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The Adhesion Activity to Monocytes Against *Streptococcus mutans* with Exposure Steeping Robusta Coffee Beans

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Abstract

Aim and Objectives: The aim of the study is to analyze the adhesion activity to monocytes against *Streptococcus mutans* with exposure steeping robusta coffee beans *in vitro*. **Materials and Methods:** Peripheral blood sampling of healthy people 6 cc added anticoagulants. Peripheral blood sampling of healthy people 6 cc added anticoagulants and Ficoll-Hypaque, then centrifugation and suspended in medium Roswell Park Memorial Institute (RPMI) 1640. The cells were placed in 96-well microtiter plate 8×10^5 cells/well for 45 min at 37°C and washed 4 times with the medium. Attached is a monocyte. Furthermore, the isolate of monocytes and treated with the appropriate group. The control group – monocytes untreated; *S. mutans* group – monocytes + *S. mutans*; coffee group 2.5% – steeping coffee beans 2.5% + *S. mutans*; coffee group 5% – steeping coffee beans 5% + *S. mutans*; and coffee group 10% – steeping the coffee beans 10% + *S. mutans*. After incubated 24 h, fixed with methanol, performed Giemsa staining, and analyzed adhesion activity of under a light microscope With magnification 400 × on to 100 monocytes. Data were analyzed using analysis of variance (ANOVA) followed by least significant difference (LSD) test. **Results:** There were significant differences between the study groups. Steeping robusta coffee beans improve adhesion *S. mutans* in monocyte cells, while the largest adhesion at a concentration of 5%. **Conclusions:** Certain concentrations of steeping robusta coffee beans consumption increase adhesion activity to *S. mutans*.

Keywords: Adhesion, coffee beans, dental caries, phagocytosis, *Streptococcus mutans*

INTRODUCTION

Adhesion of *Streptococcus mutans* to monocyte cells is important because *S. mutans* can enter bloodstream and cause various systemic diseases. When entering *S. mutans* into the bloodstream, leukocyte cells will be responded. One of the immunocompetent cells which acts to prevent the spread of *S. mutans* to the systemic is monocyte cells. One that is thought to increase the adhesion of *S. mutans* to these monocytes is coffee beans. The role of coffee beans on dental caries is important because almost everyone in the world likes coffee, while coffee beans are rich in bioactive content which is thought to be used to prevent and treat dental caries.

Lately, dental caries is a risk factor for systemic diseases. Bacteria (*S. mutans*) derived from dental caries play a role in the occurrence of endocarditis.^[1,2] Dental caries bacteria was found in coronary atherosclerotic plaque specimens in patients who died of a heart attack.^[3] *S. mutans* is a facultative anaerobic, Gram-positive cocci-shaped bacteria commonly

found in the human oral cavity and is a significant contributor to tooth decay.

The immune response of dental caries among others related to cytokines is expressed odontoblast layer, such as interleukin (IL)-1 β , IL-1 α , and tumor necrosis factor-alpha (TNF- α). It said the *S. mutans* is not an immunogen; therefore, *S. mutans* is an antigen that can be cross-reactions with the heart muscle, namely, cardiolipin (diphosphatidylglycerol); a phospholipid that is found in the membrane mitochondrial. At present, the prevention and treatment of a disease are more aimed at the host response. Apart from humoral, cellular responses also an important play role in dental caries. Humoral response was played by IgG and IgA, and the most cellular role is phagocytosis. The immune responses such as monocytes

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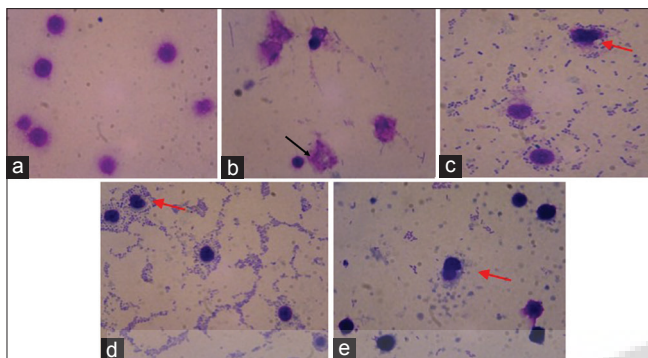


Figure 1: The activity of *S. mutans* adhesion on monocytes after exposure with Robusta coffee beans.

