DENTINO
JURNAL KEDOKTERAN GIGI
Vol VI. No 2. September 2021

THE EFFECT OF KARAMUNTING (*Rhodomyrtus Tomentosa*) LEAF EXTRACT ON THE NUMBER OF MACROPHAGES IN PULP INFLAMMATION

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ABSTRACT
Background: *Reversible pulpitis is an inflammation of dental pulp caused by the opening of the pulp due to cavities. One of the procedures in exposed pulp treatment is direct pulp capping using calcium hydroxide. However, this material has side effects, its high pH can cause necrosis, and due to that, a safer alternative material is needed. Karamunting leaf extract contains phenolic compounds, flavonoids, tannins, and saponins which have immunomodulatory properties that play an important role in healing exposed pulp.* ***Objective:*** *To determine the effect of karamunting leaf extract on the number of macrophages in pulp inflammation.* ***Methods:*** *This was a true experimental research with posttest-only group design, using simple random sampling that consist of 24 male Wistar rats which later be divided into 3 groups. The perforated rat dental pulp was then treated with karamunting leaf extract as a treatment group, calcium hydroxide as a positive control group, and not given any application (without drug) as a negative control group. The samples were analyzed histologically on the 3rd to 7th day after the application, inflammatory response occurred in all groups.* ***Results:*** *The two-way ANOVA results showed that there was a significant difference between the karamunting leaf extract group, the group that was not given drug, and the group given calcium hydroxide with a value p<0.05.* ***Conclusion:*** *Based on the research conducted, it is concluded that the administration of karamunting leaf extract can reduce the number of macrophages in pulp inflammation.*

Keywords: *calcium hydroxid, exposed pulp, karamunting leaf, macrophages, pulp inflammation.*
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INTRODUCTION

Caries is an infectious disease in the hard tissue of teeth caused by dentin and email demineralization, resulting in damaged and exposed of pulp tissue. Caries prevalence in Indonesia based on 2018 Basic Health Research (RISKESDAS) data was 57,6%. The exposed pulp makes the microorganism entering and infecting the tissue around it. Then, microorganism will cause the body defend itself by doing resistance that caused inflammation in the pulp or reversible pulpitis.[1,2]

Reversible pulpitis can be handled by the direct pulp capping treatment using calcium hydroxide. Calcium hydroxide has its character which is antibacterial and can form dentin bridge. But, resistant with a few microorganism and can cause abscess. Calcium hydroxide also can cause necrosis, so it needs other alternative material that non-irritating and non-toxic with affordable price.[3,4]

Karamunting is a plant originated from Kalimantan that has benefits such as anti-inflammation and antibacterial. Flavonoid, phenolic, tannin and other compounds in the karamunting leaves can optimize the inflammation process by inhibiting arachidonic acid change into prostaglandins and leukotrienes. It causes the disturbance in inflammation cells chemotaxis to tissue and reducing macrophage cell number. Based on the 2019 research by Ramadhiani, the effective dose of Karamunting leaves extract in the inflammation process is 800 mg/kgBB.[5,6]

Macrophage is leukocytes that play a role in phagocytes or cellularly digest the interfering materials. Macrophage is in the tissue for 5 until 6 hours after inflammation response. Macrophage will massively replace neutrophils on the third day in the inflammatory phase and start decreasing on the seventh day because it is already entering the proliferation phase. Macrophage can devour and

eliminate extracellular particles, damaged and dead cells, also pathogen bacterias.[7,8]

METHODS AND MATERIALS

The method in this research was the true experimental with the posttest-only with control group design. The making of extract and the treatment of Wistar rat were conducted in the pharmacology laboratory Medical Faculty Airlangga University Surabaya, also the making of the preparation was conducted in the Research Center Dentistry Faculty Surabaya. This research has already passed the ethical test by Ethical Commitee of Lambung Mangkurat University Banjarmasin with the statement letter No. 096/KEPKG-FKULM/EC/I/2020.

The Karamunting leaves powder mixed with the solvent that was 96% ethanol and water by 1:2 ratio. The result of the mixture of solvent and powder, sifted and dried. Then, the sieve result washed again using 1 liter of 96% ethanol solution. The Karamunting leaves that have been shredded were moved into a closed container and left in the place that protected from the light and cold for 24 hours. To get a thick extract, the mixture was distilled at low pressure (so the ethanol solvent in the extract is free) using the rotary evaporation machine at the 40-45ºC temperature, after that put into the waterbath.

