THE EFFECT OF MAULI BANANA (Musa acuminata) STEM EXTRACT ON MACROPHAGE CELL NUMBER IN PULP INFLAMMATION
(In Vivo Study In Male Wistar Rat (Rattus norvegicus) Teeth)

Nadia Dewi Astuti, Maharani Laillyza Apriasari, M. Yanuar Ichrom Nahzi
Faculty of Dentistry, University of Lambung Mangkurat

ABSTRACT

Background: Reversible pulpitis is a mild to moderate inflammatory condition of the pulp which often given pulp capping therapy as their treatments. Commonly used or “gold standard” material for pulp capping therapy is calcium hydroxide, but this material has side effect as an active therapeutic or chemical agent that can cause necrosis due to high pH. Mauli banana stem extract contains tannin and saponin compounds which have immunomodulatory properties that play a role in the pulp inflammatory process by increasing the number of macrophage. Objective: To prove the effect of mauli (Musa acuminata) banana extract gel concentration 37.5% upon macrophage cell count in pulp inflammation at day 3 and compare it with calcium hydroxide (Ca(OH)₂).

Methods and Materials: This research was a pure experimental study with post-test only control group design. This research used 3 treatment groups, which were a group of mauli banana group of mauli banana extract gel 37.5% as treatment group, a group of calcium hydroxide as positive control group and a group of Hydroxypropyl Methyl Cellulose (HPMC) gel as negative control.

Research Result: One Way Anova test results obtained p value = 0.0001 which means that there was a significant difference. Data analysis continued with Post Hoc Least Significant Difference (LSD) test which showed that there was significant difference between mauli banana extract gel concentration 37.5% with calcium hydroxide group with p value = 0,001 and with HPMC gel group p value = 0, 0001 (p <0,05), and there was no significant difference between the calcium hydroxide group and HPMC gel with p = 0,054 (p> 0,05).

Conclusion: Provision of mauli banana extract gel 37.5% concentration can increase the number of macrophage cells on pulp inflammation on the 3rd day.

Keyword: mauli banana extract gel 37.5%, pulp inflammation, calcium hydroxide, macrophage and pulpitis reversible

INTRODUCTION

Reversible pulpitis is a mild to moderate inflammatory condition of the pulp caused by stimuli such as cold or sweet. The prevalence of reversible pulpitis disease in RSKGM-FKGUI in 2009-2013 is 42.1%. The management for reversible pulpitis is by giving the pulp capping treatment. The most commonly used pulp capping material is calcium hydroxide (Ca(OH)₂), but this material has the potential to cause side effects because of its property as an active therapeutic or chemical agent. Natural ingredients can be used as an alternative to pulp capping material to reduce the side effects of calcium hydroxide. One of the ingredients that can be used is mauli banana stem extract gel derived from mauli banana: typical plant of South Kalimantan. Previous research has shown that mauli banana extract gel (Musa acuminata) 37.5% concentration is not toxic, has antibacterial, antioxidant and immunomodulatory activity by increasing the number of macrophage cells on day 3. Until now there is no proof that mauli banana (Musa acuminata) extract gel concentration of 37.5% can be used as pulp capping material.

Apriasari et al, (2014) study mentions that mauli banana extract application to BHK (Baby Hamster Kidney) fibroblast cell 21 is not toxic. Research by Fadhilah et al, (2013) mentions that mauli banana stem extract can act as antibacterial against Streptococcus mutans. Research of Puspitasari and Apriasari, (2017) mentions that mauli banana stem
extract has antioxidant properties. Research of Apriasari et al, (2016) also mentions that mauli banana stem extract with a dose of 37.5% has immunomodulatory properties characterized by an increase in the number of macrophage cells on day 3. Gel extract of mauli banana stem with concentration of 37.5% can be used as an alternative material of pulp capping. 4,5,7,8

Calcium hydroxide pulp capping material can maintain the vitality of the pulp, stimulates dentin bridge formation, and antibacterial. 9 Lack of calcium hydroxide that has high solubility in water and glycerol, can trigger the occurrence of microleakage. Calcium hydroxide does not adapt well to dentin. Long-term use of calcium hydroxide can cause necrosis because of the high pH. 9,10,11,12

In the treatment of pulp capping, there will be a healing process that begins with an acute inflammatory phase, which cause neutrophils in the area of the injury phagocytosed foreign body. 9 The remaining cells will be phagocytosed by macrophages, which are the second defense cell. Macrophages are derived from monocytes in the blood vessels that migrate to the inflamed region. Administration of mauli banana immunomodulatory stem gels can increase the number of macrophage cells on day 3rd, so that the inflammatory phase process becomes faster. A decrease in the number of macrophage cells indicates that the inflammatory phase ends and continues into the proliferative stage. 11,13

From the above background, it is necessary to further research the use of mauli banana stem extract gel. It is necessary to do research on the effect of mauli banana stem (Musa acuminata) extract gel with a concentration of 37.5% as the pulp capping material to a macrophage cell number on pulp inflammation at day 3rd.

