THE EFFECT OF STEEPING ROBUSTA COFFEE BEANS ON MONOCYTES: EXPRESSION OF IL-1β AND TNF-α AGAINST *Streptococcus mutans*

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ABSTRACT: Adhesion, IL–1β, TNF–α are components that affect in inflammation. So, the effect of steeping green and black Robusta coffee beans to adhesion of *Streptococcus mutans* on this components. This study used monocytes isolated from healthy human peripheral blood using Ficoll-Hypaque centrifugation method. Monocytes were divided into eight groups, i. e. (i) Control group (untreated monocytes), (ii) *S. mutans* group (monocytes + *S. mutans*), (iii) Black Coffee 2.5 % group (monocytes + black coffee beans 2.5 % + *S. mutans*), (iv) Black Coffee 5 % group (monocytes + black coffee beans 5 % + *S. mutans*), (v) black Coffee 10 % group (monocytes + black coffee beans 10 % + *S. mutans*), (vi) Green Coffee 2.5 % group (monocytes + green coffee beans 2.5 % + *S. mutans*), (vii) Green Coffee 5 % group (monocytes + green coffee beans 5 % + *S. mutans*), (viii) Green coffee 10 % group (monocytes + green coffee beans 10 % + *S. mutans*). *S. mutans* adhesion on monocytes was analyzed using histochemistry method, while immunocytochemical staining was used for analyzing IL–1β and TNF–α. Cells counting was done per 100 monocytes under a light microscope with 400 x magnification. Data were analyzed using ANOVA followed by LSD test. Results showed that steeping green and black Robusta coffee beans increased the adhesion of *S. mutans* on monocytes, but it decreased of IL–1β, TNF–α expression (P <0.05). In conclusion, steeping of Robusta coffee beans increased adhesion and decreased IL-1β, TNF-α against *S. mutans*.

Index terms: Black coffee, cytokine, green coffee, immunocytochemical, inflammation.

1 INTRODUCTION

Coffee contain several substances such as minerals and chemicals. Some of those are Ca, K, Fe, P, Ni, Mg, and Cr, as well as polyphenols, caffeine, melanoidins, and carbohydrates (SCALBERT & GARY, 2000, MUSSATTO et al., 2011; VIGNOLI et al.; 2011). Chemical content of coffee such as flavonoids, xanthine, antioxidants, alkaloids, polyphenols act as anti–inflammatory, antibacterial; platelet aggregation inhibits the growth of *Streptococcus mutans* (NAMBOODIRIPAD, 2009; ELEX MEDIA KOMPUTINDO, 2010; MULATO & EDY, 2015).

Robusta coffee beans have the function of anti-inflamatory activity based on previous studies. It increased the cells viability, inhibited to growth *S. mutans*, decreased inflammatory cell count (*in vivo*). Robusta coffee also increased the number of fibroblast cells and decreased the expression of IL-1α *in vitro* and *in vivo* (DEWANTI, 2016).

Among those inflammatory activities is phagocytosis. It is one of the immune system against pathogens such as *S. mutans*. The process of phagocytosis as follows: (i) the recognition, which is a process in which foreign microorganisms or particles are detected by phagocyte cells. (ii) the movement (chemotaxis), phagocyte cells move toward the pathogen. (iii) the adhesion, pathogen will attached to the receptors on the phagocyte cell membrane. (iv) the ingestion, the process of ingesting pathogens into the cytoplasm, which will enter the cytoplasm inside a vacuole-like bubble called the phagosome. (v) the digestion, lysosomes containing destructive enzymes such as acid hydrolase and peroxidase, will fuse with phagosomes to form phagolysosomes, then digest foreign matter. Sixth, the secreting, the remaining product of foreign particles that are not digested will be excreted by phagocyte cells (ABBAS et al., 2015).

