keywords: Arabica Coffee, Direct Pulp Capping, Inflammation, Lymphocytes, Pulpitis, Robusta Coffee
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INTRODUCTION

The prevalence of caries and pulp disease is still quite high in the world, according to Riskesdas (2018), the prevalence of dental caries in Indonesia reaches up to 88.8%. Dental caries is a disease caused by bacteria that affect the calcification of the tooth and pulp layers. Bacteria will easily enter the pulp if there is very deep dental caries, tooth fracture that reaches the pulp, and pulp perforation due to dental restoration procedures. Pulpitis is inflammation of the pulp caused by incipient dental caries and can be cured if dental caries is removed with proper restoration. Clinically, pulpitis is divided into two, namely reversible pulpitis and irreversible pulpitis.[1,2]

One of the diseases included in reversible pulpitis is pulp hyperaemia. Reversible pulpitis should be treated as soon as possible to maintain pulp vitality by pulp capping. Pulp capping treatment consists of direct pulp capping and indirect pulp capping. Direct pulp capping is used directly over exposed pulp during cavity preparations. [3]

The pulp capping material generally used in dental practice is calcium hydroxide. However, recent studies has demonstrated its inability to maintain vitality due to tunnel defects in its attachment to dentin, which make it easier for bacteria to penetrate and slow down the pulp

healing response. [4,5]

The various side effects caused by these ingredients are significant, so natural alternative ingredients are needed in the form of Robusta and Arabica coffee beans. One of the chemical constituents of Robusta and Arabica coffee beans contains flavonoid compounds and chlorogenic acid which have immunomodulatory properties that play a role in the pulp inflammation process by increasing of the lymphocyte cells count.[6]

Research conducted by Wijaya, et al. proved that arabica coffee bean extract study has the ability to inhibit Lactobacillus acid. Previous research conducted by Ari, robusta coffee bean extract showed the best immunomodulatory effect ever done (2017). Based on the description above, we want to study in vivo to determine the effect of Robusta coffee bean (Coffea Canephora var. Robusta) extract gel compared to Arabica coffee bean (Coffea Arabica L) extract gel 95% concentration for direct pulp capping material on the of lymphocyte cells count in the dental pulp of wistar mice.[7,8]

The purpose of this study was to analyse the effect of Robusta coffee bean (Coffea Canephora var. Robusta) extract gel compared to Arabica coffee bean (Coffea Arabica L) extract gel as a direct pulp capping material on the count of lymphocyte cells in the dental pulp.

MATERIALS AND METHODS

This study was approved by the the Faculty of Dentistry ULM ethics committee No. 050/KEPKG- FKGULM/EC/IV/2022. This research method is true experimental with post test with control group design by using simple random technique. The sample used in this research were 24 wistar mice. The inclusion criteria in this study were healthy male wistar mice, 3-4 months old, and body weight of 200-250gr. Exclusion criteria were dead mice, wistar mice that were dull, bald, and shed, and there was a weight loss of more than 10% after the adaptation period in the laboratory. Robusta and Arabica coffee samples were extracted using ethanol and water solvents, the ratio between 96% ethanol and water was 600ml : 400ml.

The samples were divided into 6 treatment groups, which consist of a group of mice given Robusta bean extract gel on the 3rd day, a group of mice given Robusta coffee bean extract gel on the 5th day, a group of mice given Arabica coffee bean extract gel on the 3rd day, a group of mice given Arabica coffee bean extract gel on the 5th day, the group of mice were given a placebo gel on the 3rd day, and a group of mice which were given a placebo gel on the 5th day.

The mice were given intramuscular anesthesia with a solution of ketamine (Ketalar®, Warner Lambert, Ireland) (65mg/kg body weight) and xylazine HCl (Rompun®, Bayer, Leverkusen, Germany) (7mg/kg body weight) dissolved in sterile phosphate buffered saline (PBS). Class I cavity prepared on the occlusal surface in perpendicular direction to the axis of the tooth until the pulp is exposed which is indicated by the presence of a pink spot on the roof of the pulp. Preparation used a hand piece with a round bur (0.9mm diameter) and irrigate the cavity with sterile saline solution, then dry it cotton pallet. Tested materials were applied to the bottom of the cavity or the roof of the pulp with a ball applicator, estimated at the end of the probe, after that it was closed using GIC, while the placebo gel group was immediately closed using only GIC.

