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THE EFFECT OF MAULI BANANA (*Musa acuminata*) STEM EXTRACT GEL APPLICATION WITH 37.5% CONCENTRATION ON FIBROBLAST CELL COUNT

(In Vivo Study On Wound Healing Process of Male Wistar Rat (*Rattus norvegicus*) Buccal Mucosa)

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ABSTRACT

Background*: Wound healing is the body process to improve tissue intergrity caused by wound. Mauli banana stem is a potential plant which can be used as a medicine to accelerate wound healing. Mauli banana stem extract has some contents such as tannin and saponin which has immunomodulatory properties. Previous study states that mauli banana stem extract gel with 37,5% concentration can accelerate the healing of traumatic ulcers.* ***Purpose****: To analyze the effect of mauli banana stem extract gel application with 37,5% concentration on fibroblast cell count in wound healing process of oral mucosa wistar rat on the 7th day.* ***Material and Methods****: This study was a true experimental design with posttest only control group design. It consisted of three treatment groups: a group which given mauli banana (Musa acuminata) stem extract gel with 37,5% concentration, hydroxypropyl methylcellulose gel (negative group) and patent drug gel containing Aloe vera (positive group).* ***Result****: The mean value of fibroblast cell count in wound healing process on the 7th day of the treatment group (28,57), negative group (20,14), and positive group (23). One-way Anova's test had shown a significant difference. Post Hoc LSD test showed a significant difference between mauli banana stem extract gel with 37,5% concentration with hydroxypropyl methylcellulose gel and patent drug gel containing Aloe vera.* ***Conclusion****: The application of mauli banana stem extract gel with 37,5% concentration can increase fibroblast cell count on the 7th day in wound healing process of oral mucosa.*

Keywords*: Fibroblast cell, mauli banana stem extract gel with the concentration of 37,5%, wound healing of oral mucosa*

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INTRODUCTION

Wound healing is a body process to improve tissue intergrity and functional capacity caused by wound.[1] Mauli banana stem is a potential plant used as wound healing medicine.[2] Mauli banana stem extract (EBPM) has some contents such as tannin and saponin which are immunomodulatory agents.[2] On the study from Apriasari et al (2017), it is stated that mauli banana stem extract (EBPM) gel with 37,5% concentration can accelerate the healing of traumatic ulcers.[3] Until now there has been no study that explains the effect of mauli banana stem extract (EBPM) gel with 37,5% concentration on the fibroblast cells count in the oral mucosa incision wound.

One of the patent drug that oftenly used by the community as a wound healing medicine is a patent

drugs gel containing Aloe vera.[4] Aloe vera is commonly used to variety of wound including mucosal wound. [4] On the study from Zahroh et al (2014), it is stated that Aloe vera has some contents such as accemanan and saponin that has antioxidant and immunomodulatory activity that can stimulate macrophage, lymphocytes, interleukins, cytokines and can increase the fibroblast cells count on the 7th day of wound healing process.[5] The disadvantage of patent drugs gel containing Aloe vera is expensive and hard to find outside Java.[2] This is why people are considering to use traditional medicine. One of the traditional medicines that can accelerate wound healing is mauli banana stem.[2]

Mauli banana (*Musa acuminata*) stem is a plant from South Kalimantan that has some contents such as saponin, tannin, ascorbic acid, lycopene, β-carotene, flavonoids, total flavonoids, and alkaloids.[6] Apriasari et al (2014) in her study was stated that highest contents of mauli banana stem is tannin.[6] Tannin has some antimicrobial activities and antioxidants which at low concentrations can inhibit bacterial growth and act as antifungal agents at higher concentrations.[6] The study from Puspitasari et al (2017) was also stated that mauli banana stem extract (EBPM) has been proven to be able to increase the number of macrophages, so that the mauli banana stem extract (EBPM) can be categorized as immunomodulatory plants. Because of its immunomodulatory properties, this drug is recommended as medicinal plant used to accelerate the healing of traumatic ulcers in oral mucosa.[7]