Regarding the process of Phagocytosis is inflammation. Accordingly, important chemical mediator in inflammation is TNF–α and Il–1β. TNF–α ascachectin is a strong proinflammatory cytokine and plays role in the immune system. Inflammation must occur, but it also causes damage to cells because it can release of chemical mediators, phagocytic enzymes (phagocyte

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oxidase, inducible nitric oxide synthase, and lysosomal protease), free radical compounds and superoxide (BRADLEY, 2008; HANA & JAN, 2013; CHARLES, 2011). The Il–1β family has been extensively reviewed in various literature having many roles in acute and chronic inflammation. The cytokine interleukin–1β (IL–1β) like TNF–α is a plays role as a mediator of the inflammatory response that plays an important role in the host's response. However, it also causes damage during chronic diseases and acute tissue injury (CHARLES, 2011; GLORIA & DAVID, 2011).

The aim in this study is to analyze the effect of black and green of steeping robusta coffee beans on adhesion of *S. mutans* on monocytes and the expression of IL–1β, TNF–α in monocytes.

2 MATERIAL AND METHODS

This research was approved by the Ethical Committee Faculty of Dentistry, University of Jember Indonesia (077/UN25.8/KEPK/DL/2018). The material of this research were as follows: Peripheral blood collection, *S. mutans* (from Microbiology Laboratory, Faculty of Dentistry, University of Jember), Ficoll-hypaque (Sigma), HBSS (Hank's Balanced Salt Solution/Gibco), RPMI (Gibco), Immunostaining KIT (Daco), PBS (Phosphate Bufer Saline/Sigma), DAB (Diamonobenzinidine/Daco), HRP (horseradish peroxidase).

The following is the methods of the research. Peripheral blood from healthy people as much as 6×10^{-3} L was mixed with anticoagulant (heparin). Then, the blood was layered on Ficoll–hypaque and centrifugated (198.968 rad s^{-1}, 30 min, 26 °C). The monocyte layer was then taken and add with HBSS in the ratio of 1:1. The next step after pipetting, it was centrifugated (178.024 rad s^{-1}, 10 min, 26 °C). The supernatant was discarded and add with HBSS, Fungizone 5×10^{-6} L, and Penstripe 2×10^{-5} L, then it was incubated for 24 h at room temperature. Monocytes were then layered inside the culture dish and added with RPMI. Afterward, cells were placed on 24–well microtiter plate 8×10^5 cells/well, then it incubated for 45 min 37 °C, then it was washed 4× with HBSS medium. Monocytes were divided into eight groups, i. e. (i) Control group (untreated monocytes), (ii) *S. mutans* group (monocytes + *S. mutans*), (iii) Black Coffee 2.5 % group (monocytes + black coffee beans 2.5 % + *S. mutans*), (iv) Black Coffee 5 % group (monocytes + black coffee beans 5 % + S.mutans), (v) black Coffee 10 % group (monocytes + black coffee beans 10 % + *S. mutans*), (vi) Green Coffee 2.5 % group (monocytes + green coffee beans 2.5 % + *S. mutans*), (vii) Green coffee 5 % group (monocytes + Green coffee beans 5 % + S.mutans), (viii) Green Coffee 10 % group (monocytes + green coffee beans 10 % + *S. mutans*). All groups were incubated for 24 h at room temperature. Monocytes were made preparates and fixation with methanol. *S. mutans* adhesion on monocytes was analyzed using histochemistry method (Giemza staining), while immunocytochemical staining was used for analyzing IL–1β and TNF–α. Immunocytochemical analysis were carried out in the following ways: the preparation was soaked in blocking solution with peroxidase at room temperature for 10 min, then incubated in back–ground sniper (protein blocking solution) for 10 min at room temperature. Primary antibodies were added 2×10^{-5} L, incubated at 25 °C for 60 min and washed with PBS. Secondary antibodies were added, incubated and washed with PBS. Preparation added with Trek Avidin–HRP reagent, washed with PBS, preparation with DAB chromogen substrate, washed with tap water. Hematoxylin mayer (counterstain) was added to the preparation, then incubated for 1 min to 3 min, then washed under tap water and dried. Cells counting was done per 100 monocytes under a light microscope with 400× magnification. Data were analyzed using ANOVA followed by LSD test.