MATERIALS AND METHODS

This research used pure experimental method (true experimental) with posttest-only design with control group design. The samples used in this study were Wistar rats (Rattus norvegicus) males. The number of samples were obtained using the Lemeshow formula. 14 The inclusion criteria in this study were male sex, weighed 240-320 grams, 3-4 months old and in healthy condition, characterized by movements of rat such as eating, drinking, no injuries or disabilities.

Preparation of mauli banana stem extract gel concentration of 37.5%

Samples of mauli banana stem were extracted by washing them with flowing water and cut them into small pieces and then dried in the oven at 40-60 degrees for 3 days. After dried, the samples were weighed to obtain 450 mg sample in weight. It was continued with the smoothing using blender and re-weighing until 250 grams of samples were obtained. The steps then followed by the extraction process.

The method used in this research was maceration method, by soaking the banana stems that had been dried and cut. Ethanol solvent 70% to 1 cm above the sample surface. Immersion was done for 3 x 24 hours while occasionally stirred, while being filtered everyday. The result would be evaporated by rotary vacuum evaporator with heated temperature of 40-50°C, then evaporated again with water bath until obtained thick extract and then weighed until 38.15 grams extract were attained. The next step was performing ethanol free test with the addition of Potassium dichromate (K2Cr2O7). The extract was stated to be free of ethanol if there was no discoloration. The prepared and ethanol-free extracts were made into a 37.5% concentration gel with hydroxypropyl methylcellulose (HPMC). The extraction process of mauli banana stem extract of 37.5% concentration was done by dissolving aquaedest for 15 minutes, kept for 24 hours, then used the next day. HPMC was mixed with propylene glycol and Tween 80, stirred rapidly. Maudi banana stem extract was added and stirred rapidly, then added candy oil. Aquadest added up to a weight of 80 grams. Mauli banana stem extract gel concentration of 37.5% has become the color of blackish brown and bitter taste.

Rats Teeth Capping Pulp Procedure

Before the study, the proposal had been submitted to the Animal Care and Use Committee at the Faculty of Dentistry of Lambung Mangkurat University and declared to be eligible based on rats according to the population characteristics of 18 rats adapted for 7 days, the rats were adapted for 7 days in the cage. The experimental animals used were grouped by simple random sampling method, then divided into 3 groups, each consisting of 6 Wistar rats. Rats were anesthetized with an intramuscular injection of a xylazine 7 mg and 65 mg ketamine mixture, then injected 1 ml / kg body weight of the rat. On the occlusal surface of the maxillary molars, class I cavity preparation was performed using a low-speed contra-angle with a round diamond bur (0.6 mm diameter) without opening the pulp chamber. The cavities were irrigated with sterile saline solution and dried with cotton pellets. Treatment was given to each group, group 1 (negative control) of rat tooth was given HPMC gel application, group 2 (control treatment) of rat teeth given mauli banana (Musa acuminata) stem extract gel at concentration 37.5% and group 3 (control positive) of rat tooth given calcium hydroxide. Once applied, the cavities were restored with a glass ionomer cement trap. All the mice were sacrificed on the 3rd day, then the jawbone in the Interdental area of the maxillary molars was taken.
Histopathology Preparation
Preparation of the preparat were began by inserting a piece of tissue into a fixation solution (formalin buffer 10%) for ± 4 days at room temperature. Continued with decalcification process using 2% nitric acid solution for ± 10 days at room temperature then washed with running water.

Proceeding to the process of progressing for ± 18 hours. The next stage was the embedding process of the specimen. After completion, the tissue was sliced in series with a microtome with a thickness of ± 6 μm parallel to the long axis of the tooth. The cutting using microtome were done by putting the tissue in a warm water with 40°-50°C on waterbath, then it was placed on glass object and continued by giving the label. After that the paraffin was melted by placing the glass object above the hotplate and the preparation was ready for the staining.

To see the presence or absence of macrophage cells in the dental pulp, staining with Haematoxylin-eosin (HE) was performed. When the staining had been considered good, the preparations dried at the bottom using a tissue, labeled and lastly the glass object was covered with a glass deck.

Observation and Calculation of Macrophage Cells Number
Observations were done by light microscope (Olympus, United States) equipped with a digital camera with 400 times magnification. The histopathologic image showed the number of macrophage cells with an oval or rectangular shaped core located eccentrically 10-30 μm in diameter on the 3rd day. The macrophage cell number of the inflamed Wistar rat tooth pulp was observed on the 3rd day and compared between each positive, negative and treatment control using two fields of view. The results of the two field calculations were summed and obtained the average value which was the result of calculation for one subject of research.