Notes: Adhesion activities *S. mutans* on monocytes (red arrow), monocytes that were lysis (black arrow). (a) Control groups. (b) *S. mutans* groups. (c) Robusta coffee beans 2,5% groups (d) Robusta coffee beans 5% groups (e) Robusta coffee beans 10% groups

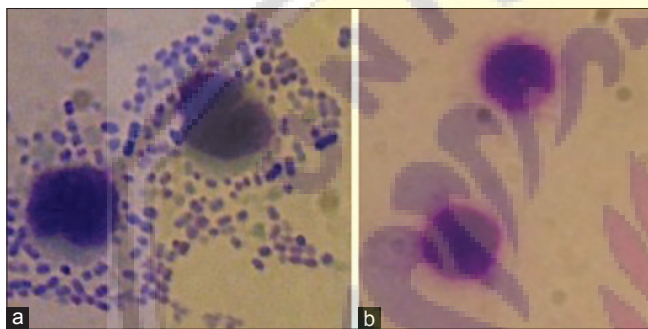


Figure 2: (a) Cells monocytes are active adhesion. (b) Not being active adhesion

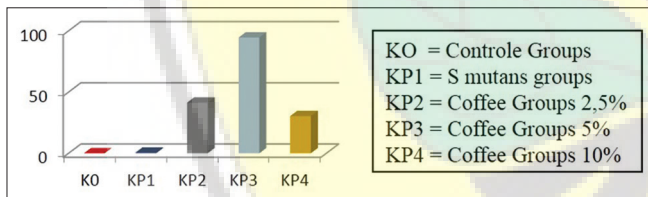


Figure 3: Adhesion activity *S. mutans* to monocytes cells on treatment with steeping Robusta coffee beans

phagocytosis of dental caries associated with IL-1 β , IL-1 α , and TNF- α . The initial process of phagocytosis is characterized by the adhesion of microorganisms on the cell surface. *S. mutans* comes into contact with such as monocytes. On the other hand, cavities should be prevented and treated for dental diseases most frequently encountered with the main causes of *S. mutans*.^[4-7] The elements of a mobile that works on nonspecific immune system, which can be grouped in mononuclear phagocytes (monocytes and macrophages) and granulocytes (neutrophils, eosinophils, and basophils). These cells derived from multipotent hematopoietic stem cells located in the bone marrow fetal liver. Based on the functions, these cells are grouped into phagocytes, cells mediators, and lymphocytes. The role of these cells in the immune response can be seen in phagocytic activity and inflammation that are

part of the innate immune response.^[8] Meanwhile monocytes are known to have receptors that can recognize *S. mutans*.

Thus, it is said that coffee is thought to inhibit dental caries by means of modulating the immune response. The paradigm change which stated that the replacement network turns into tissue regeneration, causing material use medical approach geared to the improvement of biological tissue. Biocompatibility indicates that this material can be accepted by the body. However, the coffee clearly meets these requirements. Several studies proved their coffee inhibition zone against *S. mutans*.^[9-13]

The coffee plant is one commodity that is developed in Jember. Coffee chemical constituents, such as flavonoids, xanthine, antioxidants, alkaloids, and polyphenols, may serve as anti-inflammatory, antibacterial, and platelet aggregation. Polyphenol bioavailability of coffee has also been studied.^[14-19]

The aim of this study is analyzing adhesion activity to monocytes against *S. mutans* with exposure steeping robusta coffee beans *in vitro*.

SUBJECTS AND METHODS

This research has received a certificate from the ETHIC Faculty of Dentistry, University of Jember. Steeping coffee beans is made by mixing 200 cc boiling water with 6 g of coffee beans. Blood is obtained from a healthy woman (not suspected of suffering from systemic abnormalities, no carious teeth, and no calculus), not pregnant, not smoking, and not consuming alcohol. Peripheral blood sampling 6 cc added anticoagulants. Ficoll-hypaque (Sigma) was layered, centrifugated and suspended in medium Roswell Park Memorial Institute 1640 (Gibco). Added Hank's Balanced Salt Solution/Gibco 1:1, pipetted, centrifugated. The cells were placed in 96-well microtiter plate 8 \times 105 cells/well for 45 min at 37°C and washed four times with the medium. Attached is monocyte. Furthermore, the culture of monocytes and treated with the appropriate group (each consists of four samples). The control group – monocytes untreated; *S. mutans* group – monocytes + *S. mutans*; coffee group 2.5% – steeping coffee beans 2.5% + *S. mutans*; coffee group 5% – steeping coffee beans 5% + *S. mutans*; and coffee group 10% – steeping the coffee beans 10% + *S. mutans*. staining, and analyzed adhesion activity of under a light microscope with magnification 400 \times on 100 monocytes. The data used in this study were tested for normality and homogeneity using the Kolmogorov–Smirnov test and Levene test. Hence, if the data are too large, the difference from the numbers obtained is not used. Data were analyzed using analysis of variance (ANOVA) followed by least significant difference (LSD) test.