3 RESULTS AND DISCUSSION

Results of this study showed in figures and tables. Figure 1 showed *S. mutans* looked around the monocytes. Adhesion (Figure 1 and Figure 2). Analysis with ANOVA and LSD showed a difference ($P < 0.05$) (Table 1). Whereas LSD analysis ($P < 0.05$) there was a significant difference between the control group and the black and green coffee group and between the *S. mutans* group and the black and green coffee groups. On the other hand, it was no significantly different between groups of the black coffee and the green coffee. The higher concentration of steeping black and green robusta coffee beans, the more number of *S. mutans* were attached on monocytes.

The result of IL–1β and TNF–α expression described as brown in cytoplasmic of monocytes, but also expressed on extracellularly, so next it must be analyzed to know level of these cytokines (Figures 3 and Figure 5). ANOVA ($P < 0.05$) showed that a significant difference between groups, while LSD (Table 2 and Table 3) showed that no significantly different between the black coffee and the green coffee ($P < 0.05$).

So the higher of the concentration of

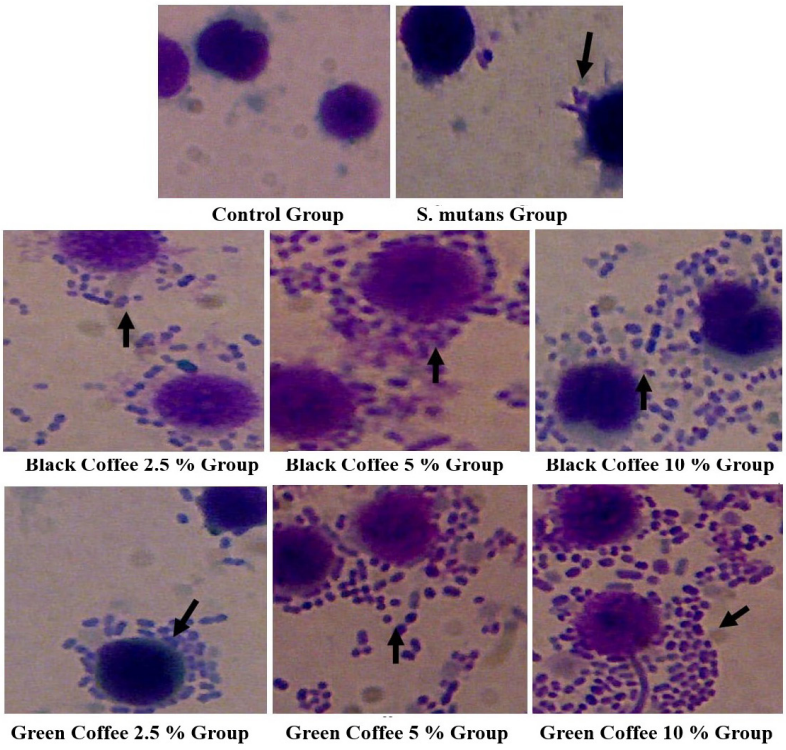

FIGURE 1 - Adhesion activities *S. mutans* in monocytes after exposed by steeping of green and black and green robusta coffee beans. Analyzed with a light microscope with magnification 1 000 x. Adhesion activities were shown with *S. mutans* that surround a monocyte cells (black arrow). Monocytes were lysis (red arrow).

TABLE 1 - Summary of ANOVA Adhesion activities S. mutans on monocytes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	95 400.000	7	13 628.571	18 690.612	.000
Within Groups	17.500	24	.729		
Total	95 417.500	31			

