The effect of 25% Mauli banana stem extract gel to increase the epithel thickness of wound healing process in oral mucosa

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ABSTRACT

Background: Mauli banana is a special plant of South Borneo that can be used as alternatif medicine for wound healing wound healing. Recent studies showed that Mauli banana stem contained some compound such as flavonoid, saponin, and tannin that had antibacterial and antiinflamation effect, and can accelerate the wound healing. Purpose: This study was aimed to know the effect of 25% Mauli banana extract gel to the epithel thickness of wound healing process in oral mucosa. Method: It was the real experimental with post test only control group design. It used 36 Sprague dawley rats that divided into 3 groups: the negative control group by giving aquadest, the positive control group by giving drug contain Aloe vera, and the treatment group by giving 25% ethanol extract of Mauli banana stem. Biopsy was done on day 3, 5, 7 and the preparat was made to measure the thickness of oral mucosa epithel by Image J software. Result: The result showed that 25% ethanol extract of Mauli banana stem can increased the thickness of oral mucosa epithel on third day (51.26 µm), fifth days (108.49 µm), and seventh day (170.66 µm). The top thickness of mucosa epithel was on the seventh day. Two-ways Anova and Post Hoc LSD (p<0.05) showed the significant different between aquadest and 25% ethanol extract of Mauli banana stem. 25% ethanol extract of Mauli banana stem and drug contains Aloe vera are the aqual of meaningfull. Conclusion: 25% ethanol extract of Mauli banana can increase the epithelial thickness of wound healing proces in oral mucosa.

Keywords: epithel; Mauli banana stem extract; oral mucosa; wound healing

INTRODUCTION

Over the last few decades the role of medicinal plants as a main ingredient in health preservation and management of disease receive a great attention. The treatment by using medicinal plants is increasingly favored because it has generally less side effects than the chemical drugs. One of the traditional materials that is used for the treatment is banana. Banana have a lot of varieties, including Mauli banana which is a typical banana of south Borneo. The stem of Mauli banana is empirically known having an efficacy as a medicinal plant which can accelerates the wound healing process. The people of Hulu Sungai Utara, South Kalimantan often use Mauli banana stems to accelerate the skin wound healing. Mauli banana as a medicinal plant which is used as an alternative medicine for healing wounds are containing some type of phytochemical compounds, such as saponin, tannin, flavonoid, ascorbic acid, lycopene and β - carotene. The function of tannin as an antibacterial can reduce inflammation and increase the epithelium forming and cause the vasoconstriction effect on blood vessels. It is containing flavonoid as antioxidant, antibacterial, anti-inflammatory and analgesic. The flavonoid itself can increase the process of inflammation and inducing the epithel formation. The study of Apriasari et al., showed that the ethanol of 25% Mauli banana stem extract is bacteriosid and fungisid. The extract of 100% Mauli banana stems can accelerate the back wound healing process of mice by increasing of macrophages. The phytochemical content of flavonoids and tannin in the banana stems has immunostimulatory effects.
to enhance the activity and the number of macrophages. The functions of activated macrophages are secreting the cytokin producing the growth factor. The growth factors play a role in the formation of new cells that are important for wound healing and epithelium formation. Base on Apriasari et al., Mauli banana stem extract could increase the number of microphage in wound healing on day 3. Another study showed that application of Mauli banana stem extract can heal the wounds without leaving a scar tissue and the healing speed took place as similar as the application of Aloe vera.

The processes of wound healing include the inflammatory phase, proliferation, and remodelling. In the inflammatory phase there will be a process of phagocytosis and inflammatory reactions that take place within a few minutes. The proliferative phase begins approximately 4 days after the injury and finishes up on 3-4 weeks or more. This phase is characterized by reepithelization, fibroblast proliferation, and angiogenesis. The reepithelization stage of wound healing is very important because it serves to restore the integrity of the oral mucosa. The faster the reepithelization the sooner the wound is closed so the wound healing is also becoming fast. The previous study suggests that the process of epithel formation is reaching the peak on day 7. The next phase is the remodeling which is the final phase in the process of wound healing.

Based on the background, the Mauli banana stem has several contents of active compound; tannin and flavonoids which are very important in increasing the wound healing process. Information of the use Mauli banana stem extract as an ingredient to accelerate the wound healing process is fewer compared to Aloe vera that already widely known by the society. This study was aimed to determine the effect of 25% Mauli banana extract gel to increase the epithel thickness of wound healing process in oral mucosa.