The experimental animal in this research is male Wistar rats with 250-300 grams weight and 3-4 months old, as many as 24 rats in total with healthy condition. The Wistar rats that have been matched with the criteria, were adapted for 1 week in the laboratory environment. Then, the experimental animals were divided into 3 groups that were the Karamunting leaf extract (treatment) group, the calcium hydroxide group (positive control), and the non-medication group (negative control) with every 2 different days and consisting of 4 Wistar rats in each group.

Before the treatment conducted, the rats injected with anesthetic fluid in the intramuscular with dissolved danxylazine HCl in phosphate buffered saline. In the right molar tooth in the occlusal maxillary conducted class I cavity preparation as deep as the drill head using the handpiece with round bur at 3000 rpm/s speed until reaching the pulp. After perforated, the cavity irrigated with sterile saline solution and dried using cotton pellet. If there is a bleeding, it can be stopped using the tip of paper point.

The treatment group 1 (n=8) was applied the 800 mg/kgBB dose of Karamunting leaves extract (*Rhodomyrtus tomentosa*), the positive control group 2 (n=8) was applied calcium hydroxide (Ca(OH)2) and the negative control group 3 without medication. The material application was conducted using the ball applicator on the pulp surface. After that, the glass ionomer cement (GIC) used as a spill material in the cavity. The experimental animals in the group 1, 2 and 3 were killed on the third and seventh day after the treatment by diethyl ether inhalation. The Wistar rats that have been killed, then, the bone in the area of right molar in the upper jaw was taken. After that, the carcass of the experimental animals were buried by ± 75 cm depth. The right molar of upper jaw pieces put into the 10% formalin buffer solution, then continued to the decalcification phase with 2% nitric acid solution, before continuing to the processing phase, the tissue pieces washed with running water.

The next phase was the specimen embedding. The tissue was sliced ± 5 μm thick using a microtome. In the waterbath, the pieces of tissue sheets will float in the warm water at the 40-50ºC temperature. In order to prevent the wrinkling, after that, the tissue was placed in the object glass. The preparation that was ready to be colored will be labeled. The colored preparation was dried using tissue in the back area, then the object glass will be closed using deck glass and labeled, then continued by observing the macrophage cells in the optical microscope with 400 times zoom.

RESULTS

Based on the data of the macrophages cell number in the Wistar rats pulp inflammation on the third and seventh day, the average macrophage cell number in the Karamunting group, the calcium hydroxide group and the non-medication group as follows.

Table 1. The Average (Mean ± SD) Macrophage Cell Number in the Wistar Rats Pulp Inflammation

Group	Mean ± SD Cell Number	
	3rd Day	7th Day
Karamunting Leaves Extract	12,20 ± 1,30	8,20 ± 0,83
$Ca(OH)_2$	10,00 ± 0,70	7,60 ± 1,39
Without Medication	7,20 ± 0,83	9,40 ± 0,54

Table 1 shows the average and deviation standard calculation result of macrophage cell number in each group. The average number of macrophages in the Karamunting leaf extract treatment group is 12,20 ± 1,30 on the third day and 8,20 ± 0,83 on the seventh day. The average number of macrophages in the calcium hydroxide group is 10,00 ± 0,70 on the third day and 7,60 ± 1,39 on the seventh day. The average number of macrophages in the non-medication group is 7,20

± 0,83 on the third day and 9,40 ± 0,54 on the seventh day.

The conducted data analysis in this research is the Shapiro-Wilk normality test because the sample total is less than 50 samples. The obtained Shapiro-Wilk normality test result is normally distributed with p value = 0,196 because the significance value (p>0,05), then, the data were continued to be tested with Levene's variance homogeneity test.

The macrophage cell significance number that was obtained from the Levene's test is 0,223. It is said that the data is homogenous variance because the significance value (p>0,05) which means the data originated from the population with the same variance. Because the calculation result of all data was homogenous, then continued to the two-way Annova parametric test with 95% confidence level.

The two-way Annova parametric test shows that there is a significant difference in the treatment groups with p value=0,000 and p value=0,000 in the day of treatment which results in H0 rejected because of the decreased macrophage number after the karamunting leaf extract applied.

DISCUSSION

The result of the Post Hoc Banferoni test shows that there is a significant difference between the administration of the karamunting leaf extract and the calcium hydroxide group, and between the karamunting leaves extract and the non-medication group.