The process of wound healing is divided into 3 phases, inflammation, proliferation, and remodeling.[8] The first phase is the inflammatory phase. This phase occurance up to the 4th day of healing process. In this phase, neutrophils will remove dead tissue, after that it will be passed to macrophages that have higher phagocytic ability that neutrophil.[9] Next, is the proliferation phase which occurs at day 4 until day 14.[10] In this phase, there is granulation tissue formation that fills the wound which consist of fibroblast cells. Fibroblast cells appear on the 4th day and reach it's peak on the 7th day.[10] Fibroblasts play an important role in the wound healing process, as they actively move from the tissue around the wound into the wound area, then proliferate and excrete some substances that play a role in the reconstruction of new tissue.[1,10] The last phase is the remodeling phase that takes place on the 21st day to one or two years, the new skin reinforcement phase with the activity of remodeling collagen and elastin on the skin.[8]

Based on the description above, it is known that mauli banana stem extract with 37.5% concentration can accelerate the healing of traumatic ulcers, but it is not known how the effect of mauli banana stem extract (EBPM) gel with 37.5% concentration to fibroblast cells on oral mucosa incision wound. Therefore, researchers are interested in doing study to determine the effect of mauli banana stem extract (EBPM) gel with 37.5% concentration to fibroblast cells on wistar rats oral mucosa wound on the 7th day.

MATERIALS AND METHODS

This study is true experimental design with posttest only with control group design. The samples used in this study were males wistar rats (*Rattus norvegicus*).

The number of samples obtained by the unpaired numerical comparative formula[11], which is 21 wistar rats. The inclusion criteria in this study were male sex, age of rats 2-3 months, and the condition of healthy or normal rats, characterized by movements of rats such as eating, drinking, no injuries or disabilities. Exclusion criteria in this study were dead wistar rat and rat that looked ill (inactive motion, didn't want to eat, and hair dull / fall).

Preparation of mauli banana stem extract gel with 37,5% concentration

Samples of mauli banana stem were extracted by washing them with flowing water and cut into small pieces, then dried in the oven at 40-60 degrees for 3 days. After being dried, the samples were then weighed to attain 450 gram samples and followed by the extraction process.

The method used is maceration, by soaking the banana stems that have been dried and cut. Banana stems soaked with ethanol solvent 70% to 1 cm above the sample surface. Immersion is done for 3 x 24 hours while occasionally stir, where everyday is filtered. The result will be evaporated by vacuum rotary evaporator with heating temperature of 40-50^{o}C, and then evaporated again with waterbath until obtained thick extract and weighed extract obtained weight 38,15 gram. The next step is ethanol free test with the addition of Potassium dichromate (K2Cr2O7). If there is no discoloration, the extract of mauli banana stem is free of ethanol. The prepared and ethanol-free extracts were made into a 37.5% concentration gel. The process was started by diluting the extract with aquadest until it dissolved for 15 minutes, kept it for 24 hours, and

used for the next day. Hydroxypropyl methylcellulose (HPMC) was mixed with propylene glycol and Tween 80, with rapid stirring. Mauli banana stem extract was added and stirred rapidly, then added with candy oil. Aquadest was added up to 80 grams. Mauli banana stem extract gel concentration of 37.5% had been done with the color of blackish brown and bitter taste.

Treatment on wistar rats

Prior to the study conducted, it has been submitted to the ethics committee (Animal care and Use Comitee) at the Faculty of Dentistry, Lambung Mangkurat University and declared to be eligible based on certificate of ethical eligibility number: 001/KEPKG-FKGULM/VIII/2017. The experimental animals used were grouped by simple random sampling method, then divided into 3 groups, each consisting of 7 wistar rats. Number of rat that resemble the population characteristics were 21 tails, then adapted for 7 days in the cage. The rats were being fed and given drink 3 times a day. Prior to the study, the Wistar Rat was preoccupied for 12 hours. Rat were taken randomly and measured on the right buccal mucosal part of the rat to make the treatment. Sedative action was by using inhaled diethyl ether until the rat found asleep. The incision wound was made with length of 10 mm and depth of 1 mm in the right buccal mucosal part of the rat using scalpel and blade no. 11 sterile, the blood that comes out was cleaned with aquadest and cotton. The treatments given to each group were EBPM 37,5% gel group (treatment group), HPMC gel group (negative group), and Aloe vera gel group (positive group) with a wound of 3x1 days weighing 0.05 grams for 7 days. The tested wistar rat were fed again with the Hi-Pro-Vite feeding container by placing in small containers and giving it every morning, afternoon, and evening. Drinks were given in 300 ml bottles that equipped with small pipes and filled with boiled water.