MATERIALS AND METHODS

The experimental animals in this study were 2-3 month male white rats (Rattus norvegicus) Sprague Dawley, 200-250 g body weight, with active movement and in a good condition. This research was purely experimental with post test design and control group. The sampling technique was done at randomly sampling, as many as 36 rats were divided into 3 groups: group 1 (negative control) was given distilled water, group 2 (positive control) given a medicine which is containing Aloe vera, and group 3 (treatment) was given ethanol of 25% Mauli banana extract gel. The observation of this research was done on the day 3, 5 and 7.

The fetching of Mauli banana stem was conducted in the Pertanian Pembangunan Negeri School, Banjarbaru. The making of Mauli banana stem extract was done at the Faculty of Mathematics, Universitas Lambung Mangkurat by maceration method with ethanol, followed by the ethanol-free test. The next step was the making of carbopol gel in the water, and followed by adding the propylene glycol. The next phase was adding hydroxypropyl cellulose medium (HPMC) and Mauli banana stem extract until reached a concentration of 25% in a gel form.

The research procedure on day 1 for all groups was done by inhalation anesthetic with diethyl ether, and then conducted by making incision wound on the left buccal mucosa 10 mm throughout and 1mm in depth. The blood that comes out during the manufacture of wound was sterilized cleaned and dried. In that area, performed water application for negative control group, while, the medicine with Aloe vera was given for positive control group, and 25% Mauli banana extract gel for the treatment group. Each treatment was carried for two times a day at 8.00 and 16.00 WITA. A decapitation for all groups was done on day 3, 5, and 7, followed by biopsy of the wound. The next stage was making blood smear with staining of hematoxylin eosin (HE).

The blood smear was observed using Olympus light microscope with a magnification of 100x a single field of view. The epithel thickness was measured by using the Software Image J. The epithel area in the visual field was divided into 10 areas and separated by the measuring line. The measurement of epithel thickness was performed on each measuring line then summed and calculated the average value that was a measurement for a single subject research.

The obtained data were tested for its normality by the Shapiro-Wilk and Levene’s test of homogeneity. If the data were normally distributed and homogeneous (p>0.05), it would be analyzed by two-way Anova with a confidence level of 95%, followed by LSD Post Hoc test.

RESULTS

In Table 1, it showed that in the negative control group (distilled water), positive control group (Aloe vera) and 25% Mauli banana extract gel group there was an enhancement of epithel thickness on day 3rd, 5th, and 7th. For each group, the lowest epithel thickness was on the 3rd day and the highest epithel thickness was on the 7th day. The increasing of epithel thickness in the negative control group was lower than the positive control group and the 25% Mauli banana extract gel (Figure 1).

The results of the analysis with two-way Anova based on the time variables of observation obtained a significant value 0.000 (p<0.05), which means that there were significant differences for each group.

Table 1. The average of reepithelizations thickness for each treatment group

<table>
<thead>
<tr>
<th>Day</th>
<th>The average of thickness reepithelizations (µm) ± SD</th>
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<tbody>
<tr>
<td></td>
<td>Control negative</td>
</tr>
<tr>
<td>3</td>
<td>33.70 ± 6.58</td>
</tr>
<tr>
<td>5</td>
<td>74.00 ± 6.98</td>
</tr>
<tr>
<td>7</td>
<td>115.55 ± 9.22</td>
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phytochemical compounds such as flavonoids and tannins in the extract gel of Ambon banana stem can accelerate the wound healing process with several activities. Those activities include affecting the inflammatory cells, increasing the reepithelization, the process of angiogenesis, and the formation of connective tissue in the skin so, it can be used as an alternative wound closures. When the injury occurred, there would be continuity losing or damaging the epithel tissue. The broken epithel tissue is going to have a reepithelization process which is a process of epithel cell reparation so, the wound will be closed. Reepithelization is a stage where wound will be healed; it includes migration, mitosis, and differentiation of epithelial cells. These stages will restore the lost mucosal integrity. Reepithelization will occur through the movement of epithel cells from the network edge to the network breaks free. The faster the reepithelization, the sooner the structure of the oral mucosa epithelium reaches normal circumstances.6,12

