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INTRODUCTION

Periodontitis is an inflammation of the periodontal tissues, where the disease is caused by various factors, one of which is the invasion of anaerobic bacteria. The bacteria that cause periodontitis consist of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides forshytus*. Clinically, periodontitis is characterized by changes in the color and volume of the marginal gingiva, bleeding on probing (BOP), the presence of a periodontal pocket, loss of attachment and alveolar bone loss. Periodontitis occurs because of an invasion of the bacteria that will cause an inflammatory response in periodontal tissues.[1] Neutrophils are the first inflammatory mediators that migrate from blood vessels to the periodontal tissues to phagocytes bacteria and other foreign objects which will then be slowly replaced by macrophages.[2]

Macrophages are inflammatory cells that have another name monocyt when they are in vascular and become macrophages when migrating to tissues. Macrophages are the largest cells compared to other leukocyte cells with a diameter of 12-15 µm, the nucleus is slightly oval or horseshoe shaped and has fine chromatin grains. Macrophage cytoplasm looks grayish blue. Macrophages are in the tissues within 5 to 6 hours after an inflammatory response.[3] Macrophages work by phagocytic bacteria and produce various kinds of inflammatory mediators such as cytokines, *Interleukin-1ß (IL-1ß), Interleukin-2 (IL-2), Interleukin- 6 (IL-6), Interleukin-10 (IL-10), Interferon Gamma (IFN-γ), Tumor Necrosis Factor Alpha (TNF-α), Transforming Growth Factor Beta-*

12 (TGF-ß12) and Prostaglandin E2 (PGE2).[4,5,6] Inflammatory mediators if present in high amounts in the tissues, can cause damage to the periodontal tissue and cause resorbtion of alveolar bone.[7,8]

Inflammatory symptoms in periodontitis can usually be used for Non-steroidal anti-inflammatory drugs (NSAIDs), one of which is ibuprofen. Ibuprofen is a drug that affects analgesic, anti-inflammatory and antipyretic. The mechanism of action of ibuprofen is by inhibiting the cyclooxygenase 1 and 2 enzymes (COX 1 and COX 2) to reduce the number of prostaglandins and prostacyclin which are inflammatory mediators in periodontal tissues.[9,10,11]

Propolis kelulut is a typical South Kalimantan's bee product. Propolis consists of the content are flavonoids, phenols, aromatic compounds, amino acids, minerals, vitamins A, E and B complex.[12,13] The content of propolis is almost 50% is a flavonoid compound consisting of acacetin, quercetin, naringenin and galangin.[14,15] Based on these compound components, flavonoids propolis can be used as antiinflammation.[12,16,17] The flavonoid content in propolis works as an anti-inflammatory by reducing the number of inflammatory mediators namely macrophages, cytokines and inhibiting the production of IL-1, IL-6, TNF-α and TGF -ß12 by suppressing the signal path p38, Mitogen-Activated Protein Kinase (MAPK), Jun N-terminal Kinases 1/2 (JNK 1/2) and Nuclear Factor Kappa-B (NF-kB). MAPK and JNK 1/2 are protein molecules that work in tissues to control and signal inflammatory mediators to come to the affected area. NF-kB plays a role in regulating the expression of genes involved in the inflammatory process. If the pathway is blocked, the macrophages which are inflammatory mediators will decrease in number. This study aims to determine the effect of the extract of flavonoid propolis kelulut (Trigona spp) at a dose of 0.5 mg on the number of macrophage cells in periodontitis.[12,18,19]

MATERIALS AND METHODS

The research was carried out after the issuance of a research permit and ethics decision by the Health Research Ethics Committee of the Medical Faculty of Lambung Mangkurat University with No. 119 / KEPKG-FKGULM / EC / I / 2019. This study used a pure experimental method with a post-test only with control group design. Making propolis extract was carried out at the Faculty of Matematika Ilmu Pengetahuan Alam, University of Lambung Mangkurat Banjarbaru, the treatment of experimental animals was carried out at the Balai Veteriner Banjarbaru and the making of histological preparations was carried out at RSUD Ulin Banjarmasin.

Propolis extract has been done by the extraction method. The raw material was soaked with 95% ethanol for 5 days. The material has been put into a rotary evaporator until it has a tenth part of the extraction shrinkage, then placed on a water-bath to evaporate ethanol and got a thick extract. The extract obtained was then fractionated to obtain the pure content of flavonoids. Flavonoid fractionation was carried out using aquades, n-hexane and ethyl acetate. The extract was dissolved in 10 parts of distilled water and then put into a separating funnel and added with n-hexane solvent. Stirring is done using a separating funnel, then two non-mixed layers have been formed. The aquades section has been taken and ethyl acetate was added in a separating funnel, then shaken and two layers were formed. The ethyl acetate layer was evaporated to obtain a pure flavonoid fraction.

