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Original Article

The Effect of Ramania Leaves Extract Gel (*Bouea macrophylla Griff*) on the Number of Osteoblast (*in vivo* Study of Post Extraction in Wistar Rats (*Rattus norvegicus*))

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ABSTRACT

Ramania leaves extract contains secondary metabolite compounds such as flavonoids, steroids, phenols and terpenoids which can be used as an alternative medicine for socket wound healing. Flavonoids have anti-inflammatory and antioxidant properties that can accelerate wound healing and can stimulate the formation of osteoblasts. Analyzing the differences in the number of osteoblasts in the negative control group, positive control given aloe vera gel 15% and ramania leaf extract gel 15% on day 5, 7 and 14. This study used true experimental design with posttest-only control design, using 27 rats divide into 3 groups. The experimental animals were sacrificed on days 5, 7 and 14 for histopathological observations. Two-Way ANOVA test results obtained p value = 0.013 <0.05, which indicates that there is a significant difference between the use of 15% ramania leaf extract gel 15% and the number of osteoblasts on days 5, 7 and 14. Bonferroni's Post Hoc test showed a difference. The number of osteoblasts was significant among the negative control group with ramania leaf extract gel 15%. Ramania leaf extract gel 15% was proven to be able to increase the number of osteoblasts and has the potential as an alternative medicine for socket healing after tooth extraction.

Key words: Bouea macrophylla Griff, Flavonoids, Osteoblast, Ramania leaf extract gel, Secondary metabolite, Tooth extraction.

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INTRODUCTION

Tooth extraction is one of the most common treatments in dentistry. Indonesia is considered to have a very high prevalence of extraction, which is 79.6% of the population. According to Basic Health Research (RISKESDAS) of South Kalimantan Province in 2018, injuries due to oral surgery were 0.13% while injuries due to tooth extraction reached 8, 46%.[1,2] Tooth extraction is an act of removing the tooth completely from the alveolar bone which causes damage to the hard and soft tissues in the oral cavity.[3] The process of tooth extraction will leave a cavity and a scar in the area of tooth extraction. When there is damage to soft tissue and hard tissue, the body's mechanism will restore the components of the damaged tissue by creating a new and functional structure so that the damaged tissue after tooth extraction will experience healing.[4,5]

Healing after tooth extraction consists of three phases, namely the inflammatory phase, the proliferative phase, and the remodeling phase.[6] The healing process after tooth extraction begins with the presence of a blood clot that clogs the broken blood vessel to stop the bleeding. In the inflammatory phase, the wound area will be dominated by inflammatory cells, namely neutrophils and macrophages that have a phagocytic function and the release of growth factors that will stimulate the proliferation and differentiation of osteoprogenitor cells into osteoblasts on the 5th day after tooth extraction.[7] The proliferative phase is characterized by the formation of new blood vessels and the proliferation of fibroblasts which will synthesize collagen for the formation of new tissue, the epithelialization process which will close the wound area, and the healing of bone tissue.[8,9,10,11] The success rate of bone healing is indicated by the formation of the new bone matrix which is secreted by osteoblasts. Osteoblasts and osteoclasts are cells that are responsible for bone formation.[12] Osteoclasts play a role in the process of bone resorption and osteoblasts will form a new bone matrix by continuously secreting type 1 collagen, releasing calcium and phosphate ions to increase bone density in the proliferative phase on the 7th day and the 14th day. An increase in the number of osteoblasts makes healing after tooth extraction faster. In the remodeling phase, the damaged bone returns to its pre-damaged state by forming new bone.[13–15] The healing process after tooth extraction can prevent further damage to the bone. Alveolar bone damage after tooth extraction can reduce the success of dental treatments such as the use of dentures and dental implants which causes reduced prosthesis support.[16] The socket healing process can be accelerated by patented medicines made from Aloe vera with a mixture of other ingredients such as hyaluronic acid, glycyrrhetic acid, and polyvinyl-pyrrolidone. Aloe vera plays a role in helping the healing process and hard tissue by reducing inflammation due to trauma after tooth extraction which will accelerate the wound healing process and can stimulate osteoblasts in the formation of new bone so that it will repair bone damage that occurs.