A few hours after injury that was leading to tissue destruction, the epithel cells of the wound edges slowly began to migrate to the injured area. The wound edge of epithelium composed of basal cells detached from its base and became loose knot and then enlarged and moved to fill the wound surface. The epithelium began to closely migrate to the edge of the wound within 24 hours after injury. It was caused by an inflammatory phase, a process of phagocytosis and cleaning the debris, so the reepithelization process is less.6,11

On the 3rd day, the epithel thickness was continuing increase. In Table 1 there was a difference between the increasing of epithelium gel thickness of the Mauli banana stem extract with negative control. The thickness of the epithelium in the group of 25% Mauli banana extract gel was higher than the negative control. This was caused by the wound healing has already entered the stage of proliferation on the 3rd day. The phagocytosis process of strange particles by inflammatory cell in 25% ethanol extract of Mauli banana stem was more rapidly, thus it made the proliferation of epithel cell was getting faster anyway. The epithel cells near the wound area were rapidly divided and migrated as intersect movement one another. The migrated epithel cells were getting change of its shape into a more columnar and increasing its mitotic activity.15,16

On the 5th day the thickness of the epithelium was increased. The inflammatory phase had begun to decline and epithel layer was thickened. The increment of epithel thickness was influenced by several growth factors that helped in increasing and stimulating the migration and epithel cells mitosis. Those factors that affect reepithelization are fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor-α (TGF-α), and epidermal growth factor (EGF).15

On the 7th day, the epithel thickness was in the highest position, at that time the inflammatory phase started to stop and a lot of fibroblast were migrating to the wound area and the collagen and fibroblasts were being attached to the edges of the wound, so that the epithelium can be

**DISCUSSION**

The phytochemical content which is owned by Mauli banana stem extract and patent medicines with Aloe vera can accelerate the wound healing process. The Mauli banana stem gel extract is containing some type of phytochemical compounds, such as antibacterial such as tannins which reduces inflammation and increase the process of reepithelization and cause the vasoconstriction effect on blood vessels. The content of flavonoids as anti-inflammatory helps to accelerate the inflammatory process and may induce reepithelization so the wound healing is getting faster.12

It is supported by a previous study conducted by Prasetyo6 which stated that some of the content of

**Figure 1.** The thickness of epithelial mucous on seventh days by 100 x magnification. (a) negative control; (b) positive control; (c) the extract of Mauli banana stem.
thickened, especially on day 7-14. The epithel thickness had appeared on day 1 and then increased on day-3 and more increased on day 7, since the formation of epithel was started from the wound forming until the wound healing process has stopped and the wound was closed. The increasing of epithel thickness might indicate the healing process can take place more quickly and the inflammatory processes became more shortly.

The measurement results of epithel thickness in Figure 1 showed the positive control group and the group 25% ethanol extract of Mauli banana stem had the same thickness. The epithel thickness of the negative control group was thinner than group of 25% ethanol extract of Mauli banana stem. This was due to the containing flavonoid and tannin in the 25% Mauli banana extract. Tannin as antibacterials can minimize the microorganism’s infections and suppressed the infections which can inhibit the reepithelization process. The containing of phytochemical flavonoid accelerated wound contraction and reepithelization. The flavonoid had immunostimulatory effects to increase the phagocytosis activation by macrophages and increase the inflammation process. The increment of macrophage affected the growth factor production which contributed the process of proliferation thus help to accelerate the reepithelization process. This result is similar with the process of wound healing in mice using gel extract of Ambon banana stems. Reepithelization in Mauli banana stems group was higher than the negative controls group until the 7th day, however there was no difference between control group and gel extract of Mauli banana stems group. It was the same as effect of Ambon banana stems extract.

Compared to the positive control group, the group of 25% Mauli banana extract gel had the same epithelium thickness. This was due to a patent medicine with Aloe vera that also stimulated the wound healing by stimulating the anti-inflammatory and increasing the inflammatory cells activity. It was helping the reepithelization phase. The epithel thickness on negative control group was thinner than 25% ethanol extract of Mauli banana stem and a positive control, since the aquades is sterile water and does not have the active ingredient. The absence of active ingredients was causing many microorganisms and damaged cells that should be cleaned up, so the healing process goes slowly. After the epithel got the maximum thickness, the mitotic activity of epithel cells would decrease and the already formed tissue began to enter maturation phase.

Based on the results, it can be concluded that 25% Mauli banana extract gel can increase the epithel thickness in the wound healing process in oral mucosa.

REFERENCES