

THE EFFECT OF RAMANIA LEAF
(*Bouea macrophylla* Griff)
EXTRACT GEL ON THE NUMBER
OF FIBROBLAST CELLS OF
INCISION IN MALE WISTAR RATS
(*Rattus norvegicus*)

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Submission date: 13-Jun-2023 02:05PM (UTC+0700)

Submission ID: 2115080599

File name: 3._Jurnal_Nasional_13095-35857-2-PB.pdf (511.78K)

Word count: 2926

Character count: 14441

**THE EFFECT OF RAMANIA LEAF (*Bouea macrophylla* Griff) EXTRACT GEL
ON THE NUMBER OF FIBROBLAST CELLS OF INCISION IN MALE
WISTAR RATS (*Rattus norvegicus*)**

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INTRODUCTION

Wound is a condition in which the unity of a tissue has loss or discontinuity. Wound generally occurs due to surgery or trauma.¹ A research conducted by Med Market Diligence in 2009 on the incidence of wounds recorded 110.30 million cases of surgical wounds and 1.60 million cases of trauma wounds in the world.² Incidence of wounds in the field of oral surgery can be caused by tooth extraction and soft or hard tissue surgery. Basic Health Research (Rikesdas) in 2018 recorded 19% of cases of dental extraction in Indonesia, specifically there were 17.8% of these cases in South Kalimantan. According to the same report, there are 0.3% oral surgery cases in Indonesia and 0.1% oral surgery cases in South Kalimantan.³

The process of wound healing in the oral cavity is the same as the process of wound healing in other body parts. The process of wound healing consists of three main phases, namely the inflammation, proliferation, and remodelling

phases. After the inflammation phase, the wound will undergo a proliferation process marked by the appearance of angiogenesis, granulation tissue formation, collagen deposition, and epithelialization. The proliferation phase will show an increase in the number of cells and the appearance of fibroblasts which is one of the wound healing factors.^{1,4} Fibroblasts generally experience a peak on the 7th day and decrease on the 14th day.^{1,5} Fibroblasts will migrate to the damaged tissue and undergoes proliferation, then synthesizes collagen which then produces granulation tissue to unite the wound.⁶ The disturbed proliferation phase will result in a longer wound healing process and can cause chronic injury.⁷

Wound healing aims to restore the function and shape of tissue to normal conditions with minimal complications. The wound healing process requires a sterile condition so that is often given drugs that contain anti-inflammatory, antibacterial, and antiseptic to optimize the wound healing

process.¹ The administration of inappropriate wound healing drugs results in inhibition of wound healing thereby increasing the costs required for management of infected wounds.^{7,8}

An alternative that can be chosen as a wound healing drug is the use of herbal medicines because it has minimal side effects and more affordable price.⁹ One of the plants in South Kalimantan that can be used as herbal ingredients is ramania (*Bouea macrophylla* Griff).¹⁰ This is because ramania leaf has secondary metabolites such as flavonoids, steroids, phenols, and terpenoids.¹¹ Flavonoids can act as immunomodulators and activate macrophages which release growth factors and stimulate migration and proliferation of fibroblasts.⁵

According to research that was conducted by Rahman et al (2017), ramania leaves contain 167.06 µg / mg of flavonoid compounds so that ramania leaf extract has the potential to be used as an adjuvant therapy for wound healing.¹² The process of wound healing can occur physiologically and is influenced by several factors such as age, hormones, stress levels, nutrient intake, systemic diseases, consumption of drugs, alcohol and cigarettes. These factors sometimes can cause the delayed of wound healing process. This condition requires adjuvant therapy in wound care management to optimize the wound healing process.^{13,14} Ramania leaf extract in this study will be made into the form of gel. Gel is a drug that is often used for topical administration. The selection of gel preparations is done because the gel provides optimal local effect and better absorption.¹⁵

MATERIALS AND METHODS

This research has passed the ethics feasibility test published by the Ethics Commission of the Faculty of Dentistry, University of Lambung Mangkurat, Banjarmasin through a certificate No. 059/KEPKG-FKG/UM/EC/2020.

This research was a pure experimental study (true experimental) with a post-test only and control group design. The research sample was 24 wistar rats (*Rattus norvegicus*). The inclusion criteria for the sample were male Wistar rats weighing 200-250 gr, aged 2-3 months, moving actively, and having a good appetite.

