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The effect of *musa acuminata* stem in increasing macrophage and neovascular cells of healing process

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ABSTRACT

Introduction: *Musa acuminata* stem has both of immunostimulatory and immunosuppressant effects that are influenced by their concentrations. The study aimed to analyze the effect of Mauli banana stem extract (EMBS) to increase the number of macrophage and neovascular cells in traumatic ulcer healing.

Methods: *Rattus norvegicus* of male Wistar strain were used as model of traumatic ulcer. The left buccal mucous was biopsied, then used the histopathology method to find the number of macrophage and neovascular cells. **Results:** EMBS gel was effected when administered at a concentration of 37.5% because it significantly increased the number of macrophage and neovascular cells in traumatic ulcer healing on the 3rd day to 5th days. **Conclusion:** It can be concluded that EMBS within concentration of 37.5% can accelerate ulcer healing through the increasing number of macrophage and neovascular cells.

Keywords: Condensed tannin, macrophage, *Musa acuminata*, neovascular, Terpenoid saponin

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INTRODUCTION

Ulceration of the oral cavity is one of the most common disorders in the oral cavity. This disorder is characterized by the existence of lesions such as ulcers which lose their epithelial layers exceeding the basal membranes.¹⁻³ Ulceration in the oral cavity becomes the main reason for patients to consult a dentist. The ulceration may be recurrent, rare occurred, or being permanent ulcerations.⁴

Ulceration in the oral cavity will interfere with the process of mastication, resulting in the disorders of nutrient intake. The therapy of ulceration in the oral cavity is essentially symptomatic to reduce inflammation, pain, and accelerate wound healing.⁵

Previous research indicates that gel extract of mauli banana stem (EMBS) can accelerate and increase epithelium thickness of the buccal mucosal wound healing in mice.⁶⁻⁸ EMBS has bioactive materials of mauli banana consisted of 67.59%

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of tannin, 14.49% of saponin, 0.34% of alkaloids, 0.44% of ascorbic acid, 0.25% of flavonoids, and 0.006% of lycopene.⁶ Condensed tannins can induce tyrosine phosphorylation of insulin receptor on the cell surface of 3T3-L1 adipocytes *in vitro*, so it can activate phosphatidylinositol 3-kinase pathway (PI3K), which plays a role in cell proliferation.^{9,10} Terpenoid saponins will be captured by the G protein receptor on macrophages, then through a process that produces a protein kinase C, it activates NFκB, thereby increasing the activity and number of macrophages.¹¹

Condensed tannins and terpenoid saponins are immunomodulators that can increase the activity and the number of macrophages.⁹⁻¹¹ Previous research has also proven that the gel extract of mauli banana stem can increase the amount of HIF-1α, Hsp90α, and VEGF.⁸ Based on these facts, the study aims to analyze the effects of the gel extract of mauli banana stem to increase the number of macrophages and neovasculars on mucosal healing in traumatic ulcers.

METHODS

This experiment was a true experimental research. The experimental units were 40 rats of *Rattus norvegicus* of male Wistar strain. There were the traumatic ulcer models. The biopsy was taken from the ulcer in the left buccal mucous. The criteria of rats were healthy, weighed 250-300 grams, and aged 2-3 months. Ethical approval was obtained at the Faculty of Dentistry, Airlangga University No 56/ KKEPK.FKG/ VI/ 2015.

Mauli banana plant was taken after producing banana fruit. The maceration method was used, followed the ethanol-free examination. The gel compositions of ethanol extract of Mauli banana stems were consisted of 3 levels i.e of 25%, 37.5%, and 50%. The extracts were mixed with 15% HPMC, 1% Tween 80, 8% Propylene glycol, 5 drops of peppermint oil and distilled water until reaching concentration of 100%. Materials to make the preparation and staining of histopathology were 10% BNF, alcohol, xylol, paraffin, and Haematoxylin Eosin (HE) staining.