This study used 36 male wistar (Rattus novergicus) rats with a weight of 200-250 grams and 3-4 months of age. The rats were then divided into 9 treatment groups, giving flavonoid propolis extract on day 1.3 and 5, the treatment group-administered ibuprofen gel (positive control) on day 1.3 and 5 and negative control group on day 1, 3.5. Mice were made into peridontitis by binding to the cervical mandibular incisors using 3.0-size ligature silk suture for 7 days. Before binding, rats are anesthetized first with ketamine HCl 0.2 ml / 200 grBB to facilitate binding. After the clinical signs of periodontitis were seen, the mice were treated. Flavonoid propolis extract was given topically in gingiva rats at a dose of 0.5 mg. Ibuprofen gel was administered topically to the gingiva at a dose of 9 mg / kgBW, then gingival tissue was taken on days 1.3 and 5.

The gingival tissue was fixed in a 10% BNF solution for 48 hours, then cut 0.5 cm thick. The tissue pieces are dehydrated with alcohol and then embedding is done by planting on paraffin blocks. Paraffin block tissue is cut by 4-5 µm thick, then Papanicolaou is stained.

Macrophage cells were then observed under the Olympus light microscope with a magnification of 400x with 10 fields of view. The data obtained was analyzed by the normality test with Shapiro-Wilk, homogeneity test with Levene 's Test, then One Way ANOVA test and followed by Bonferroni Post Hoc test.

RESULTS

The average results of macrophage cell number in the group given flavonoid propolis extract, ibuprofen gel and negative controls on days 1.3 and 5 can be seen in table 1. The treatment group with day 1 flavonoids propolis extract had an average value of 7.275. The number of macrophage cells began to increase in number on the 3rd day with an average value of 8.025. The decrease in the number

of macrophage cells occurred on the 5th day with an average value of 5.825. The positive control group with the administration of ibuprofen gel on day 1 had an average value of 6.125. The third day there is also an increase in the value, with an average of 9.175 and the fifth day the average value decreases to 4.325. The average score in the negative control group on day 1 was 16.75. The negative control group on day 3 experienced an increase in the number of macrophage cells with an average value of 17.275 and on the 5th day there was a decrease in the number of macrophage cells, with an average value of 16.275.

Table 1. The mean and standard deviation of the number macrophage cells in periodontitis rats based on the treatment group.

Treatment Group	Mean±SD		
	Day-1	Day-3	Day-5
Flavonoid propolis kelulut 0,5 mg	7.275± 3.5236	8.025±0.9639	5.825±1.1758
Ibuprofen gel 9mg/kgBB	6.125±0.9946	9.175±2.6775	4.325±4.5375
Negative Control	16.75±7.6129	17.275±7.661	16.275±7.4141

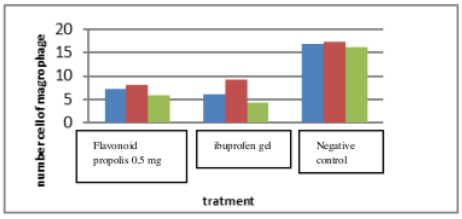

Figure 1. Average graph of the number macrophage cells in the group of periodontitis rats in each treatment

The data was evaluated using *Shapiro Wilk's* normality test and Levene's Test homogenity in all treatment groups and obtained p> 0.05 which stated that all data were normally distributed and homogeneous. The data was then followed by the One Way ANOVA parametric test and p = 0.001.

The results of the Post Hoc Bonferroni test showed that there were significant differences in the number of macrophage cells between the 1.3.5 day propolis group and the negative control group at 1,3,5 days. There was a significant difference between the ibuprofen gel group at day 1,3,5 and the negative control group at day 1,3,5, and there were significant differences between the negative control group and the 1,3,5 day propolis group and ibuprofen day 1,3,5. This shows that the group given flavonoid propolis extract influences the number of macrophage cells.

DISCUSSION

This study has proven that given flavonoid propolis extract with 0.5 mg dose has affect to decrease on the number of macrophage cell in wistar rats with periodontitis. The group given flavonoid propolis extract on day 1 was seen starting to increase in the number of macrophage cells. These cells initiate to gather in the inflammatory area within 5-6 hours after an inflammatory response. The increase in the number of macrophage cells begins to occur because propolis has the ability as an immunomodulator to enhance the ability of the immune system to the performance of immune system mediators.[5,18]

The third day after the administration of flavonoids propolis showed a higher increase than the previous day due to the immunomodulatory power present in propolis. This is under research by Kalsum et al, 2017 which states that in addition to being anti-inflammatory, propolis has properties as an immunomodulator to increase the ability of phagocytes to macrophages, the increasing number of macrophage cells can accelerate the inflammatory phase and wound healing. Then, inflammatory cells number will be decrease. and repair the tissue.[5,18] Nalim, 2004 explains that flavonoids propolis can increase the body's immune system, one of which is macrophages which massively phagocytes to weaken the cell walls of foreign objects that are harmful to the body.[14]