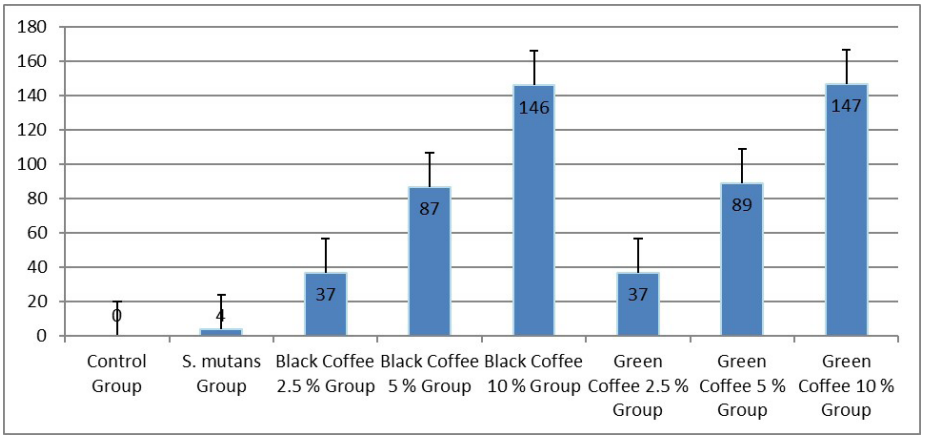

FIGURE 2 - Diagram of Adhesion activities *S. mutans* in monocytes by steeping of green and black Robusta coffee beans.

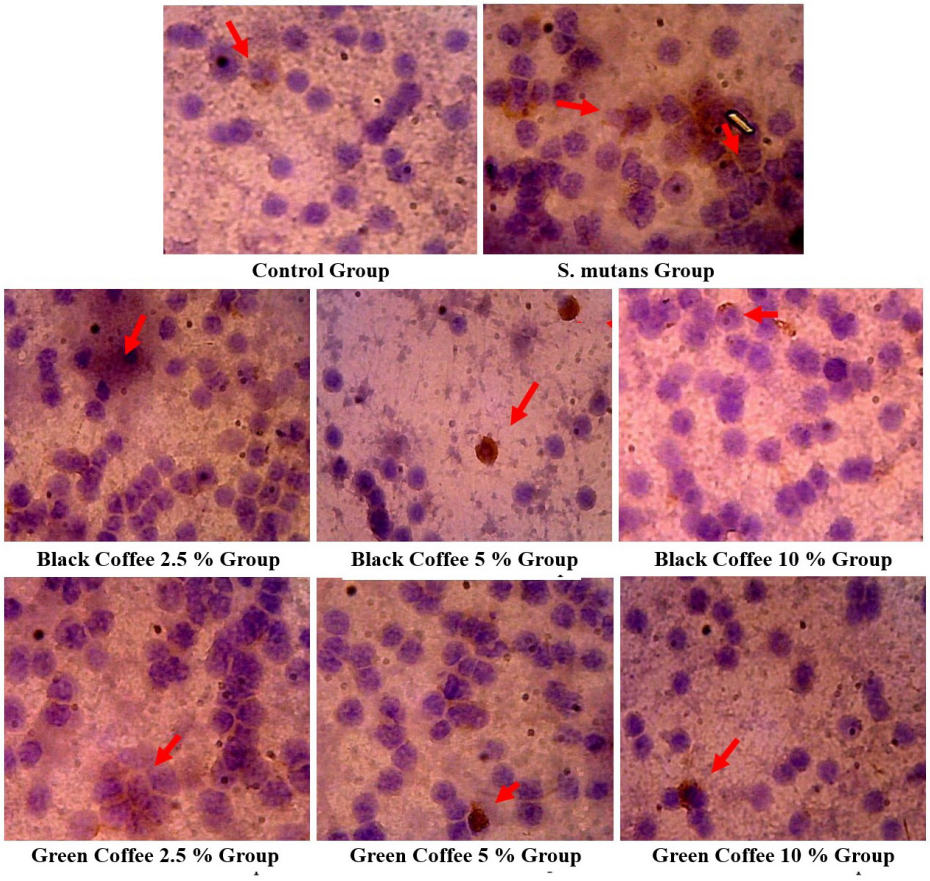

FIGURE 3 - Monocytes that express IL–1β are brown (black arrow). Analyzed with light microscope with magnification 1 000 x. Monocytes were lysis (red arrow).