The experiment used 40 rats, divided into 4 groups of treatment i.e, the rats administrated gel only as a negative control group (K), rats administrated with 25% of EMBS (P1), rats admin-

istrated with 37,5% of EMBS (P2), and rats administrated with 50% of EMBS (P3). Each group was consisted 10 rats. The EMBS was applied topically every 6-8 hours per day.

The treatment of experimental rats was started by giving inhaled ether anesthesia, then the left buccal mucous was injured with biopsy punch with a diameter of 6 mm and a depth of 1 mm, and then it was taken with a scalpel. The left buccal mucous of the rats was biopsied on the 3rd and 5th day after being decapitated. The tissue was soaked in 10% buffered formalin (pH 7.4), and then it was made into preparations. The next phase were done histopathology tests by using Haematoxylin Eosin (HE) staining technique to see the number of macrophage and neovascular cells. The number of macrophage and neovascular cells were calculated in three different field locations using a light microscope (Olympus, United States) under 400x magnification and subsequently averaged.

RESULTS

The results of statistical analysis using ANOVA and Post Hoc LSD examinations indicated that there was a significant difference ($p < 0.05$) in the number of macrophages based on the time of EMBS gel administration in all groups, except in the group of rats administration with EMBS gel at a concentration of 0% (negative control) on the 3rd day compared to the 5th day ($p=0,12$). In the treatment groups of EMBS gel of 25% ($p = 0.006$), EMBS gel of 37.5% ($p = 0.003$), and EMBS gel 50% concentration ($p = 0.002$). In this study, there were increased numbers of macrophages on the 3rd day compared to the 5th day in all groups of EMBS gel. The highest increasing number of macrophages occurred in the EMBS gel of 37.5% concentration, i.e. from 10 to 13 cells (Table 1).

Based on time variable of EMBS gel application, on the 3rd day there was a significant difference between the number of macrophages of EMBS gel of 0% concentration (negative control) and all treatments, except for the EMBS gel of 25% concentration. On the 3rd day, there was a significant difference between the number of macrophages of EMBS gel of 25% concentration and concentrations of 37.5% and 50%. On the 3rd day there was a significant difference between

Table 1. The number of macrophage cells in traumatic ulcer healing on 3rd until 5th days

Group	Time		p
	3 days	5 days	
0% EMBS	5.0±1.22 (3-6)	6.4±1.14 (5-8)	0.12
25% EMBS	5.8±0.84 (5-7)	8.4±0.89 (7-9)	0.006*
37.5% EMBS	9.6±1.52 (8-11)	12.4±1.14 (11-14)	0.003*
50% EMBS	9.0±1.58 (7-11)	12.0±2.24 (9-15)	0.002*
p	0.000*	0.000*	0.000*

Notes: * significant with $\alpha=0,05$

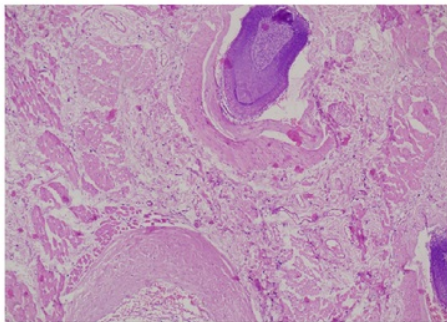


Figure 1. The results of histopathology test showed the indicate of macrophage and neovascular cells on the 3th days

the number of macrophages of EMBS gel of 50% concentration and the EMBS gel of 37.5% concentration (Fig.1).

Based on time variable of EMBS gel application, on the 5th day, there was a significant difference on the number of macrophages between EMBS gel of 0% concentration (Negative control) and EMBS gel of 25%, 37.5% and 50% concentrations. On the 5th day, there was a significant difference on the number of macrophages between EMBS gel of 25% and EMBS gel of 0% concentration (Negative control), also with EMBS gel of 37.5% and 50% concentrations. On the 5th day, there was a significant difference on the number of macrophages between EMBS gel of 37.5 % concentration and all treatments, except with EMBS gel of 50 % concentration. On the 5th day, there was a significant difference on the number of macrophages between EMBS gel of 50 % and all treatments except with EMBS gel of 37.5% concentration (Fig.2).