The Making Ramania Leaf Extract Gel

The procedure of the research was started by making ramania leaf extract using maceration method. Ramania leaves were washed by water until clean. 4 kg of Ramania leaves were then dried using an oven at 50°C for 4 hours. The leaves were mashed with a blender. The simplicia powder obtained was sieved with a mesh. 650 grams of simplia powder macerated with 95% ethanol solvent. The ratio of powder to solvent was 1:5. Maceration was carried out for 3 days and

replacement of 95% ethanol solvent was done every 24 hours. After 3 days, the filtering and filtrate were taken. The filtrate was concentrated with a rotary evaporator at 50°C until a thick extract was obtained then the extract was evaporated at a water bath at 50°C and 100% pure ramania leaf extract was obtained. The ramania leaf extract was then mixed with a gel base to make the gel. 5 gr Ramania leaf extract mixed with base gel as much as 95 gr, 10 gr extract mixed with base gel as much as 90 gr, and 15 gr extract mixed with gel base as much as 85 gr to obtained ramania leaf extract gel with the concentrations of 5%, 10% and 15%.

The Preparation of Animal Testing

The animal was adapted for 7 days by being given standard food and drink in a laboratory setting. Rats were anesthetized intraperitoneally with ketamine 40-100 mg/kg BW combined with xylazine dose of 5-10 mg/kg BW. The hair in the back area of the wistar rat was shaved first with a length of 5 cm and height of 3 cm, then it was disinfected using 70% alcohol. Incision wounds were made on the backs of rats 2 cm long and up to the subcutis using a blade no. 15. The excreted blood was cleaned using cotton that was soaked in water. Rats were divided into 4 groups: the treatment groups which were given ramania leaf extract gel with the concentrations of 5%, 10%, 15% and the control group which were given placebo gel. Each group consisted of 3 rats. The extract gel was applied 1 time a day in a one-way motion and the cotton bud applicator position was rotated, the wound was then covered with gauze.

The Making and Observation of Histological Preparations

The euthanasia procedure was carried out according to the guidelines of The AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. On the 7th and the 14th day, all of the rats in each group were up for euthanasia by intraperitoneal local anaesthetic mixed of ketamine 40-100 mg/Kg BW and xylazine dose 5-10 mg/Kg BW, then wait until the rat became unconscious and tissue retrieval was performed. Intake of tissue in wistar rats was carried out by excision. Excised area was the area along the wound border with a length of 3 cm, width 0.3 cm and depth of subcutis. Wistar rats that have taken tissue then were buried with a depth of 75 cm. Each excised specimen of each treatment was taken to make histopathological preparations of wistar rat incision.

Histopathological preparations were prepared by tissue fixation using a 10% Neural Formalin Buffer solution (BNF). The tissue was then cut to a size of 10 mm using a scalpel and then dehydrated. The tissue was made into paraffin blocks and stored in a freezer. The paraffin block was cut using a microtome with a thickness of 5 microns. The

results of the pieces were placed in a Waterbath then the shape was tidied up and placed on the glass object. Preparations that have been made were then stained by haematoxylin eosin (HE) staining method. Preparations were observed using a microscope equipped with optilab, 400x magnification in 5 visual fields.

RESULTS

The statistical results show the data of all groups were normally distributed and the variance of data was homogeneous. Two-way Anova statistical test results obtained p value = 0.640 > 0.05 which showed no significant difference between the use of ramania leaf extract gel to the number of fibroblast cells on the 7th and 14th days. The Post Hoc Bonferroni test showed a significant difference in the number of fibroblast cells on the 7th and 14th days between the control group and the ramania leaf extract gel treatment group at a concentration of 15%.

The results of the average number of fibroblasts on the 7th and 14th days of wistar rat incision can be seen in Table 1. The graph of the average number of fibroblasts on the 7th and 14th days of wistar rat incision can be seen in Figure 1.