TABLE 2 - Summary of ANOVA Monocytes that express IL–1β

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15 382.500	7	2 197.500	155.575	.000
Within Groups	339.000	24	14.125		
Total	15 721.500	31			

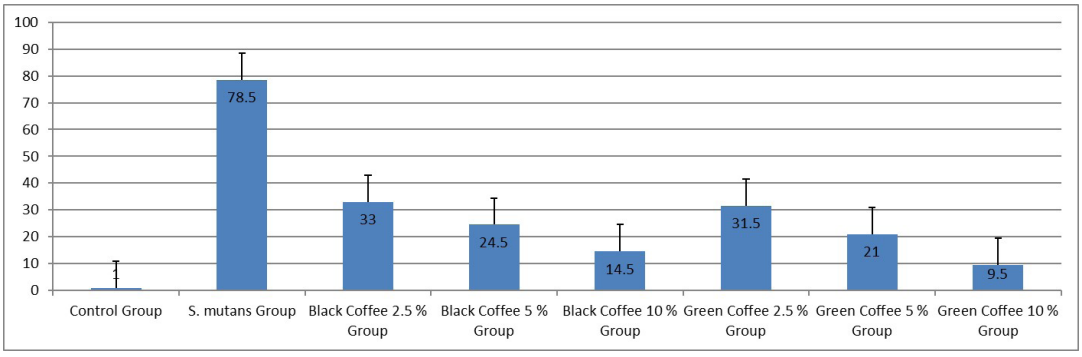

FIGURE 4 - Diagram of IL–1β of monocytes after exposed by steeping of green and black Robusta coffee beans and *S. mutans*.