RESULTS

ANOVA analysis results Various concentrations of robusta coffee beans increase adhesion of *S. mutans* to monocyte cells [Figures 1-3]. The results of this study can be seen in the

following figure. In the picture also seen several monocytes that lysis, especially in the *S. mutans* groups [Figure 1b].

DISCUSSION

Until now, various studies proved that the positive and negative role of coffee on health. One of the negative effects of steeping coffee beans is causing discoloration of the teeth. In addition, it causes side effects, namely, hypertension (still under debate). On the other hand, Song *et al.* proved that coffee intake is a risk indicator for tooth loss. Therefore, scientists are still trying to isolate bioactive ingredients that do not have negative effects, but the presence of various bioactive ingredients is thought to also be complementary.

In this study, we proved that in gated adhesion activities of coffee groups 10% lower than the ingestion activity coffee groups 2.5% and coffee groups 5% [Figures 1-3], allegedly because of the number of bacteria have been reduced in number. *S. mutans* groups many died allegedly due to the load cell is too heavy because of the many bacteria [Figure 1b]. Besides that, there is no additional protection on the cell. In contrast to coffee groups that get additional protection from bioactive components of coffee beans. Coffee Groups 5% seen monocytes enlarged allegedly not optimally protected by coffee beans because the dose is small. Hence, after menfagosit monocyte cell lysis approached, while the coffee groups 5%, and coffee groups 10%, monocyte cells appear normal because these cells are protected by coffee beans (concentration greater than *S. mutans* groups). Besides, in coffee groups, 10%, also seen the number of bacteria less allegedly concentration of coffee beans was higher than other research groups. This was presumably due to the content of the coffee beans that also have antibacterial, so many dead bacteria.

Other ingredients coffee beans are flavonoids and caffeine allegedly acted as an immunomodulator. This flavonoids have the ability to increase the body's immune system. In the roasting process of chlorogenic acid which is an antioxidant, it will synthesize with proteins and polyphenol compounds to form melanoidin. In addition, coffee beans also contain trigonelline which serves to prevent attachment of *S. mutans* on the tooth surface.^[15,16] A study of cellular immune function performed *in vivo* in mice proves that flavonoids can stimulate lymphocyte proliferation, increases the number of T-cells, and increases the activity of IL-2. Flavonoids could potentially work against the lymphokines produced by T-cells that would stimulate the phagocytic cells, including monocytes to perform phagocytosis response.^[15,16] Monocytes have receptors that can recognize *S. mutans*. The main receptor known to participate in the fight against *S. mutans* is dectin-1, Toll-like receptor-2 (TLR2), and TLR4. Dectin-1 induces phagocytosis whereas activation of TLR2 induces the production of cytokines.^[19,20] The coffee beans are thought to bind to receptors on monocytes thereby affecting protein transcription and cell nucleus, subsequently increased receptor activity Dectin-1, TLR2, and TLR4 neutrophil cells further increasing activity in

recognizing *S. mutans*, thus the more the number of monocytes that active.^[20,21]

Based on the results of this study, we recommend choosing green coffee beans because they do not cause black teeth. Sugarless coffee will be better because it can prevent the occurrence of dental caries. However, for connoisseurs of black coffee, we recommend gargling to prevent the black color. In the future, we will use these coffee beans to make mouthwash and toothpaste for the prevention of dental caries.

CONCLUSION

Certain concentrations of steeping Robusta coffee beans consumption increases adhesion activity to *S. Mutans*, where the highest concentration of 5% increases adhesion activity of monocytes.

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Conflicts of interest

There are no conflicts of interest.

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