The Making of Oral Mucosa Preparations

Preparation of the slide was made after the mucosal mouth tissue of the wistar rat incised. The tissue was fixed by submersing the tissue in a container of fixation fluid (formalin buffer) for 24 hours. The tissue was loaded in cassette embedding and then rotated with a tissue proccessor for approximately 14.5 hours with a 10% BNF solvent. The tissue was made to paraffin blocks by filling it with paraffin liquid and attached until cool down. The tissue was cut with a 5µm microtome. The pieces were placed on the object glass. Staining was performed by using Hematoxylin Eosin (HE) and mounted.

Observation and Counting of Fibroblast Cell

Observations was using a light microscope (Olympus, United States) equipped with a digital camera with 400 times magnification. The predetermined region of the right buccal mucosa is calculated by the number of fibroblast cells by means of specified fibroblast cells that are present in all the outer boxes and performed in three field locations. The results of the calculation of the three field of view are summed and obtained the average value which is the result of calculation for one study subject. The data has been tabulated, then analyzed with SPSS application.

RESULTS

The study result obtained average value of fibroblast cell count at day 7 was highest in the group of EBPM 37,5% gel. The least average value of the fibroblast cells count is in the group of HPMC gel. The result average value on fibroblast cells count in all treatment groups can be seen on Figure 1.

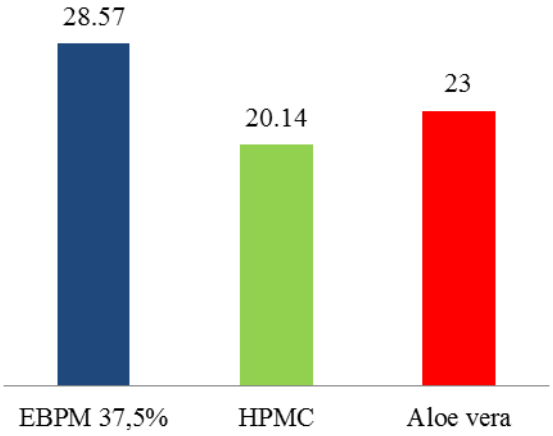

Figure 1. Average Number of Fibroblast Cells On Wounded Wistar Rat Mucosal Wound on Day 7 In Treatment Group of EBPM 37.5% Gel, Negative Group of HPMC Gel, and Positive Group of *Aloe vera* Gel on Diagram.

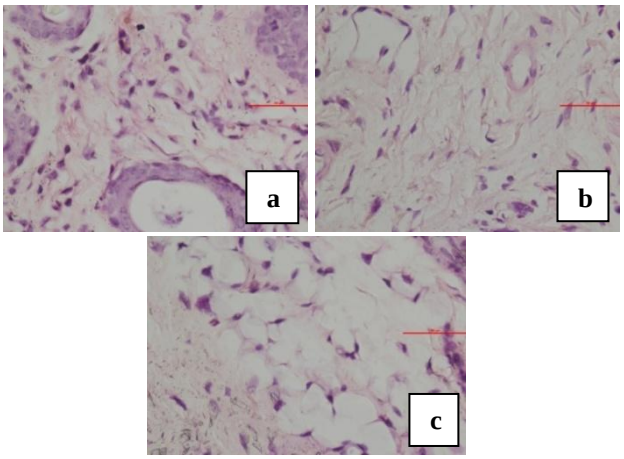

Figure 2. Histopathology of Fibroblast Cells on Mucosal Wound Injuries Healing on 7th Day In EBPM 37,5% Gel Group (a), HPMC Gel Group (b), *Aloe Vera* Gel Group (c) and Observed Using an Olympus Light Microscope At 400 Times Magnification.

Figure 2 shows more fibroblast cells in the EBPM 37.5% gel compared with the HPMC gel group and the *Aloe vera* gel with fibroblast cells which is slightly on the histopathologic picture of buccal mucosal healing on day 7th.