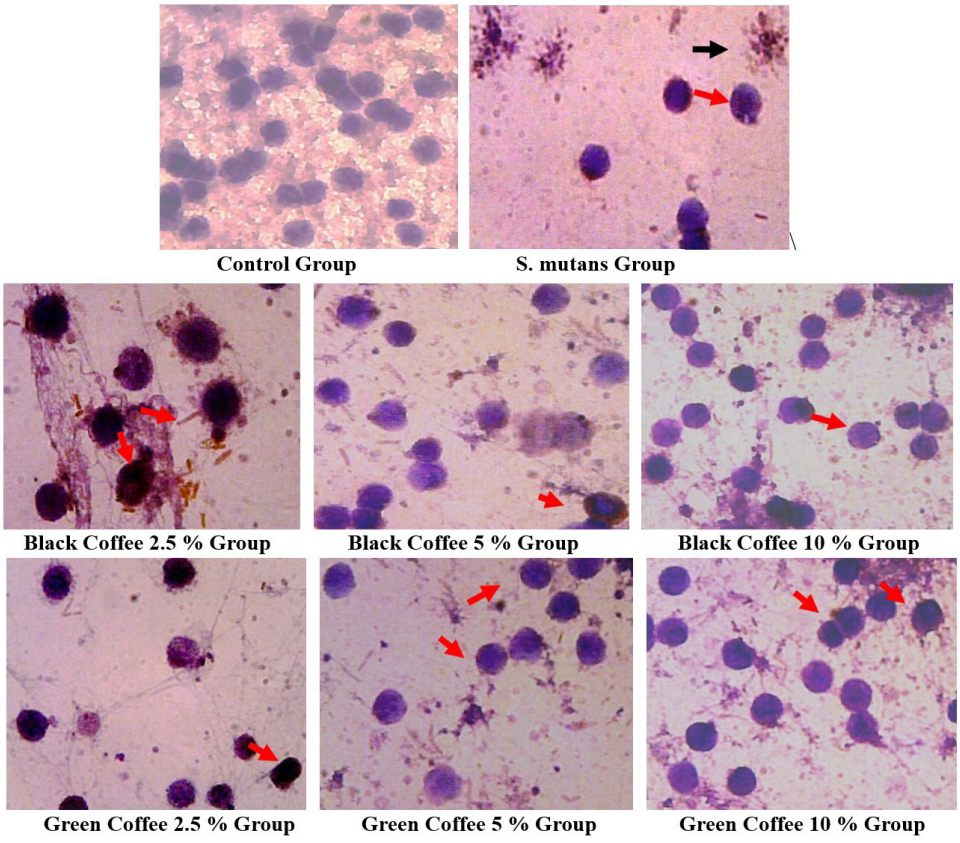

FIGURE 5 - Monocytes that express TNF–α are brown (red arrow), monocytes lysis (black arrow). Analyzed with a light microscope with magnification 1000 ×.

TABLE 3 - Summary of ANOVA Monocytes that express TNF–α

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15 201.617	7	2 171.660	2 752.202	.000
Within Groups	18.938	24	.789		
Total	15 220.555	31			

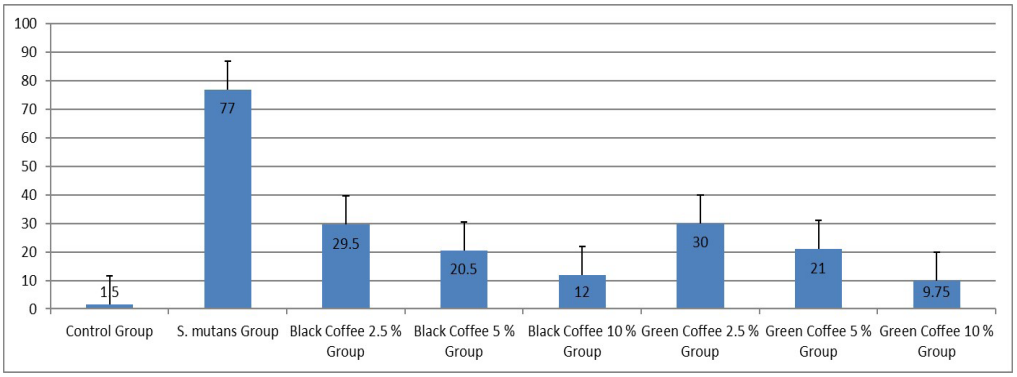

FIGURE 6 - Diagram of TNF–α of monocytes after exposed by steeping of green and black Robusta coffee beans and *S. mutans*.

steeping green and black Robusta coffee beans caused decreasing of IL–1β, TNF–α (Figure 2 and Figure 4). Steeping green and black Robusta coffee beans caused decreasing of inflammation against *S. mutans*.

The result showed that *S. mutans* group appears fewer adhesion activities than the coffee group and many monocytes were lysis, it suspected monocytes cells were not against to *S. mutans* with a maximum. Cell damage can be caused by cell–derived NO resulting in cellular respiration disorders, cell function and proliferation (cytostatic). NO will bind to Ferrum and prevent Ferrum from leaving the cell causing host cell damage (cytocidal) (ALLAIN et al., 2011). The coffee groups were proved that the higher the concentration, the higher the adhesion activity of *S. mutans* in the monocytes cell. Besides that, very few monocytes cells undergo lysis. That process was thought to be suspected by the bioactive components of coffee beans that also have antibacterial, and it could maintain cells survival. Robusta coffee beans could increase cells viability (DEWANTI, 2003). Antioxidants can inhibit the action of cytokine–induced NO synthase (iNOS) enzymes through iNOS control of mRNA and inhibit the transport of arginine by the control mechanism of CAT–2 mRNA (cationic amino acid transporter–2 mRNA) (FRANCESCHELLI et al., 2019).