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but the content of hyaluronic acid in some people can cause local irritation in the form of redness, swelling, and itching. To minimize the side effects, one alternative can be used, namely medicinal herbs.[17,18]

Medicinal herbs are often used in the health sector, which is proven to be efficacious and has low side effects compared to synthetic drugs.[19] One of the medicinal herbs used is ramania (*Bouea macrophylla* Griff). Ramania is a typical plant of South Kalimantan that can be used as herbal medicine for wound healing because it has secondary metabolites such as flavonoids, steroids, phenols and terpenoids. The largest content in ramania leaves is flavonoids, which is 167.06. Flavonoids can stimulate the formation of osteoblasts, they are osteoconductive, and they can suppress the formation of osteoclasts so that the number of osteoblasts will increase to accelerate the formation of new bone.[20] Terpenoids, phenols, and steroids in ramania leaves have antioxidant properties that can counteract free radicals in the body so that they can suppress bone damage caused by free radicals. [1,13,21] Ramania leaves which are used as medicinal plants are extracted and then made into gel preparations. A gel is a preparation that is often used for topical administration, the gel has the first-pass metabolism, which means it can absorb into the tissue quickly and provide an optimal local effect.[13] The concentration of the gel used in this study was a concentration of 15%. The choice of concentration was based on a previous study conducted by Humaira (2021) and Rizkia (2021) that a 15% concentration of ramania leaf extract gel (RLEG) which was applied topically to the incision wound of wistar rats was more effective in wound healing than the smaller concentrations of 5% and 10%.[1,22] Based on the description of the background above, it is known that flavonoids can stimulate the formation of osteoblast cells for bone formation and it is not known how the effect of giving ramania leaf extract gel (*Bouea macrophylla* Griff) with a concentration of 15% on the number of osteoblasts, therefore it is necessary to research the effect of ramania leaf extract gel (*Bouea macrophylla* Griff) with a concentration of 15% on the number of osteoblasts on the 5th, 7th and 14th days after tooth extraction of wistar rats (*Rattus norvegicus).*

MATERIALS AND METHODS

This research is a true experimental design with post-test with control group design. The implementation of this research has been approved by the Health Research Ethics Commission, Faculty of Dentistry, Lambung Mangkurat University with a statement of ethical feasibility number 054/KEPKG-FKGULM/EC/III/2021. The material used in this study was ramania leaf which was previously determined at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA). Ramania leaves were made into simplicia powder and then macerated with 95% ethanol as solvent. Simplicia powder and solvent are mixed evenly using a magnetic stirrer so that the solvent enters the entire surface of the simplicia powder and evens out the solution concentration. Filtering and changing the 95% ethanol solvent was done every 24 hours. After 72 hours, filtering was carried out and the filtrate was obtained and then solvent evaporation was carried out using a rotary evaporator. The results were placed in a water bath to obtain a thick extract of 100% pure ramania leaves followed by an ethanol-free test. The thick extract of ramania leaves was then made into a gel preparation by mixing 10 grams of HPMC.[21,23]

The sample of this study used 27 male wistar rats aged 2-3 months with a bodyweight of 250-300 grams which were divided into 3 treatment groups, namely the untreated group, the group given *Aloe vera* gel, and the group given the 15% concentration of Ramania leaf extract gel.[3]

The study began with the adaptation of mice for 7 days. Before tooth extraction, the rats were injected with ketamine general anesthetic intraperitoneally, then the lower left incisor of the wistar rat was extracted with a needle holder, causing post-extraction injuries. The post-extraction socket was irrigated with aquadest solution and then 15% ramania leaf extract gel was applied, the untreated group (negative control) and the Aloe vera gel group (positive control). The application of the treatment was carried out every day.[4,24] Wistar rats were euthanized on the 5th, 7th, and 14th days and then the tissue was cut to make histological preparations with haematoxylin eosin (HE) staining. The number of osteoblasts was counted using an optilab microscope in 3 fields of view with 400x magnification. The data were tested using the Shapiro-Wilk normality test and the homogeneity test using Levene's Test. The data obtained were normally distributed and homogeneous ($p>0.05$) were processed by using the parametric Two-way ANOVA test and followed by the Post Hoc Bonferroni test to see which group had the most influence.[1,3,22]