RESULTS
Based on data of macrophage cell count on inflammation of 3rd day Wistar rat pulp, the mean value of macrophage cell count in the gel group of 37.5% of mauli banana stem extract, HPMC gel group and Ca(OH)₂ group, are as follows:

Figure 1. The Mean Value Of Macrophage Cells On the 3rd Day Wistar Rat Pulp Inflammation In Treatment Group Of Mauli Banana Stem Extract 37.5%, HPMC Gel Group And Ca(OH)₂ Group Graph.

Based on Figure 1, it can be concluded that the mean value of macrophage cell number was the highest in the gel of mauli banana stem extract concentration of 37.5% group, while the mean value of macrophage cell number is the lowest in the HPMC gel group. The macrophage cell histopathologic overview on pulp inflammation at day 3 in the group of mauli banana stem extract gel 37.5%, HPMC gel group and Ca(OH)₂ group can be seen in Figure 2.

Figure 2. The macrophage cell histopathologic overview on pulp inflammation at day 3 in the group of mauli banana stem extract gel 37.5%, HPMC gel group and Ca(OH)₂ group which observed using Olympus light microscope with 400 times magnification.

Data analysis in this study used Shapiro-Wilk normality test. The result showed that in group of mauli banana stem extract concentration of 37.5% group had p value = 0.875, while hydroxypropyl methylcellulose (HPMC) gel had p value = 0.316, and Ca(OH)₂ group have p value = 0.926. The results obtained from the normality test showed that all data was normally distributed (p> 0.05). Next, the data homogeneity was tested using Levene's test, getting significance value of p = 0.138 (p>
0.05) which indicates that the data have homogeneous variances.

One-way Anova test results, mean and deviation standard can be seen in table 1 below:

Table 1. One-way Anova test results, mean and standard deviation on macrophage cell count.

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>Significance</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>EBPM Gel 37.5%</td>
<td></td>
<td>20.17 ± 5.981</td>
</tr>
<tr>
<td>2.</td>
<td>HPMC</td>
<td>0.0001</td>
<td>6.17 ± 2.229</td>
</tr>
<tr>
<td>3.</td>
<td>Ca(OH)₂</td>
<td></td>
<td>10.83 ± 2.041</td>
</tr>
</tbody>
</table>

One Way Anova test results obtained p = 0.0001 which means that there was a significant difference from the mauli banana stem extract gel concentration of 37.5%, the calcium hydroxide Ca(OH)₂ group and the HPMC gel group on the number of macrophage cells in the inflammation of the pulp Wistar rat teeth. The data analysis continued with the advanced test of the Post Hoc Least Square Differences (LSD) test to determine which groups made a significant difference. Results obtained from the LSD Post Hoc test can be seen in table 2 as follows:

Table 2. LSD Post-Hoc Test

* = There was a significant difference (p <0.05)

Based on the result of the Post Hoc LSD test on the above table, it can be seen that there is a significant difference in the average number of macrophage cell between mauli banana stem extract gel 37.5% and Ca(OH)₂ treatment group and there is significant difference compared to HPMC gel group (p <0.05). In the treatment group between Ca(OH)₂ and HPMC gel, there was no significant difference (p> 0.05).

DISCUSSION

Based on the result of the statistic test on the research of mauli banana stem extract gel concentration of 37.5% which used as pulp capping material on Wistar rat teeth, it can be seen that the macrophage cell number on the 3rd day had significant difference compared to group of calcium hydroxide (Ca(OH)₂) and HPMC gel group. This is because mauli banana stem extract gel 37.5% concentration works as immunomodulator.

Puspitasari and Apriasari, (2017) mentioned tannin, treprenoid, and saponin compounds as immunomodulators. Tannins have properties as antioxidants. Plants containing antioxidants are potential immunomodulators. Apriasari et al, (2014) study mentions that mauli banana stem extract has tannin compound as much as 67.594% and saponin compound as much 14.494%. The other content of the compounds in the mauli banana stems gel extract are flavonoids, ascorbic acid, β-carotene and lycopene. This causes the largest number of macrophage cells are found in the banana extract gel group of Mauli banana stem concentration of 37.5%, 7,7,15

Macrophage cells are chronic inflammatory cells. In the inflammatory stage, the initial symptoms of inflammation are indicated by the secretion of various mediators such as TGF-β, coagulation system, inflammatory cells and endothelial cells.15 In acute inflammatory phase, the cells that migrate to the injury site are initiated by neutrophils. This cell is dominant within 24-36 hours after injury. Neutrophil cells function to eliminate irritated and damaged tissue through phagocytosis.9,17