Bioactive components of coffee beans were flavonoids, cafein, chlorogenic acid, and alkaloids (RAMANAVICIENE et al., 2003). These components were alleged had role as an immunomodulator. In studies of other natural ingredients that contain flavonoids have the ability to improve the immune system. A study of in vivo cellular immunity function in mice proves that flavonoid compounds can stimulate lymphocyte proliferation, increase T–cell count and increase IL–2 activity. Flavonoids potentially work against lymphokines produced by T cells that will stimulate phagocyte cells including monocytes to perform phagocytic responses (SHEN & JU-HUA, 2018). Monocytes have receptors that can recognize *S. mutans*. The major receptors known to play a role against S, mutans is Dectin–1, TLR2, and TLR4. Dectin–1 induces phagocytosis whereas TLR2 induces activation of cytokine production (NETEA et al., 2006; DENNEHY et al., 2009). Coffee beans are thought to bind to receptors on monocyte cells that affect the transcription proteins and cell nuclei, subsequently increasing activity of Dectin–1, TLR2, and TLR4 receptors of monocytes cells thus increasing activity in recognizing *S. mutans*, thus increasing the number of monocytes cells active. Also, monocytes cells release cytokines such as IFNγ, IL–1β, and TNF–α which are known to be factors that trigger adhesions, especially IL–1β known as immunoregulators can stimulate the expression of intercellular adhesion molecule–1 (ICAM–1), ICAM–1 causes monocyte to easily adhesion (THICHANPIANG et al., 2014).

4 CONCLUSION

Steeping of Robusta coffee beans increased adhesion and decreased IL-1β, TNF-α against *S. mutans*. If the concentration is higher, so the adhesion activities is higher. So, steeping of green and black Robusta coffee beans reduce inflammation caused by *S. mutans*.

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6 REFERENCES

ABBAS, A. K,; LICHTMAN, A. H.; POBER, J. S. **Celluler and moleculer immunology, 8th Ed**. Philadelphia: W.B. Saunders Company, 2015. p. 76–78

ALLAIN A. V.; HOANG, V.T.; LASKER, G.F.; PANKEY, E.A.; MURTHY, S.N; KADOWITZ P.J..Role of nitric oxide in developmental biology in plants, bacteria, and man. Curr Top Pharmacol. v. 15, n. 2, p. 25–33, 2011.

BRADLEY, JR.TNF–mediated Inflammaton Disease. **J.Pathol**. Great Britain, v. 214, n. 2, p. 149–160, 2008.

CHARLES, A. D. Interleukin–1 in the pathogenesis and treatment of inflammatory diseases. **Blood**. US, v. 117, n. 14, p. 3720–3732, 2011.

DENNEHY, K. M.; WILLMENT, J. A.; WILLIAMS, D. L.; BROWN, G. D. Reciprocal regulation of IL–23 and IL–12 following co–activation of Dectin–1 and TLR signaling pathways. **European Journal of Immunology**. Germany, v. 39, n. 5, p. 1379–1386, 2009.

DEWANTI, I. D. A. R.; I DEWA, A. S.; PUJIANA, E.; ROEDY, B. Robusta coffee beans decrease of inflammation in dental caries. In: International Conference on Medicine and Health Sciences , 1., 2016, Jember. Proceeding International Conference on Medicine and Health Sciences, Jember: East Java: Aston Hotel: University of Jember, 2016, v. 1, 173–176 p.

DEWANTI, I. D. A. R.; I DEWA, A. S.; PUJIANA, E. L.; RISTYA, W. W. E.; ERAWATI, W.; ROEDY, B.; DYAH, S.; SUNLIP, W. Robusta coffee beans (*Coffea canephora*) decrease IL-1α (Interleucine-1α) expression and increases the number of fibroblasts in dental pulp of wistar rats. **J. Math. Fund. Sci**. Indonesia, v. 51, n. 1, p. 68–76, 2019.

ELEX MEDIA KOMPUTINDO. Khasiat Bombastis Kopi [Efficacy of bombastic coffee]. Jakarta: PT Elex Media Komputindo, 2010. 4–6 p. [In Bahasa Indonesia]

FRANCESCHELLI, S.; GATTA, D. M. P.; PESCE, M.; FERRONE, A.; QUILES, J. L.; GENOVESE, S.; EPIFANO, F.; FIORITO, S.; TADDEO, V. A.; PATRUNO, A.; RILLI, A.; FELACO, M.; SPERANZA, L. Modulation of CAT-2B-Mediated L-Arginine Uptake and Nitric Oxide Biosynthesis in HCT116 Cell Line Through Biological Activity of 4′-Geranyloxyferulic Acid Extract from Quinoa Seeds. **Int J Mol Sci**. v. 20, n. 13: 3262, 2019.