RESULTS

The results of the study entitled "The Effect of Ramania (Bouea macrophylla Griff) Leaf Extract Gel on the Number of Osteoblasts (In vivo Study Post Tooth Extraction of Wistar Rats (Rattus norvegicus)). The results of the calculation of the average and standard deviation of the number of osteoblasts after tooth extraction of wistar rats can be seen in table 1.

Figure 1 shows the number of osteoblasts on day 5, 7, and 14 after tooth extraction of wistar rats in each treatment group was different. On average on the 5th, 7th, and 14th showed an increase in the number of osteoblasts. Based on these results, the highest increase in the number of osteoblasts on the 14th day, respectively was found in the group given the 15% concentration of ramania leaf extract gel, positive control, and negative control. The lowest number of osteoblasts on day 5, respectively starting from the negative control group, the positive control group, and the 15% concentration of ramania leaf extract gel. The results of this study proved that the 15% concentration of ramania leaf extract gel was able to increase the number of osteoblasts from day 5 to day 14 compared to the positive control group and the negative control group.

The data obtained in the calculation of the number of osteoblasts showed that the data were normally distributed in all groups ($p>0.05$). The results of the homogeneity test using Levene's Test showed $p>0.05$, which means the data varies homogeneously. The data obtained have met the requirements for using the Two-Way ANOVA test. The results of the Two-Way ANOVA data analysis can be seen in table 2.

Table 2 shows the treatment group and the effect of day $p=0.000$, which means there is a significant difference in the number of osteoblasts between groups and groups on the 5th, 7th, and 14th. The interaction between treatment groups and days is 0.013 ($p<0.05$), which means there is an effect on the interaction between the treatment group giving the gel with the day of treatment on the number of osteoblasts

Table 1. Average (Mean±SD) Number of Osteoblasts in Socket Post Tooth Extraction Wistar Rat (Rattus norvegicus).

Group	Mean ± Standard Deviation Osteoblast		
	Day 5th	Day 7th	Day 14th
C (-)	4,00 ± 1,00 (2-4 cells)	12,67 ± 1,52 (11-14 cells)	24,00 ± 1,52 (23-26 cells)
C(+)	6,00 ± 1,00 (5-7 cells)	18,33 ± 2,08 (16-20 cells)	33,33 ± 1,52 (32-35 cells)
RLEG 15%	7,33 ± 1,52 (6-9 cells)	21,00 ± 2,00 (12-16 cells)	34,00 ± 2,00 (32-36 cells)

Table 2. Results of Two-way ANOVA Test Number of Osteoblasts After Wistar Rat Teeth Extraction.

Source	Mean square	Sig.
Group	121.593	.000
Day	1396.148	.000
Group * Day	11.259	.013

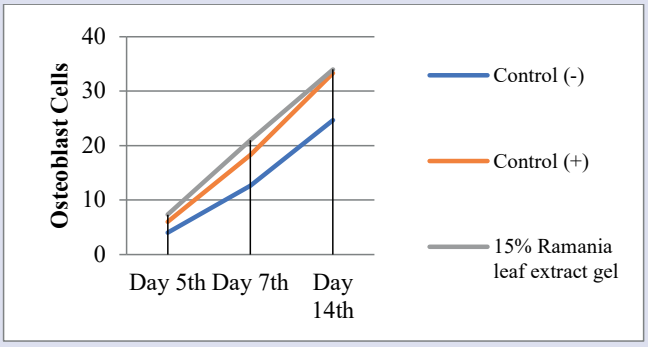

Figure 1. Diagram of the Average Number of Osteoblast Cells in Sockets After Removal of Wistar Rats on Days 5, 7 and 14.