Unphagocytized injury or irritants by the neutrophils will be phagocytosed by macrophage cells. Research of Dwintanandi et al, (2016) and in research of Enggardipta et al, (2016) mention that macrophage cells have been found in the injury site from day one. This is because macrophages are a normal host cell of the pulp. As many as 8% of the cell population in the pulp are macrophages, T lymphocytes and dendritic cells. Activated macrophages can secrete cytokines TNF-α, IL-6, IL-12 and IL-1β. At the time of injury, two to three days after the monocyte injury in the blood vessels migrate to the tissues and differentiate into macrophages that function to continue the process of phagocytosis performed by neutrophils. Macrophage are found in the area of injury during inflammatory phase until the healing phase. Macrophages function to initiate proliferation by releasing cytokines and initiating tissue formation by producing TGF-β. The presence of injury causes endothelial cells to release proinflammatory cytokines (CCL-2, IL-6, IL-8) that induce chemotaxis monocytes. Other factors that recruit monocytes to the area of the injury are TGF- β, PDGF, and VEGF, and TGF-α.9,11,17

Saponin and tannin compounds play a role in increasing TGF-β expression. TGF-β function to stimulate monocyte migration to the injury area where cytokines (IL-4, IL-10 and IL-13) cause monocyte differentiate into macrophages.17,18

Besung’s (2006) study states that triterpenoid
saponin is captured by macrophages through protein receptors G (a protein embedded in the cell surface precisely on the cell membrane). Protein G with GDP present in the cell membrane will approach that ligand (triterpenoid). GDP will be replaced by Guanosine 5 Triphosphate (GTP) so that the G protein becomes active. Protein G bond with GTP to adenyl cyclase (effector protein) that will activate adenyl cyclase and convert Adenosine Triphosphate (ATP) to Cyclic Adenosine Monophosphate (cAMP) as a second messenger. Cyclic Adenosine Monophosphate (cAMP) activates protein kinase A (PKA). Protein kinase A (PKA) phosphorylates many types of proteins and activates them, triggering a cellular response.19

The inflammatory process is complete when macrophages have decreased in number which means it has entered the initial proliferation stage, which is then replaced by granulation tissue with fibroblast cells and blood vessels. The fibroblast cells will form collagen, which will subsequently undergo mineralization to form a reparative dentin. Reparative dentine is formed due to tooth structure destruction causing odontoblastic cell death, resulting in a odontoblast-like cell originating from differentiated pulmonary progenitor cells. This will trigger the release of growth factors, by the dentine matrix as a signal of reparative dentine formation.21,22

The positive controls in this study were Ca(OH)₂ which is the “gold standard” of pulp capping material that triggered the formation of sclerotic dentine, reparative and antibacterial dentine.23,24 The advantage of calcium hydroxide is having good antibacterial ability. Studies show that bacteria are reduced to infected pulp after one hour of calcium hydroxide is applied. One of the disadvantages of calcium hydroxide is tunnel defects. The already established reparative dentine was depleted and sometimes fibroblasts and capillaries are present in the defect. Fibroblasts and capillaries play a role in dentin bridge formation.24

Other researchers have found that the quality of the reparative dentine improves as dentine bridges become thicker.24 The average number of macrophage cells of the treatment group with Ca(OH)₂ in this study ie 10.83. In the study of Dwintanandi et al, (2016), it is mentioned that the number of 3-day macrophage cells in the Ca(OH)₂ treatment group increased. Based on the Post Hoc Least Square Differences (LSD) test, there was a significant difference of the significant value between the mauli banana stem extract gel 37.5% and Ca(OH)₂ is 0.001 (p <0.05). This is because mauli banana stem extract gel has immunomodulatory compounds that can increase macrophage activation and phagocytosis, so the number of macrophage cells in the gel group extract is greater than Ca(OH)₂ on day 3. Graham et al, (2006) and Hilton, (2009) suggest that high calcium hydroxide pH causes irritation of the pulp, thus stimulating improvement through proteins such as Bone Morphogenetic Protein (BMP) and Transforming Growth Factor-Beta One (TGF-β1). Bone Morphogenetic Protein (BMP) and Transforming Growth Factor-Beta One (TGF-β1) signal the odontoblast-like cells to differentiate. Differentiated odontoblasts will trigger the formation of reparative dentine. This causes the number of macrophage cells in the Ca(OH)₂ group were greater than the number of macrophage cells in the HPMC gel group. The number of macrophage cells in the HPMC group had a lower mean than the mauli banana stem extract gel group of mauli and calcium hydroxide ie 6.17. This is because the HPMC gel has no active substance.23,24

It can be concluded that mauli banana stem extract gel concentration of 37.5% as pulp capping material of wistar rats can increase macrophage cell number on the 3rd day of pulp inflammation.

BIBLIOGRAPHY