Table 1. The average (Mean±SD) number of fibroblast cells of incision in wistar rat

Group	Mean ± SD Cells Number	
	7th Day	14th Day
Placebo gel 0,57 cells)	10,66 ± 1,52 (9-11 cells)	7,66 ± (6-8
Ramania leaf extract gel concentration of 5% cells)	13,33 ± 0,57 (12-14 cells)	8,33 ± (7-9
Ramania leaf extract gel concentration of 10% cells)	13,66 ± 1,52 (12-15 cells)	9 ± (7-11
Ramania leaf extract gel Concentration of 15% cells)	16,66 ± 1,15 (15-17 cells)	12 ± (10-14

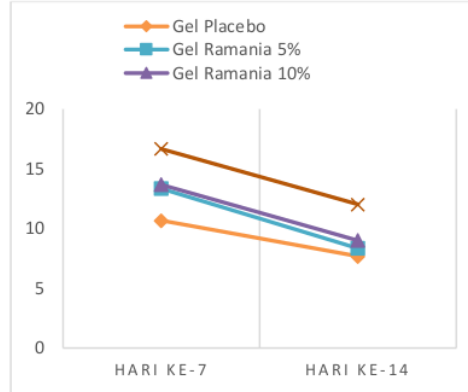


Figure 1. The average number of fibroblast cells of incision in wistar rat on the 7th and the 14th day in each group.

Based on the graph in Figure 1, it can be concluded that the higher the concentration of the extract gel, the higher the number of fibroblast cells will increase in wistar rat incision wounds. The highest average number of fibroblast cells in a consecutive order were the ramania leaf extract gel with concentration of 15%, 10%, 5% and the placebo gel.

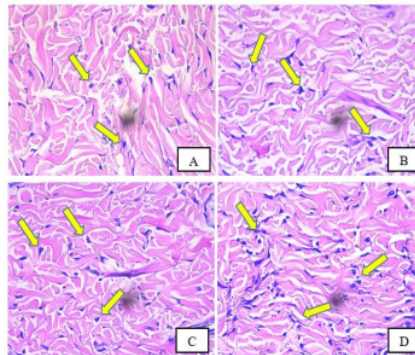


Figure 2. (A) Placebo gel on the 7th day (B) Ramania leaf extract gel concentration of 5% on the 7th day (C) Ramania leaf extract gel concentration of 10% on the 7th day (D) Ramania leaf extract gel concentration of 15% on the 7th day

Figure 2 showed a histopathological picture of fibroblast cells belonging to a group of wistar rats with incision wounds on the 7th day. Based on the average calculation on that day, the group of wistar rats with incision wounds given placebo gel had 9-11 fibroblast cells, while the group of wistar rats with incision wounds which were given ramania leaf extract gel with a concentration of 5% had 12-14 fibroblast cells, a concentration of 10% had 12-15 fibroblast cells, and the 15% had 15-17 fibroblast cells.

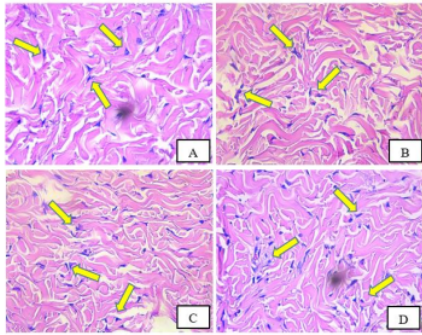


Figure 3.
 (A) Placebo gel on the 14th day (B) Ramania leaf extract gel concentration of 5% on the 14th day (C) Ramania leaf extract gel concentration of 10% on the 14th day (D) Ramania leaf extract gel concentration of 15% on the 14th day

Figure 3 showed a histopathological picture of fibroblast cells belonging to a group of wistar rats with incision wounds on the 14th day. Based on the average calculation on that day, the group of wistar rats with incision wounds that was given placebo gel had 6-8 fibroblast cells, the group of wistar rats with ramania leaf extract gel with a concentration of 5% had 7-9 fibroblast cells, the group of a concentration of 10% had 7-11 fibroblast cells, and the 15% had 10-14 fibroblasts.

DISCUSSION

The results of this research showed an increase in the average number of fibroblasts and comparison between groups showed the highest average number of fibroblasts was found in the group of ramania leaf extract gel with a concentration of 15% with an average of 16.66 cells, followed by a group of ramania leaf extract gel concentration of 10% with an average of 13.66 cells, a group of ramania leaf extract gel concentration of 5% with an average of 13.33 cells and a placebo gel group with an average of 10.66 cells. This research is in accordance with the research by Ardiana et al (2015) which states that the application of binahong gel (*Anredera cordifolia*) can increase the number of fibroblast cells on the 7th day.¹⁶