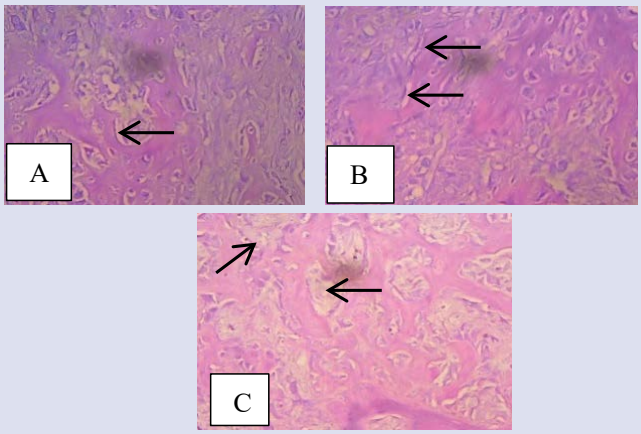

Figure 2. Histopathological description of wistar rat (*Rattus norvegicus*) osteoblast cells in the group: (a) negative control, (b) positive control and (c) ramania leaf extract gel with a concentration of 15% on day 5 after tooth extraction.

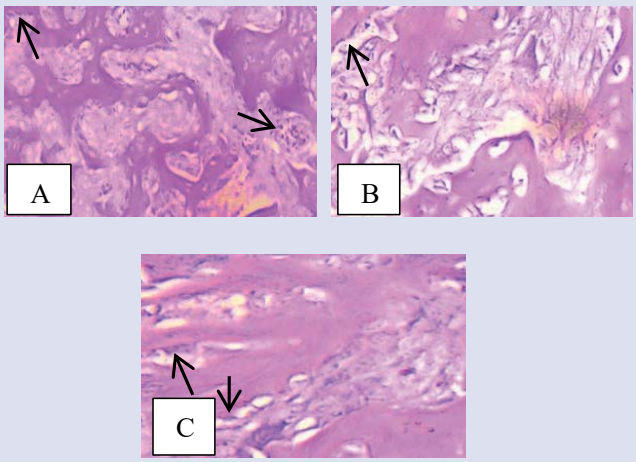

Figure 3. Histopathological description of wistar rat (*Rattus norvegicus*) osteoblast cells in the group: (a) negative control, (b) positive control, and (c) ramania leaf extract gel with a concentration of 15% on day 7 after tooth extraction.

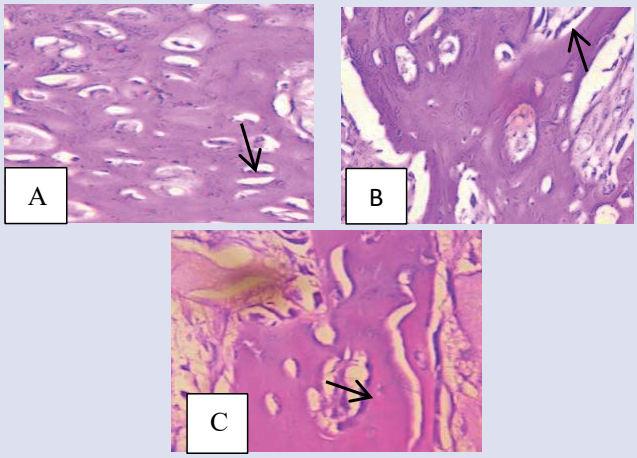

Figure 4. Histopathological description of wistar rat (*Rattus norvegicus*) osteoblast cells in the group: (a) negative control, (b) positive control, and (c) ramania leaf extract gel with a concentration of 15% on day 14 after tooth extraction.

Data analysis was continued by the Post Hoc Bonferroni test to determine the treatment group and the day that gave a significant difference in the number of osteoblasts. Based on the Post Hoc test the treatment group showed that there was a significant difference in the number of osteoblasts between the negative control group, positive control group, and the Ramania extract gel with a value of 0.000 ($p<0.05$). In the positive control group, Aloe vera gel, and the 15% concentration of Ramania leaf extract gel group, there was no significant difference with $p=0.171$. Based on the Post Hoc test in the day group, the number of osteoblasts between the 5th, 7th, and 14th days was 0.000 ($p<0.05$), so there was a significant difference in the number of osteoblasts on the 5th, 7th, and 14th.