GLORIA, L. C.; DAVID, B. Understanding the mechanism of IL–1β secretion. **Cytokine Growth Factor Rev**. , v. 22, n. 4, p. 189–195, 2011.

HANA, Z.; JAN, H. TNF–a signalling and inflammation: Interactions between old acquaintances. **Inflamm. Res**. Switzerland, v. 62, n. 7, p. 641–651, jul. 2013.

MULATO, S.; EDY, S. **Kopi, Seduhan, & Kesehatan** [Coffee, Brewing & Health] Jember: Indonesian Coffee and Cocoa Research Center, 2015. 27–44 p. [in Bahasa Indonesia]

MUSSATTO, S. I.; CARNEIRO, L. M.; SILVA, J. P. A.; ROBERTO, I. C.; TEIXEIRA, J. A. A study on chemical constituents and sugars extraction from spent coffee grounds. **Carbohydrate Polymers**. UK, v. 83, n. 2, p. 368–374, 2011.

NAMBOODIRIPAD, P. C. A.; KON, S. Can coffee prevent caries?. **J Conserv Dent**. India, v. 12, N. 1, p. 17–21, 2009.

NETEA, M. G.; Neil, A. R. G.; CAROL, A. M.; STEVEN, B.; CLAIRE, C.; GERBEN, F.; RICHARD, P. H.; GWYNETH, B.; H. BLEDDYN, H.; TREES, J.; LIESBETH, J.; ED. T. BUURMAN; KARLIJN, G.; DAVID, L. W.; RUURD, T.; ALISTAIR, M.; DONNA, M. M.; FRANK, C. O.; JOS, W. M. V. D. M.; ALISTAIR, J. P. B.; BART, J. K. Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll–like receptors. **Journal of Clinical Investigation**. US, v. 116, n. 6, p. 1642–1650,. 2006.

NUHU, A. A. Bioactive micronutrients in coffee: recent analytical approaches for characterization and quantification. **ISRN Nutr**. UK, v. 2014, p. 1–13, 2014.

SHEN, R.; JU-HUA, W. The effect of icariin on immunity and its potential application. **Am J ClinExpImmunol**. US, v. 7, n. 3, p. 50–56, 2018.

OLIVEIRA, M.; SUSANA, C.; SIMONE, M.; CLAUDIA, A.; FILIPA, D.; SANDRA, R.; EULALIA, M.; CRISTINA, D. M.; M. BEATRIZ, P. P. O. Intra and interspecific mineral composition variability of commercial instant coffees and coffee substitutes: contribution to mineral intake. **Food Chemistry**. *Netherlands, v.* 130, n. 3, p. 702–709, 2012.

RAMANAVICIENE, A.; MOSTOVOJUS, V.; BACHMATOVA, I,; RAMANAVICIUS, A. Anti-bacterial effect of caffeine on Escherichia coli and Pseudomonas florescens. **Acta Medica Lituanica**. Lithuania, v. 10, n. 4, p. 185–188, 2003.

SCALBERT, A.; GARY, W. Dietary intake and bioavailability of polyphenols. **J. Nutr**. *US,* v. 130, n. 8, p. 2073S–2085S, 2000.

THICHANPIAN, P.; HARPER, S. J.; WONGPRASERT, K.; DAVID O. BATES, B. O. TNF-α-induced ICAM-1 expression and monocyte adhesion in human RPE cells is mediated in part through autocrine VEGF stimulation. **Molecular Vision**. China, v. 20, p. 781–789, 2014.

VIGNOLI, J. A.; BASSOLI, D. G.; BENASSI, M. T. Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. **Food Chemistry**. Netherlands, v. 124, n. 3, p. 863–868, 2011.