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PMN Leukocytes and Fibroblasts Numbers on Wound Burn Healing on the Skin of White Rat after Administration of Ambonese Plantain Banana

Aryenti[1*)], Suryadi[2], Harijadi[3], Juniarti[4], and Yuhernita[4]

1. Department of Anatomy, Faculty of Medicine, Universitas YARSI, Jakarta 10510, Indonesia
2. Department of Anatomy, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
3. Department of Pathology Anatomy, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
4. Department of Biochemistry, Faculty of Medicine, Universitas YARSI, Jakarta 10510, Indonesia

*)*E-mail: aryenti@ymail.com*

Abstract

A study of ambonese plantain banana (*Musa paradisiaca* var *sapientum* Lamb) treatment in burn wound healing on the skin of white rats (*Rattus novergicus*) has been conducted. The wound healing of burn injuries was evaluated by counting the number of PMN leukocytes and fibroblasts at the 7th, 14th, and 21st days following the treatment. The study showed that the decrease in number of PMN leukocytes of subjects treated with ambonese plantain banana was relatively more significant compared to both negative and positive control (Bioplacenton ®). In contrast, an increasing number of fibroblasts was significantly demonstrated at the 14th and 21st days after treatment. In conclusion, ambonese plantain banana treatment in burn injuries will provide better results compared to both positive and negative controls.

Abstrak

Penilaian Jumlah Leukosit PMN dan Jumlah Fibroblast terhadap Penyembuhan Luka Bakar pada Kulit Tikus Putih setelah Pemberian Getah Batang Pisang Ambon. Telah dilakukan uji pemanfaatan getah pisang ambon (*Musa paradisiaca* var *sapientum* Lamb) dalam penyembuhan luka bakar pada kulit tikus putih (*Rattus novergicus*). Penyembuhan luka bakar dievaluasi dengan menghitung jumlah leukosit PMN dan jumlah fibroblas pada hari ke 7, 14, dan 21 setelah perlakuan. Hasil penelitian menunjukkan bahwa penurunan jumlah leukosit PMN pada subjek yang diobati dengan getah pisang ambon relatif lebih signifikan dibandingkan dengan kontrol negatif dan positif (Bioplacenton ®). Sebaliknya, peningkatan jumlah fibroblas secara signifikan ditunjukkan pada hari ke-14 dan ke-21 setelah perawatan. Kesimpulannya, pengobatan dengan getah pisang Ambon pada luka bakar memberikan hasil yang lebih baik dibandingkan dengan kedua kontrol positif dan negatif.

Keywords: burn wound healing, fibroblasts, Musa paradisiaca var *sapientum* Lamb, *PMN leukocytes*

1. Introduction

Burn injuries resulting from the high incidence of trauma have become a serious problem for society. The wound healing is divided into three phases, i.e. inflammatory phase, proliferative phase and maturation phase. The inflammatory phase will be followed by proliferative phase, which is characterized by epithelization and angiogenesis to repair damaged blood vessels and form new ones. It also occurs concurrently with fibroblast proliferation. Subsequently, the synthesis of collagen by fibroblast occurs to form tissue structure [1-3]. The study on natural remedies for treatment of burn injuries has currently become an interesting subject for the investigators. The ambonese plantain banana *(Musa paradisiaca* var *sapientum* Lamb*)* is one of major sources and least expensive among them. Several studies have been conducted to investigate the active ingredient and the efficacy of ambonese plantain banana including the saponin, lectins, tannin, ascorbic acid, quinone and anthraquinone [4]. Additionally, it also contains glycosides, anthocyanins, flavonoid and carbohydrates [5].

Some studies reported that *M. paradisiaca* var *sapientum* Lamb has demonstrated to have antioxidant, antidiarrhea, antimicrobial, anti-sensitization, hypoglycemic and anti-allergic properties [6-8]. Moreover, it has also been reported to have anti-cancer activity on colorectal cancer and may act as a chemopreventive agent against cancer [9-10].