The results of statistical analysis using ANOVA and Post Hoc LSD examinations indicated that there was a significant difference ($p < 0.05$) on the number of neovasculars based on time variable

Table 2. The number of neovascular cells in traumatic ulcer healing on 3rd until 5th days

Group	Time		p
	3 days	5 days	
0% EMBS	4.60±1.52(3-7)	6.60±2.97(3-11)	0.216
25% EMBS	7.00±1.87(5-9)	11.25±0.96(10-12)	0.005*
37.5% EMBS	10.60±2.07(9-14)	17.60±5.03(12-25)	0.021*
50% EMBS	6.20±1.30(5-8)	9.20±2.28(7-13)	0.034*
p	0.000*	0.001*	0.000*

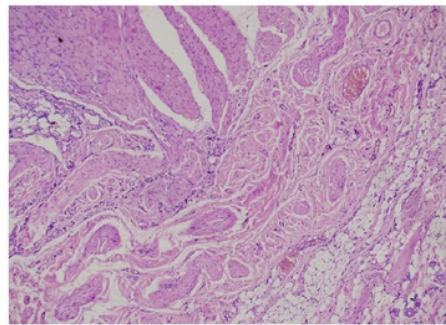


Figure 2. The results of histopathology test showed the indicate of macrophage and neovascular cells on the 5th days

of EBPM gel application in all groups, except in the group of EMBS gel of 0% concentration (negative control) on the 3rd day compared to the 5th day. In the treatment group of EMBS gel of 25% concentration ($p = 0.005$), 37.5% concentration ($p = 0.021$), and 50% concentration ($p = 0.034$). In this study, there was an increasing number of neovasculars on the 3rd day compared to the 5th day in all groups of EMBS gel. The most significant increasing number of neovasculars occurred in the EMBS gel of 37.5% concentration which was from 12 to 19 cells (Table 2).

Based on time variable of EMBS gel application, on the 3rd day there was a significant difference between the number of neovasculars of EMBS gel of 0% concentration (negative control) and all treatments, except for the EMBS gel of 25% and 50% concentrations. On the 3rd day, there was a significant difference between the number of neovasculars of EMBS gel of 25% and 37.5% concentrations. On the 3rd day, there was a significant difference between the number of neovasculars of EMBS gel of 50% concentration and of 37.5% concentration (Fig.1).

Based on time variable of EMBS gel application, on the 5th day there was a significant difference between the number of neovasculars of EMBS gel of 0% concentration (negative control) and EMBS gel of 25% and 37.5% concentrations. On the 5th day there was a significant difference between the number of neovasculars of EMBS gel of 25% concentration and of 0% concentration (negative control) and of 37.5% concentration. On the 5th day, there was a significant difference between the number of neovasculars of EMBS gel of 37.5% concentration and all treatments. On the 5th day, there was a significant difference between the number of neovasculars of EMBS gel of 50% and 37.5% concentrations (Fig 2).

DISCUSSION

Condensed tannins may bind in the α -subunit of the insulin receptor in the cell membrane in vitro and may make kinase tyrosine residues in β -subunit undergo autophosphorylation. The autophosphorylation from tyrosine kinase receptors can bind with PI3K and activate it. Active PI3K can catalyze the PIP3 phosphorylation, then the PIP3 recruit two kinase proteins which are AKT and PDK1 heading to the plasma membrane and activating AKT. The AKT activation causes Rheb become active, it then triggers mTOR.⁹ The mTOR will activate p 70 S6K and phosphorylation of 70 and 85 kDa isoform S6K.¹³ The S6K then phosphorylates transcription factors of in the nucleus, which then inhibits CDK inhibitor. As a result, there is a CDK activation and it stimulates cell proliferation of macrophages.¹⁴

