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# ROBUSTA COFFEE BEANS INCREASE LEVELS OF TNF- α AS A RESPONSE TO *Streptococcus mutans*

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## INTRODUCTION

Coffee plant is commodity exports that was encouraging because it has relatively high economic value in the world market. Coffee plantations are managed by people, and this day its grow continually in several provinces in Indonesia so expansion is increasing continuously. Coffee production in Indonesia ranks the fourth largest in the world after Colombia, Brazil and Vietnam (Zainuddin and Murtisari, 1995; Simanihuruk and Sirait, 2010). In addition, the coffee plant is one of the leading commodities developed in Jember. Chemical content of coffee such as flavonoids, xanthine, antioxidants, alkaloids, polyphenols can serve as a bitter taste, antibacterial, platelet aggregation. The bioavailability of polyphenols coffee has also been studied (Scalbert et al., 2000; Coralie et al., 2006; Naziq, 2012).

Namboodiripad and Srividya (2009) prove the existence of the inhibition zone coffee against *S. mutans.* These bacteria are structurally and antigenetically express a surface protein that are called antigen I / II, B, Sr and PAC that have a molecular weight of 185 kDa. These antigens by the researchers were assigned that play a role in the pathogenesis of dental caries, and its are effective as a vaccine in the prevention of dental caries. I/II antigens of *S. mutans* have properties of adhesive, that the bacteria are used attachment to host components during colonize and infection, so its become the focus of a number of researchers. These surface-antigen proteins have an effect in the attachment of *S. mutans* with acquired pellicles on tooth surfaces (Yuliati, 2005). The immune responses of dental caries were associated with cytokine such as IL-1 β, α, IL-1 and TNF-α that were expressed odontoblast layer. It is said that bacteria excretion a extracellular virulence-immunomodulatory protein (VIP, which has a mitogenic effect on lymphocytes, suppress the immune response of the host and induces production of IL-10, which is an immunosuppressor cytokines. So, VIP is an important virulence factor produced microorganisms and closely associated with the pathogenicity of bacteria (Gomes et al., 2006).

Thereby, it is said that coffee could be expected to inhibit dental caries by means of modulate the immune response. The paradigm change stated that the tissue replacement turns into tissue regeneration that was cause approach of medical materia that geared to a biologically tissue improvement. Biocompatible materials indicate that it can be accepted by the body (Harty and Ogston (1995), and the coffee was clearly meets these requirements.

The current utilization of the coffee plant in the modulates an immune response against *S. mutans* that is cause a dental caries that have not been studied, so arises problem how the immunomodulatory potential of robusta coffee beans against dental caries ?

The general objective of this research is to analyze the immunomodulatory potential of robusta coffee beans against dental caries, whereas the aim in particular is analyze the influence of robusta coffee beans against TNF-α levels in response to *S. mutans*.

## METHODS

The research was conducted in the Laboratory of Bioscience, Faculty of Dentistry, University of Jember. Tools and materials used; culture of *S. mutans*, steeping of robusta coffee beans, sterile-distilled water, peripheral-venous blood, ficoll-hypaque gradient, penstripe, fungizone, histopaque, RPMI, 70% alcohol, 92 well microplate, coverslip, falcon tube, heparin tube, sterile syringe filter, centrifuge, laminar flow, incubator, micropipette and others. Procedure of research was conducted include; prepare tools and materials, prepare steeping of robusta coffee beans, prepare culture of *S. mutans*, the isolation of monocytes and analyze levels of TNFα were used a techniques of ELISA.

### Prepare steeping of robusta coffee beans

20 gr of coffee beens were steeping in 200 ml boiling water. Then made a concentration of 2.5%, 5% and 10%.

### Isolation of monocytes

The steps of monocyte-isolation procedure ;

(1) The first takes the vein blood of the subject of 6 cc and then divided into two tubes with a total of 3 cc in each heparin tube.

(2) Coats the 3 ml histopaque in falcon tube.
(3) Coats the 3 ml ficoll on the top of histopaque carefully and do not mixed with histopaque.
(4) Coats the 6 ml of blood on it carefully.
(5) Centrifugation with a speed of 1,900 rpm for 30 minutes at a temperature of 26 ºC.
(6) Formed six layers, monocytes are at second layer that are the layer of the most clear.
(7) Discard the top layer, then takes layer containing neutrophils to place it in falcon tubes.
(8) Add HBSS (Hanks Balanced Salt Solution) 1: 1 and then pipetting.
(9) Centrifugation with a speed of 1700 rpm for 10 minutes at a temperature of 26 ºC. The results will form two layers.
(10) Discard the supernatant and reserving the part of the precipitate.
(11) Add HBSS as much as 2 cc.
(12) Add 5 µL of fungizone and 20 µL of penstripe per 1 cc.
(13) Pipetting and then inserts the neutrophils in each well of 100 mL carefully.
(14) Incubation for 15 minutes.