The saponin in *M. paradisiaca* var *sapientum* Lamb has been reported to have antibacterial, antibiotic, and antimicrobial effects to cause decreased numbers of inflammatory cells, such PMN leucocytes, and inhibit other pathogens including fungi, bacteria and viruses [11], while tannin has been reported to have anti-inflammatory effect [12]. The ambonese plantain banana has a better anti-inflammatory effect than chloramphenicol oinment. Moreover, the plantain has an anti-haemorrhage, antidiarrhea, and may become alternative treatment for ulcer and gout [2,11-13].

The study evaluating the effect of ambonese plantain banana on lacerated wound showed that it may have some benefits including rapid cessation of primary bleeding, faster wound healing, shorter period of inflammatory process, and histologically, it increases the production of collagen fibers and the number of fibroblasts [11,14-15].

There has been little study reporting about the benefit of the plantain banana for treatment of burn injuries. Therefore, the present study was aimed to investigate the efficacy of *M. paradisiaca* var *sapientum* Lamb on burn wound healing. The study was conducted to count PMN leucocytes and fibroblast numbers, as an indicator of burn wound healing process following the treatment of ambonese plantain banana.

2. Methods

In this study, the plantain of *M. paradisiaca* var *sapientum* Lamb was collected from Sleman, Yogyakarta. The study was an experimental study, with post-test only control group design. The study subjects included 45 white male Wistar rats aged 2 months with 300-400 gram body weight range. Subjects were obtained from the central animal house of University of Gadjah Mada, Yogyakarta. The subjects were divided into 3 groups: Group I (the negative control) was a group of rats underwent burn injuries that received no treatment; group II (the positive control) was a group of rats with topical treatment of 1% bioplancenton gel on the back skin of the rats' burn injuries; while group III (treatment group) was a group of rats treated by 20 microliters of the plantain from the stalk edge of *M. paradisiaca* var *sapientum* Lamb. Each group consists of 5 rats according to decapitation 7^{th}, 14^{th}, and 21^{st} days following treatment.

The wound model for burn injuries of forty five rats were made by using cauter at 50 ^{o}C temperature for 10 second on the back skin of the rats through 0.5 cm incisions on the either side of the vertebral column and 2.5 cm distance from both sides of ear side. Histological preservation was stained with hematoxyline-eosin (HE) and was continuously examined by counting the number of PMN leukocytes and fibroblasts.

3. Results and Discussion

The number of PMN leukocyte cell. The average number of PMN leukocytes of the group treated with the plantain of *M. paradisiaca* var *sapientum* Lamb and the control group is shown in Table 1. There the lower number of PMN leukocyte in the treatment group compared to the negative control since the 7^{th} day. On the 14^{th} and 21^{st} days, the average number of PMN leukocytes has decreased in all groups. The lowest number of leukocytes was found in the group treated with the plantain banana of *M. paradisiaca* var *sapientum* Lamb, followed by the group of positive and negative controls.

The average number of PMN leukocytes from the treatment and controls groups at each period of decapitation are shown in Figure 1. It demonstrates the effect of treatment on decreased number of PMN leukocytes, i.e. the longer the period of treatment, the more decreasing number of PMN leukocytes tend to be.

The lowest degradation number of PMN leukocytes was found in the positive control group (treated with bioplacenton), followed by the group treated with *M. paradisiaca* var *sapientum* Lamb, and the negative control group (untreated group).

Table 1. The Number of PMN Leukocytes in 10 High-Power Field (HPF)

Day	n	The number of PMN leukocytes in 10 HPF (Mean ± Standard Deviation)		
		Untreated Group	Wound model treated with bioplacenton gel	Wounded model treated with 20 microliters of the ambonese plantain banana
7	5	114.20±11.99	89.80 ± 5.93	110.60±12.21
14	5	90.20±17.56	48.60 ±10.81	31.60± 9.24
21	5	64.40±22.31	18.60 ± 8.44	9.40± 2.22

From the ANOVA results, we found significant difference ($p < 0.05$) on the number of PMN leucocytes within the decapitation periods. There was also significant difference on the number of PMN leukocytes among treatment groups and it demonstrated that the number of leukocytes in the treatment group was affected by the interaction days of decapitation ($p < 0.05$). LSD test was conducted to determine the differences among the groups and the results are presented in Table 2.