DISCUSSION

The results of this study showed that on day 5 after tooth extraction, osteoblasts began to appear in the damaged area with the lowest number of osteoblast cells in 3 visual fields consecutively in the untreated group (negative control) with an average cell count of 4, 00 cells, Aloe vera gel group (positive control) as many as 6.00 cells and 7.33 cells in the untreated group. This study is in accordance with Sa'diyah et al (2020) who stated that on the 5th only the proliferation of osteoblasts from osteoprogenitor cells began, so the number of osteoblasts on the 5th was lower than on the 7th and 14th day.[9,13]

On the 7th day after tooth extraction, the number of osteoblasts increased compared to the 5th day, with the highest number of osteoblasts on the 7th day consecutively in the 15% concentration of Ramania leaf extract gel group with an average number of cells in 3 visual fields of 21,00 cells, the Aloe vera gel group was 18,33 cells and the untreated group was 12,67 cells. The increase in the number of osteoblasts on the 7th day is because osteoblasts are still undergoing cell proliferation, this will increase the number of osteoblasts, thus accelerating bone formation.[15,17,25] The results in this study show the application of a 15% concentration of ramania leaf extract gel in the post-extraction socket was better at increasing the number of osteoblasts than positive and negative controls. This is due to the presence of secondary metabolites in ramania leaves which can be used to optimize wound healing and increase the number of osteoblasts. Ramania leaves have secondary

metabolites such as flavonoids, steroids, phenols and terpenoids. Research conducted by Rahman *et al* (2017) stated that ramania leaves macerated with ethanol solution had the largest metabolite compound, namely flavonoids as many as 167.06. Flavonoids can increase the activity of TGF-β and *bone morphogenetic protein* (BMP), which are *growth factors* that can stimulate the differentiation and proliferation of osteoprogenitor cells into osteoblasts and increase the activity of osteoblasts to synthesize collagen thereby accelerating bone repair and formation. Flavonoids can also suppress bone damage, increase the number of osteoblasts and decrease osteoclast activity by inhibiting the activity of IL-6 which is a proinflammatory cytokine that supports bone repair and formation. [13,14,17] Other metabolites contained in ramania such as steroids can suppress bone damage that occurs post tooth extraction to support bone repair and formation. while phenols and terpenoids can stimulate the differentiation and proliferation of osteoblast cells for bone formation.[25–27]

The results of this study showed an increase in the average number of osteoblasts between groups, indicating that the highest number of osteoblasts on the 14th day was found in the group given the 15% concentration of ramania leaf extract gel with an average number of cells in 3 visual fields of 34,00 cells, accompanied by 33,33 cells of *Aloe vera* gel group and 24,67 cells of the untreated group. The increase in the number of osteoblasts on day 14 was caused by active osteoblast cells in repairing damaged bone by synthesizing type 1 collagen and the activity of changing woven bone into lamellar bone was seen. This is in accordance with research conducted by Ismardianita *et al* (2017) which stated that osteoblasts increased on day 14.[28,29] Based on statistical tests, the 15% concentration of Ramania leaf extract gel and *Aloe vera* gel did not have a significant difference in increasing the number of osteoblasts on the 5th, 7th and 14th days because the positive control used was a patent drug containing *Aloe vera* which can also help the healing process, hard tissue by stimulating osteoblasts for new bone formation. Based on phytochemical tests using 95% ethanol as solvent, *Aloe vera* has a lower total flavonoid content than ramania, which is 54.95 $\mu g/mg$. [30] The low total flavonoid content in Aloe vera gel may make the 15% concentration of ramania leaf extract gel give a better effect on increase the number of osteoblasts. The results of this study were the highest number of osteoblasts on day 5th, 7th and 14th were found in the ramania leaf extract gel group with a concentration of 15%, compared to the number of osteoblasts in other groups.

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