**Analysis of levels of TNF-α**

Analysis of levels of TNF-α was used direct enzyme-linked immunosorbent assay (ELISA) technique. The supernatant was coated on the base of microtiter plate (92 well). After washing, then was reacted with antibodies of anti-TNF-α (anti-human), then was reacted with secondary antibodies labeled with enzymes that are degrade color and then was reacted with substrate chromatogenic. The product formed was measured its absorbance that was used ELISA reader.

## RESULTS

The results of measurements of average levels of TNF-α in monocytes exposed steeping coffee beans and *S. mutans* shown in Table 1 and Figure 1. The average levels of TNF-α were highest in the KP4 group (steeping the robusta coffee beans 10%) followed KP3 group (steeping robusta coffee beans 5%), KP2 group (steeping the robusta coffee beans 2.5%) KP1 group (without steeping robusta coffee beans) and K0 group (the control group).

**Table 1. Results of the calculation of the average levels of TNF-α.**

| Research groups | Number of samples | The average of TNF-α levels (pg / mL) ± standard deviation |
|---|---|---|
| K0 | 4 | 0,442 ± 0,025 |
| KP1 | 4 | 0,447 ± 0,041 |
| KP2 | 4 | 0,920 ± 0,114 |
| KP3 | 4 | 2,001 ± 0,679 |
| KP4 | 4 | 4,286 ± 3,194 |

Description :
K0 (The control group) : monocytes without treatment.
KP1(The 1st treatment group) : monocytes + *S. mutans*.
KP2 (The 2nd treatment group) : monocytes + steeping the coffee beans 2.5% + *S. mutans.*
KP3 (The 3rd treatment group) : monocytes + steeping the coffee beans 5% + *S. mutans*.
KP4 (The 4th treatment group) : monocytes + steeping the coffee beans 10% + *S. mutans*

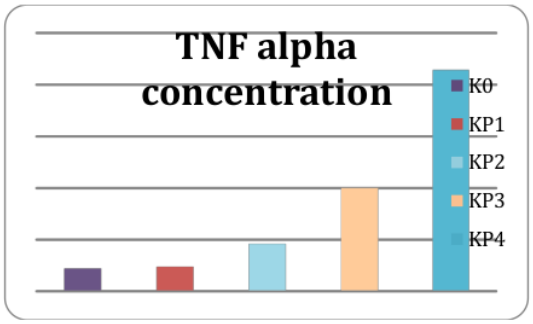


**Figure 1. Bar chart of TNF-α levels produced monocytes cells**

The results of data analysis using one-way ANOVA showed significant value of 0.002 (p <0.05), meaning that there is a significant difference in the study group as a whole. Furthermore, the results of analysis used LSD are shown in Table 2. The results of LSD test shows that only KP4 (steeping the coffee beans 10%) is significantly different from the control group (K0)

**Table 2. Results of LSD (Least Significant Difference) test**

| Research groups | K0 | KP1 | KP2 | KP3 | KP4 |
|---|---|---|---|---|---|
| **K0** | - | 0,971 | 0,611 | 0,107 | 0,000* |
| **KP1** | | - | 0,637 | 0,115 | 0.001* |
| **KP2** | | | - | 0,256 | 0,002* |
| **KP3** | | | | - | 0,023* |
| **KP4** | | | | | - |

Description :
* : Indicates a value significantly (P <0.05)

## DISCUSSION

The average levels of TNF-α were highest in the KP4 group (steeping the robusta coffee beans 10%) followed KP3 group (steeping robusta coffee beans 5%), KP2 group (steeping the coffee beans 2.5%) KP1 group (without steeping robusta coffee beans) and K0 group (the control group). The higher concentrations are used hence the higher content of active substance contained, so concentrations of steeping of robusta coffee beans are the higher hence the higher levels of TNF as well.

Steeping of coffee beans significantly affect the monocytes to produce TNF-α are being exposed by S. mutans. This was evidenced from statistical analysis of one-way ANOVA showed significant value of 0.002 (p <0.05). The concentration of steeping of

the coffee beans was influence the production of TNF-α at a concentration of 10%, whereas at concentrations of 5% and 2.5%; its influence is not significant. These are shown the results of the statistical analysis of LSD in Table 2. Coffee contains bioactive compounds such as flavonoids (Farcas et al., 2014). The flavonoid compounds known to act as an immunomodulatory agents (Yilma et al., 2013) that alter the immune response by suppression(immunosuppressive) or enhancement (immunostimulant) (Saunders Comprehensive Veterinary Dictionary, 2007).

TNF-α is an inflammatory cytokine produced by macrophages or monocytes during inflammation and that is responsible for a variety of activities in signaling cells, encouraging to necrosis or apoptosis (Idriss and Naismith, 2000). The steeping robusta coffee beans were increase the monocytes producing TNF-α that were presumed enhance the activity in signaling cells encouranging to necrosis or apoptosis during inflammatory process.

## CONCLUSION

The steeping of robusta coffee beans in concentration of 10% was increased the level of TNF-α produced by monocytes that was exposed to *S. mutans*.

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