Terpenoid saponins can increase the number and activity of macrophages, which also *Centella asiatica*'s mechanism of immunomodulatory effect through the G receptor protein. *Centella asiatica* has terpenoid saponins as dominant bioactive. This causes the G protein with GDP in the cell membrane become closer to the ligand. The GDP will be replaced by GTP so that the G protein becomes active. Next, the binding between protein G and GTP heads to *adenyl cyclase*, then it activates *adenyl cyclase C phospholipase* to be *phosphoinositol* and results in *triphosphat inositol* (IP3) and *diacylglycerol* (DAG). PIP3 will follow the pathway just as the effect of tannins administration, while DAG will produce a protein

kinase C activates I κ B kinase (IKK). Phosphorylase of IKK inhibits I κ B, where I κ B will be complex with NF- κ B in the cytoplasm. This phosphorylation marks the degradation of I κ B by the *proteasome*, which then releases NF- κ B from complex inhibition. NF- κ B protein is then freed and it then is transported into the nucleus, will next activate gene transcription that activates NF κ B.^{11,15,16}

In previous studies, *Astragalus* plants containing terpenoid saponins can increase NF κ B expression along with mRNA from IL-1 β dan TNF- α cytokines. Terpenoid saponins causes effects of immunostimulator.¹⁷⁻¹⁹ In the next process, it will trigger increasing number and activity of macrophages just as the ones occur during EMBS gel administration. This fact indicates that EMBS gel also possesses immunostimulator characteristic, because it is able to increase the number of macrophages in this study.

Macrophages play an important role in wound healing, since they produce growth factors, trigger angiogenesis and fibrogenesis. The released macrophages will phagocyte bacteria and clean up tissue debris. During the transition from the inflammatory process to the repair of wound process, the macrophages will stimulate cell migration, proliferation, and matrix network formation. The growth factors, among others, for angiogenesis include TGF β , VEGF and FGF-2.²⁰⁻²²

In a condition lack of macrophages, what happens is the inhibition of wound healing.²³ It needs a balance number of neutrophils and macrophages in wound healing. On the other hand, in a chronic inflammation condition which inhibits wound healing, there is increasing number of neutrophils which trigger macrophage excess. This situation causes severe wound damage caused by too long hypoxia.^{8,24}

Macrophages can control signals in angiogenesis process during the formation of granulation tissue which occur from the 4th until the 10th day after a wound was formed.^{21,25} Neovasculars during the process of formation of granulation tissue are controlled by many cytokines and growth factors. Normally, macrophages during wound healing process are cells which prepare angiogenesis process while stopping it.^{21,25,26} This phenomenon also occurs in the administration of *Terminalia chebula* extract which can accelerate wound healing through synergy effect of metanin

and other bioactives. The other studies indicate that the synergizing effect occurs when tannins and saponins are simultaneously given.^{27,28}

In EMBS gel of 37.5% concentration, there is a strengthening effect of bioactives of condensed tannins and terpenoid saponins, resulting in increasing number of macrophage and neovascular cells. Mauli banana stem as an immunostimulator can increase the number of macrophage cells. Macrophages play an important role in wound healing which produce growth factors and trigger angiogenesis, so it can be increasing the neovascular cells. The angiogenesis process which is a critical part in the process of wound healing. Mauli banana stem as an antioxidant requires nutrients that support cell metabolism, as well as tannins and saponins to form the granulation tissue consisting of blood vessels that need oxygen for the improvement and growth of blood vessels.

Mauli banana is the antioxidant and immunostimulator agent, so it called an immunomodulator topical drug. This research proved EMBS gel of 37.5% concentration can be recommended as the topical drug to accelerate wound healing including traumatic ulcer. It can be concluded EMBS gel within concentration of 37.5% can accelerate ulcer healing through the increasing number of macrophage and neovascular cells.

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