On the 3rd and 5th day, the mice were killed to take the maxillary left first molar and the surrounding tissue, then it was fixed (10% formalin) after which it was decalcified using 2% nitric acid. The preparation stained with haematoxylin-eosin (HE) staining to see the presence or absence of lymphocyte cells in the dental pulp. observations were made from the results of all groups histologically using a 400x magnification light microscope. The count of inflammatory cell infiltrates was calculated from 3 different fields of view.

Data analysis used the Shapiro-Wilk normality test. The homogeneity test used was Levene's Test to determine the homogeneity and differences of the 2 groups. The data obtained were normally distributed and homogeneous ($p > 0.05$) the analysis was continued with the Two-Way Anova parametric test to see the effect of giving the extract in each group and continued with the Post Hoc Bonferroni test to find out which day group and which treatment showed significant differences.

RESULTS

The mean of lymphocyte cells counts shown in Figure 1.
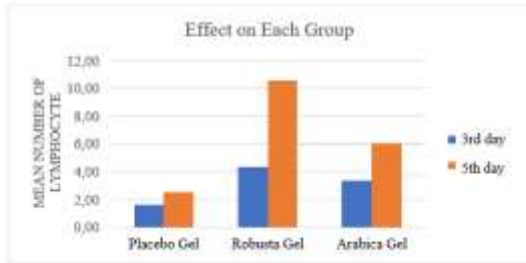
Figure 1. The mean of Lymphocyte Cells All Treatments on Days 3rd to 5th

In Figure 1. shown the largest increase in of the lymphocyte cells count in the group given Robusta coffee bean (Coffea Canephora var. Robusta) extract gel 95% compared to the group given Arabica coffee bean (Coffea Arabica L) extract gel 95% and placebo gel.

Table 1. Results of Mean Value and Standard Deviation of Lymphocyte Cells Count in Pulp Inflammation.

Treatment Standard	Days	Mean ± Deviation	Sig.
Robusta Coffee Bean Extract Gel Concentration 95%	3	4.25±0.957	
Robusta Coffee Bean Extract Gel Concentration 95%	5	10.75±0.957	
Arabica Coffee Bean Extract Gel Concentration 95%	3	3±0.816	
Arabica Coffee Bean Extract Gel Concentration 95%	5	6±0.816	
Placebo Gel	3	1.75±0.957	
Placebo Gel	5	2.5 ± 0.577	

Table 1. Shows the mean and standard deviation

the count of lymphocyte cells on the 3rd and 5th day of pulp inflammation. The results of the Shapiro-Wilk normality test based on the standard residual showed a sig. $p>0.05$ ($p=0.253$) which means that the data distribution is an normally distributed. Furthermore, the data was tested for homogeneity using Levene's test which showed the value of sig. $p>0.05$ ($p=0.852$), this indicates that the data varies homogeneously.

**Table 2.** Two Way Anova Statistical Test Results.

Source	Square	Significance Value
Treatment Group	58.042	0.000*
Day 3rd and 5th	70,042	0.000*
Treatment Group*Days 3rd and 5th	16,792	0.000*

In the Two Way Anova parametric test, the results showed that there were significant differences and interactionsin each treatment group and the 3rd and $5t^{h}$ day groups with sig values. $p=0.000$ ($p<0.05$), then continued with the Post hoc Bonferroni test to find out which groups gave significant differences in Table 3.

Table 3. *Post Hoc Bonferroni*From the Result of Lymphocyte Cells Counts Based on Treatment.