Data analysis in this study use Shapiro-Wilk normality test. The result showed that in group of EBPM 37,5% gel had p value = 0,677, group with HPMC gel had p value 0,876, and group of *Aloe vera* gel had value p = 0.333 (p> 0.05), so it can be concluded that all data is normally distributed. Next, the homogeneity test data using Levene's Test, obtained value of 0.272 (p>0.05) that the data has a homogeneous variant.

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

No.	Group	Sig.	Mean ± SD
1.	EBPM 37,5%	0,000	28,57 ± 2,968
2.	HPMC		20,14 ± 1,988
3.	*Aloe vera*		23 ± 3,108

One-way Anova test results showed significant value of p <0,05, so it is concluded that there are at least 2 treatment groups that have significant difference in the number of fibroblast cells.

Table 2. *Post-Hoc LSD Test*

Group	**EBPM 37,5%**	**HPMC**	***Aloe vera***
EBPM 37,5%	-	0,000*	0,002*
HPMC	0,000*	-	0,114
Aloe vera	0,002*	0,114	-

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

Based on the results of Post Hoc LSD test on the table above, it can be seen that there is a significant difference of the average of fibroblast cells number between the EBPM 37,5% gel group with the HPMC gel group and *Aloe vera* gel group(p <0.05). In that table it was also found that there was no significant difference in mean of fibroblast cells count in the HPMC gel group with the group of Aloe vera gel (p> 0.05).

DISCUSSION

This study aims to prove the effect of EBPM 37,5% gel to wistar rat mucosa incision wound of fibroblast cell count on day 7th. From the obtained study result, there is increase of fibroblast cell count after provision of EBPM 37,5% gel on wistar oral mucosa wound healing. This occurs because the EBPM 37,5% gel has immunomodulatory properties that will stimulate the formation of fibroblast cells.

Immunomodulator is a drug that can restore the immune system imbalance which is needed in the process of wound healing. The presence of chemical compounds that can increase the activity of the immune system is very helpful to overcome the immune system and those compounds can be obtained from plant including on mauli banana stem.[12]

On the study from Apriasari et al (2016), it is stated that EBPM 37,5% gel have high tannin contain equal to 67,59%.[3] On the study from Apriasari et al (2016) also stated besides tannin, EBPM 37,5% gel have important contents for wound healing process which are saponins, ascorbic acid, lycopene, β-carotene, flavonoids, total flavonoids, and alkaloids.[3]

Tannin in EBPM has the potential to increase angiogenesis, fibroblasts formation, collagen deposition, and improve wound contraction.[3] Tannin is an antioxidant that also induces Transformation Growth Factor-β for fibroblast proliferation. Transformation Growth Factor -β is one growth factor that will boost cell propagation or

proliferation which will be the framework for reepithelization and fibroblast proliferation.[13]

Saponins can trigger the Vascular Endothelial Growth Factor and increase the number of migrating macrophages into the wound area thus increasing the production of cytokines that will activate fibroblasts in the wound tissue.[14] Saponins also act as agents for angiogenesis by regulating Vascular Endothelial Growth Factors that increase endothelial cell mitogenic activity in vascular formation blood during the proliferative phase.[14] The function of Vascular Endothelial Growth Factor includes increased angiogenesis in wound healing.[15]

This study is based on the study from Budi et al (2016) which states that banana stem extract can accelerate wound healing that will trigger the increasing number of fibroblast cells.[16] The number of fibroblast cells can be considered as wound healing parameters. The fibroblast cells will produce collagen that will link the wound, affect the process of reepithelization, migrate and proliferate to form new connective tissue and synthesize collagen affecting the strength at the wound healing site. Proliferation of fibroblasts determines the final outcome of wound healing.[16,17]

On the study from Puspitasari et al (2017), it is stated that Aloe vera is equivalent to EBPM 25%, because it has the same contain of tannins, saponins, and flavonoids and has a same time to accelerate the wound healing.[7] Meanwhile, HPMC gel has fewer fibroblast cells because HPMC gel is a gelling agent that usually used as the basis of topical preparations and has no active ingredients, causing many microorganisms to be phagocytosed and longer wound healing.[18] It can be concluded that the application of EBPM 37,5% gel is better than *Aloe vera* gel and HPMC gel.

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