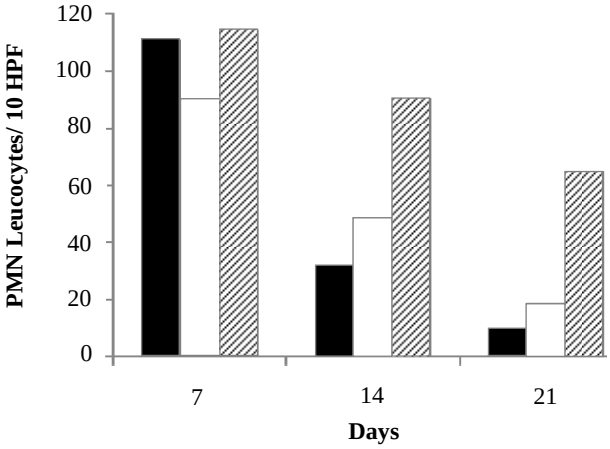

Figure 1. The Number of PMN Leukocytes in Groups Treated with *M. paradisiaca* var *sapientum* Lamb (■), Bioplacenton (□) and Negative Control (▨) Group during Wound Healing of Burn Injuries of Each Period of Decapitation

The lowest number of PMN leukocytes was found at the 7th day in the group of burn injuries wound model treated with bioplacenton gel, which was followed by the group without any treatment, and finally by the group of wound model treated with the plantain of *M. paradisiaca* var *sapientum* Lamb (Table 2). The statistic results demonstrated that there was a significant difference of PMN leukocytes number ($p < 0.05$) between the group treated with the plantain banana of *M. paradisiaca* var *sapientum* Lamb and the group treated with bioplancenton gel. However, no significant difference was found ($p > 0.05$) between the group treated with the ambonese plantain banana and the untreated group.

Number of fibroblasts. The mean value and standard deviation on the number of fibroblasts at the 7th, 14th dan 21st days in the group treated with ambonese plantain banana and the control group are shown in Table 3. The number of fibroblast of the treatment groups were increasing on the 7th day and reaching the peak on the 14th day; therefore, the fibrolast number on the 21st day was lower than the 14th day, except in the negative control group. Figure 2 demonstrates a diagram showing the number of fibroblast of the group treated with ambonese plantain banana of each period of decapitation. It shows that there was increasing number from the 7th day to 14th day. The highest increament was found in the group treated with *M. paradisiaca* var *sapientum* Lamb, followed the positive control group and finally the negative control group. Furthermore, there was a decrease on the 21st day.

Table 2. LSD Test Results on the Number of PMN Leukocytes During the Wound Healing of Burn Injuries on the Back Skin of White Rats Between the Group Treated with Plantain of *M. paradisiaca* var *sapientum* Lamb, Positive Control Group (Treated with Bioplacenton) and the Negative Control Group (Untreated Group) at the 7th Day of Study

Group	Mean ± Standard Deviation
Untreated group	$110.60^{b} \pm 12.21$
Group of wound model treated with bioplacenton gel	$89.80^{a} \pm 5.93$
Group of wound model treated with 20 microliters of ambonese plaintain banana	$114.20^{b} \pm 11.99$

Note: ^{a,b}: different letter shows significant results

Table 3. The Mean Value and Standard Deviation on the Number of Fibroblasts during the Wound Healing of Burn Injuries on the Back Skin of White Rats between the Group Treated with the Plantain of *M. paradisiacal* var. *sapientum* Lamb and the Positive Control Group (Treated with Bioplacenton Gel) and the Negative Control Group (Untreated), Under Microscopic Magnification of 400 x 10 HPF