This research revealed that the macrophage cell number in the karamunting leaf extract group on the third day has the highest average among the groups. This is caused by the active compound content and anti-oxidant in the karamunting leaf extract that plays a role in optimizing the inflammation process. Flavonoid in the karamunting leaves has a potential to stimulate the phagocytic cells, which are macrophage, to conduct the phagocytosis process. Macrophage will isolate and destroy the debris, also activates the defense. The inflammatory process and scar tissue healing can be optimized if the number of macrophages is high. Then, the macrophage cell will gradually reduce the inflammation and the tissue repaired.[9,10]

The research result on the 7th day shows that there is decreasing in macrophage cell number to the Karamunting leaves extract and calcium hydroxide (Ca(OH)2). This is caused by the tissue that experienced the inflammation phase starting to enter the transition phase to the healing phase. The decreasing of the inflammatory process can be seen from the reducing of macrophage number. If the macrophage number obtained is too high in the inflammed tissue, then this can cause the damage in the healthy tissue around the inflammation. Meanwhile if it is too low, then the body does not capable to fight the infection source.[11,12]

The phenolic and flavonoid compounds in the karamunting leaves can detain the inflammatory process through the cyclooxygenase enzyme and lipoxygenase enzyme which will make the arachidonic acid turn into prostaglandins and leukotriene. Meanwhile, tannin in the karamunting leaves is worked by detaining the excess of arachidonic acid in the inflammation cell that will cause the lack of arachidonic substrate through the cyclooxygenase enzyme and lipoxygenase enzyme. Eventually, there will be pressure in prostaglandins, prostaxillin, endeperoxidase, and leukotriene number.[13,14]

The vasodilatation of blood vessels and local blood stream will affect the inflammation cells migration. This condition will cause the disturbance in the inflammation chemotaxis to the tissue and detain the inflammation process, resulting in the decreasing of the macrophage cell number on the 7th day and optimizing the inflammatory process. The karamunting leaves also contain saponin which plays an important role in the proliferation process or scar healing, because saponin can stimulate the fibronectin synthesis by fibroblast and change the expression of the TGF-β receptor.[1,5,11]

The inflammatory process starts entering the proliferation phase when the number of macrophages decreased and has entered the early proliferation phase, which continued to be replaced by the granulation tissue with the fibroblast cells. The fibroblast cells will form collagen then continued to experiencing mineralization to the reparative dentin. The reparative dentin formed because of the damage of tooth structure which cause the death of the odontoblast cell, resulting in the forming of odontoblast like-cell originated from the differentiated pulp progenitor cell. It will trigger the release of growth factor by the dentin matrix as the reparative dentin forming signal.[11,15,16]

The post hoc test of calcium hydroxide ($Ca(OH)^2$) which is the positive control in this research shows the better average compared with the negative control group. This is caused by the calcium hydroxide works by triggering the sclerotic dentin forming, reparative dentin and antibacterial. The macrophage cell number average in the third day of calcium hydroxide group is 10 and in the seventh day, it was decreased to 7,6. This corresponds to the 2016 research by Dwintanandi et al which said that the macrophage

cell number on the seventh day in the $Ca(OH)^2$ treatment group experienced decreasing[6,12]

Based on the Post Hoc Benferroni test, it is obtained a significant difference of significance value between the Karamunting leaves group and $Ca(OH)^2$, that is 0,003 ($p<0,05$). This is caused by the Karamunting leaves has an immunomodulator compound which can increase activation and macrophage phagocytosis, resulting in macrophage cell number in the extract group is bigger than $Ca(OH)^2$.[12]

Calcium hydroxide has a high pH, resulting in irritation in the pulp and stimulate the occurence of repairment through the protein, that is Transforming Growth Factor-Beta One (TGF-β1) and Bone Morphogenic Protein (BMP) which can give sign in the odontoblast like-cell in order to differentiate. This condition will trigger the forming of reparative dentin.[2,12]

Calcium hydroxide has a good antibacterial capability. Hilton (2009) stated that the bacteria decreased in the infected pulp after 1 hour applying calcium hydroxide. One of the disadvantages of calcium hydroxide is the tunnel defects. The formed reparative dentin will be thinning and sometimes fibroblast and capillary will be found in the said defect. Fibroblast and capillary play a role in the forming of dentin bridge. Another researcher has already found that the reparative dentin quality will be better when the dentin bridge is thick.[6,12]

Based on the conducted research, it can be concluded that the application of the 800mg/kgBW dose of the karamunting leaf extract can decrease the macrophage number in the pulp inflammation.

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