The results of this research showed that the application of ramania leaf extract gel in the wound area is more effective in increasing the number of fibroblasts compared to the application of placebo gel. This is likely due to the presence of secondary metabolites in the ramania leaf which can be used to optimize the wound healing process and increase the number of fibroblasts. Based on a research that was conducted by Fitri et al (2018), ramania leaf

contain secondary metabolites such as flavonoids, phenols, steroids and terpenoids.¹¹ Ramania leaves macerated with ethanol solution contains 167.06 $\mu\text{g}/\text{mg}$ of flavonoid compounds.¹²

Flavonoids can act as immunomodulatory that can increase the production of IL-2. IL-2 production will stimulate the proliferation and differentiation phases of T cells. Differentiated T cells will turn into Th1 cells and secrete IFN- γ which has the potential to activate macrophages. Active macrophages will release several growth factors, namely PDGF, FGF, TGF- α , TGF- β and EGF which are responsible for the process of mitogenic fibroblasts that are important in the process of wound healing.¹⁷ The proliferation of fibroblasts indicates with the granulation tissue that begins to form through mechanisms that will produce three-dimensional extracellular tissue in connective tissue.¹⁵

Granulation tissue that begins to form during the proliferation of fibroblasts will produce an extracellular matrix.¹⁸ Fibroblasts with matrix metalloproteinase (MMP) will capture the fibrin matrix and then convert it to glycosaminoglycan (GAG), then the extracellular matrix will be replaced by another fibroblast product namely type III collagen.¹⁹ On days 5 to 7, the fibroblasts will proliferate actively so that the number of fibroblasts will reach the peak. The increase in the number of fibroblasts in this research is thought to be caused by the presence of flavonoid compounds in Ramania. This is in line with the research conducted by Palumpun et al (2017) regarding the application of betel leaf extract (*Piper betle*) to the number of fibroblast cells in the process of wound healing in male wistar strain rats (*Rattus norvegicus*) which stated that the flavonoids in betel leaves can increase the proliferation of fibroblasts, granulation tissue formation, as well as increasing the epithelialization and myofibroblasts activity.²⁰

Flavonoids also known to have antioxidant capabilities. The antioxidant effect on this flavonoid can help the inflammation phase by releasing free radicals and releasing oxidation by increasing the activity of the enzyme superoxide dismutase and glutathione transferase.⁷ Flavonoids will help protect the body from the excessive amounts of reactive oxygen species so that the wound healing process is not delayed and the synthesis process collagen for wound closure can happen.²¹

The results of the research on the 14th day showed a decrease in the average number of fibroblasts and comparison among groups showed the most optimal number of fibroblasts is in the group of ramania leaf extract gel with a concentration of 15%. Based on research that has been done, it can be seen that the number of fibroblast cells reach the peak on the 7th day and decrease on the 14th day. The decrease in the

number of fibroblasts on the 14th day is in accordance with research conducted by Etika et al (2017) which stated that the number of fibroblasts after the 10th day of injury will decrease because the fibroblast cells will undergo a phenotype change into myofibroblasts and become more progressive in the process of collagen and fibronectin synthesis.⁵

The results of this research showed that the ramania leaf extract gel with a concentration of 15% gave a better effect than the concentration of 5% and 10% of ramania leaf extract gel and placebo gel. This can be seen from the number of fibroblasts that has the highest increase compared to the number of fibroblasts in other groups on the 7th day and a decrease in the number of fibroblasts on the 14th day. The results of this research are supported by the research that was conducted by Dewantari and Sugihartini (2015) which stated that the higher the concentration of the extract in the gel preparation, the more the increase of the wound healing activity.²² The results of this research showed that the ramania leaf extract gel can help the wound healing process as seen from the average number of fibroblasts between groups. Meanwhile, based on statistic results, there was no significant difference in the ramania leaf extract gel group with a concentration of 5% and the concentration of 10%. This is probably due to the range of variations in the gel concentration used which is too short, a higher gel concentration is thought to have a more significant effect on the number of fibroblasts. This is supported by the research conducted by Putra et al (2017) about the effectiveness of banana leaf extract gel (*Musa paradisiaca* L.) for wound healing which stated that the low concentration of extract cause the results of research to have insignificant differences so a higher concentration is needed.²³

Based on this research, it can be concluded that the ramania leaf (*Bouea macrophylla* Griff) extract gel has the ability to increase the number of fibroblasts and has the potential as an adjuvant therapy for wound healing with the most effective concentration is the concentration of 15%.

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