(I) The Treatment	(J) The Treatment	Mean Difference (IJ)	Significance Value
Robusta Coffee Bean Extract Gel Concentration 95%	Arabica Coffee Bean Extract Gel Concentration 95%	3.00	0.000*
	Placebo Gel	5.38	0.000*
Arabica Coffee Bean Extract Gel Concentration 95%	Robusta Coffee Bean Extract Gel Concentration 95%	-3.00	0.000*
	Placebo Gel	2.38	0.000*
Placebo Gel	Arabica Coffee Bean Extract Gel Concentration 95%	-5.37	0.000*
	Robusta Coffee Bean Extract Gel Concentration 95%	-2.37	0.000*

Figure 2. Histopathological Overview of Male Wistar Mice (Rattus norvegicus) Lymphocyte Cells in group a) Robusta coffee bean (Coffea Canephora var. Robusta) extract gel 95%; b) placebo gel; c) Arabica coffee bean (Coffea Arabica L) extract gel 95% on day 3rd.
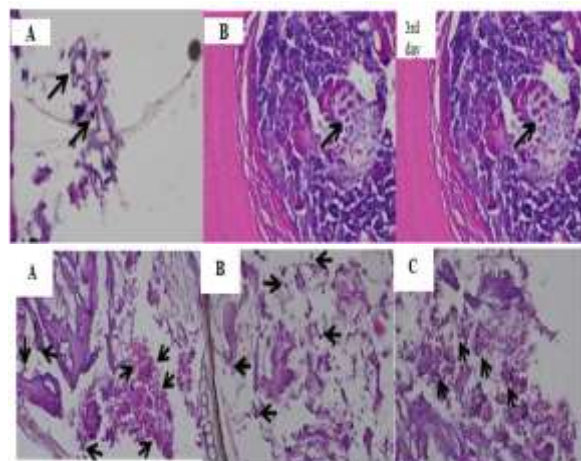
Figure 3. Histopathological Overview of Male Wistar Mice (Rattus norvegicus) Lymphocyte Cells in group a) Robusta coffee bean (Coffea Canephora var. Robusta) extract gel 95%; b) placebo gel; c) Arabica coffee bean (Coffea Arabica L) extract gel 95% on day 5th.

DISCUSSION

This study aims to prove the extract gel effect of robusta coffee bean compared to arabica coffee beans on the count of lymphocyte cells in pulp inflammation. In dentistry, pulpitis can occur due to caries and injury. Dental caries is not the only cause that damages the pulp. The exposed dentinal tubules, whether on the side of the canals, are an entry point for bacteria, toxins, and other factors that can damage the pulp. Pulp disease can also caused by trauma from hard objects, heat from cavity preparation, and the toxic effects of restorative materials.

The study group consist of 6 groups, which consist of group 1 that were given robusta coffee bean (Coffea Canephora var. Robusta) extract gel on the 3rd day, group 2 that were given robusta coffee bean (Coffea Canephora var. Robusta) extract gel on the 5th day, group 3 that were given Arabica coffee bean (Coffea Arabica L) extract gel on the 3rd day, group 4 that were given Arabica coffee bean (Coffea Arabica L) extract gel on the 5th day, group 5 that were given placebo gel on 3rd day, and group 6 that were given placebo gel on 5th day.

The results showed that the treatment group with Robusta coffee bean (Coffea Canephora var. Robusta) extract gel 95% and Arabica coffee bean (Coffea Arabica L) extract gel 95% had effect as shown by an increase in the count of lymphocytes on the 3rd day and a greater

increase in the count of lymphocytes on the 5th day compared to the placebo gel group (negative control).

Based on the research, the results showed that of the lymphocyte cells count showed a significant difference between the placebo gel treatment group, 95% Robusta coffee bean extract gel, and 95% Arabica coffee bean extract gel because the p= 0.000* (p<0.05). Based on the mean difference between the placebo gel group on 95% Robusta coffee bean extract gel (-5.37), and 95% Arabica coffee bean extract gel (-2.37), 95% Robusta coffee bean extract gel had a better effects than arabica coffee bean extract gel 95%. The results of the lymphocyte cells count showed a significant difference between the groups on day 3rd and day 5th. The mean difference of lymphocyte cells in the Robusta coffee bean extract gel (Coffea Canephora var. Robusta) was higher than the average of lymphocyte cells count in the Arabica coffee bean extract gel (Coffea.Arabica L) and were given placebo gel on days 3rd and 5th.