Day 5 of the administration of flavonoid propolis extract showed a significant decrease in the number of macrophage cells. The decrease in the number of macrophage cells was more than the negative control group (without treatment). This is under the theory which states that there is a content in propolis in the form of flavonoids. Flavonoids consist of several derivative compounds such as acacetin, quercetin, naringenin and galangin. Galangin is a flavonoid derivative compound that has the greatest anti-inflammatory activity. Flavonoid propolis works as an anti-inflammatory by inhibiting eicosanoid synthesis. This inhibition will cause a decrease in arachidonic acid in the cell's phospholopid membrane, where it will cause inhibition of inflammatory mediators such as prostaglandin, leocotrin and thromboxane.[20]

The large flavonoid content in propolis can reduce the number of macrophage cells, cytokines and inhibit the production of IL-1, IL-6, TNF-α and TGF-β12 by pressing the p38 pathway Mitogen-Activated Protein Kinase (MAPK), Jun N-terminal Kinases 1/2 (JNK 1/2) and Nuclear Factor Kappa-B which are protein molecules that work in tissues to control and signal inflammatory mediators to come to the injured area and regulate the expression of

genes involved in the inflammatory process. If the signal pathway is inhibited, the inflammatory cells automatically also decrease in the inflamed tissue.[12,18,19] This is in line with the study by Prasetyo et al., 2013 which stated that propolis has an immunomodulator effect.

Propolis can also be anti-inflammatory by inhibiting the activity of binding of deoxyribonucleis acid (DNA), Nf-kB transcription, and protein activator-1.[21] Bufallo et al., 2013 has proven that propolis can reduce the number of macrophage cells and reduce inflammatory mediators secreted by macrophages namely IL2 and NfKb.[7] Research by Rofiah, 2014 also proves the application of propolis to mice incision wounds to see the number of macrophage cells in healing wound, there was a significant decrease in the number of macrophage cells on the 5th day of administration of propolis extract.[22]

The average number of macrophage cells in the positive control group on day 1 showed an increase in cell counts because on the first-day macrophage cells began to replace neutrophils to cleanse areas of inflammation. Day 3 also showed an increase in the administration of ibuprofen gel. This is in line with the study by Izzaty et al, 2014 which stated that there was an increase in the number of inflammatory cells on days 1 and 3 after administration of ibuprofen. Increasing inflammatory cells on that day can accelerate the performance of macrophages to carry out the task of phagocytic tissue foreign matter and end the inflammatory phase.[23]

Day 5 after administration of ibuprofen gel showed a decrease in the number of macrophage cells more than the administration of flavonoid propolis extract. This is because ibuprofen is an NSAID drug that has anti-inflammatory properties. This has been proven by the Farahmand et al 2016 study, where ibuprofen gel has been tested to be used as an adjunct therapy for the treatment of periodontitis.

In the study, ibuprofen gel was used as an adjunct therapy in patients with scaling and root planning, the results showed that the addition of ibuprofen gel had a decrease in the clinical state of inflammation in the patient's gingiva, because ibuprofen has an anti-inflammatory content by inhibiting cyclooxygenase 2.,

This enzyme serves to stimulate inflammatory cells such as cytokines, hormones and growth factors. If the cyclooxygenase 2 enzyme is inhibited, inflammatory cells can automatically decrease and block alveolar bone resorption.[24] This is also in line with research by Agustin et al, 2016 which states that after ibuprofen administration there was a decrease in the number of inflammatory cells on day 5 of the process healing of wounds.

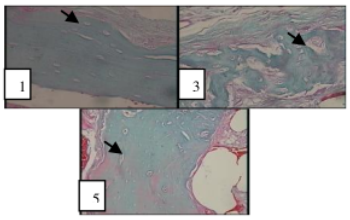

Figure 2. Macrophage cells were seen as oval with a diameter of 12-15µm on histopathological preparations for periodontal tissue of wistar rats by giving extracts of flavonoid propolis kelul on days 1.3 and 5.

Post Hoc Bonferroni advanced test results showed a significant difference between the groups given propolis extract and the negative control group.

This indicates that the administration of flavonoids propolis influences the number of macrophage cells in gingival inflammation. Similarly, the group given ibuprofen gel showed significant differences with the negative control group, which stated that the administration of ibuprofen gel also influenced the number of macrophage cells. It can be concluded that in this study there was an effect of flavonoid propolis kelulut (*Trigona spp*) with 0.5 mg dose to decrease on macrophage cell number in periodontitis and administration of flavonoid propolis kelulut (*Trigona spp*) with a dose of 0.5 mg macrophage cells on day 3 and decreased significantly on day 5, but have not been able to compare the effect of ibuprofen gel administration to decrease the number of macrophage cells.

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