The results of were continued using a post-hoc Bonferroni statistical to determine which day group and which treatment tests showed that of the lymphocyte cells count in the pulp inflammation of the Wistar mice group given Robusta coffee bean extract gel (Coffea Canephora var. Robusta) on the 3rd and 5th days gave a significant effect when compared to the count of lymphocytes in inflammation. The pulp of the Wistar mice group was given Arabica coffee bean extract gel (Coffee Arabica L) and the placebo group was gel.

On 3rd day group, it was seen that the average of lymphocyte cells count in the group of wistar mice given Robusta coffee bean extract gel was 4.25 cells, the next group given Arabica coffee bean extract gel was 3 cells, and the group given placebo gel was 1.75 cells.

On the 5th day group, the mean of count of Lymphocyte cells counts in the group of wistar mice given robusta coffee bean (Coffea Canephora var. Robusta) extract gel was 10.75 cells, the count of lymphocyte cells in the group of wistar mice given the Arabica coffee bean (Coffea Arabica L) extract gel was 6 cells, and the count of lymphocyte cells in the group given the placebo gel was 2.5 cells.

In this research, the mean count of lymphocyte cells on the 3rd and 5th days in the robusta coffee bean (Coffea Canephora var. Robusta) extract gel 95% group greater than the

Arabica coffee bean (Coffea Arabica L) extract gel 95% group, and the placebo gel group, and still increasing to 5th day. The bioactive components of coffee beans are flavonoids, caffeine, chlorogenic acid, and alkaloids. These components act as immunomodulators. According to Kim, et al (2018), bioactive components such as flavonoids and chlorogenic acid in robusta coffee beans are much greater than Arabica coffee, chlorogenic acid in robusta coffee beans is 7.0-10.5%, while Arabica coffee only contains 5-7.5%. Robusta coffee beans contain flavonoids 15-103 mg/g, while Arabica coffee beans contain flavonoids 54.16mg/g.[2] According to Prasetya et al (2021) chlorogenic acid has the ability to reduce the secretion of proinflammatory cytokines such as IL-1β, TNF-, and IL-6. These proinflammatory cytokines can increase the activation of inflammatory cells such as macrophages and lymphocytes. This is in line with the results of the study which showed that the robusta coffee bean (Coffea Canephora var. Robusta) extract gel 95% was more effective as an immunomodulator of lymphocyte cells than the Arabica coffee bean (Coffea Arabica L) extract gel 95% group.[9,10,11,12]

The group given robusta coffee bean extract gel (Coffea Canephora var. Robusta) on the 3rd day experienced an increase because it had entered the chronic inflammatory phase. This phase is characterized by an increase in the count of lymphocytes. This is in line with research by Reisa, et al. which proves that activated macrophages will release cytokines, namely IL-1 and TNF which activate lymphocyte cells.[5] According to Zayyan's research (2016) activated lymphocyte cells will then produce IFN- which will stimulate monocytes to the tissue. The mean of lymphokines count significant increase on day 5th of the experiment. This is due to day 5th the lymphocytes are activated and form lymphokines, producing interferons, and interleukins. Interleukins released by lymphocytes are used to activate macrophages for better phagocytosis.[8,10,13]

The group that were given the robusta and arabica coffee bean extract gel had a higher count of lymphocyte cells because to coffee beans contain flavonoid compounds and chlorogenic acid which have the ability as immunomodulators in helping wound healing, this is in line with research by Reisa (2020), which proves that flavonoids and chlorogenic acid as immunostimulants that can stimulate the

increase in IL-2 by increasing IFN-γ and TGF-β transcription that resulted in an increase of cytokines and IL-2 can be stimulated in the presence of costimulatory factors or mitogens. IL-2 is one of the proinflammatory cytokines that triggers an increase and activation of immune cells. Activation of immune cells by flavonoid compounds will improve cell phagocytic power so that the processes needed to eliminate bacteria and debris in the injured area can be done more quickly.[10,14,15]

Based on this research, it can be concluded that the mean of Lymphocyte cells counts in dental pulp inflammation of wistar mice given robusta coffee bean (Coffea Canephora var. Robusta) extract gel was higher than the mean of lymphocyte cells count in dental pulp inflammation of wistar mice given Arabica coffee bean (Coffea. Arabica L) extract gel and given a placebo gel on 3rd and 5th day.

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