Day	n	The number of fibroblasts in 10 HPF (Mean ± Standard Deviation)		
		Untreated Group	Wound model treated with bioplacenton gel	Wounded model treated with 20 microliters of the ambonese plantain banana
7	5	2.00±0.70	4.40±1.14	2.80±0.84
14	5	15.00±3.32	34.80±1.30	44.00±1.59
21	5	23.60±3.85	34.80±4.20	34.40±3.36

Note: n = sample size

The ANOVA results demonstrated significant difference ($p < 0.05$) on the number of fibroblasts for each period of decapitation. There was also a significant difference ($p < 0.05$) of the fibroblast numbers between both treatment groups. It also demonstrated that the number of leukocytes in the treatment group was affected by the interaction days of decapitation ($p < 0.05$). In order to evaluate the significant difference between both treatment groups, LSD test was performed as shown in Table 4.

On the 7th day, the highest number of fibroblasts was found in the group of wound model treated with bioplacenton gel, followed by the group treated with the ambonese plantain banana and finally by the untreated group. Subsequently, statistic tests were performed revealing that there was a significant difference ($p < 0.05$) of the number of fibroblast between the group treated with ambonese plantain banana and the group treated with bioplacenton gels; however, there was no significant difference ($p > 0.05$) between the group treated with ambonese plantain banana and the untreated group.

On the 14th day, the highest number of fibroblast was demonstrated in the group treated with ambonese plantain banana, followed by the group treated with bioplacenton gel and the untreated group. Afterward, statistic tests were performed and we found that there was a significant difference of fibroblast number ($p < 0.05$) among the groups.

Table 5 shows that the highest number of fibroblast was found on the 21st day in the group treated with bioplacenton gel, followed by the group treated with ambonese plantain banana and finally by the untreated group. Afterward, statistic tests were performed revealing that there was a significant difference ($p < 0.05$) between the group treated with ambonese plantain banana and the untreated group; however, there was no significant difference ($p > 0.05$) between the group treated with ambonese plantain banana and the group treated with bioplacenton gel (Table 6).

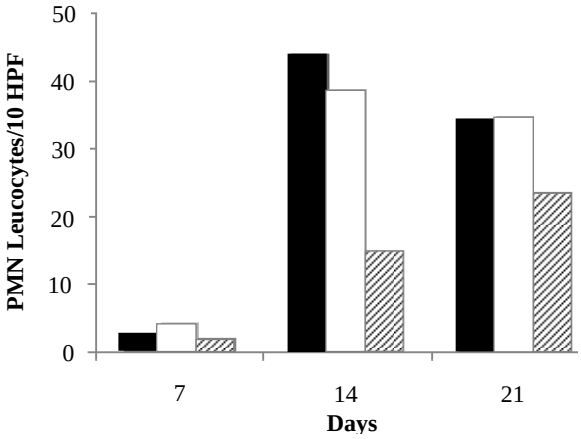

Figure 2. The Number of PMN Leukocytes in Groups Treated with *M. paradisiaca* var *sapientum* Lamb (■), Bioplacenton (□) and Negative Control (▨) Group during the Wound Healing Process of Burn Injuries

Table 4. The Results of LSD Test on the Number of Fibroblasts during Wound Healing of Burn Injuries between the Group Treated with the Plantain of *M. paradisiacal* var. *sapientum* Lamb and the Positive Control (Treated with Bioplacenton) and Negative Control Group (Untreated) on the 7th day

Group	Mean ± Standard Deviation
Untreated group	$2.00^{a} \pm 0.70$
Group of wound model treated with bioplacenton gel	$4.40^{b} \pm 1.14$
Group of wound model treated with 20 microliters of ambonese plaintain banana	$2.80^{a} \pm 0.84$

Note: ^{a,b}, different letter shows significant differences

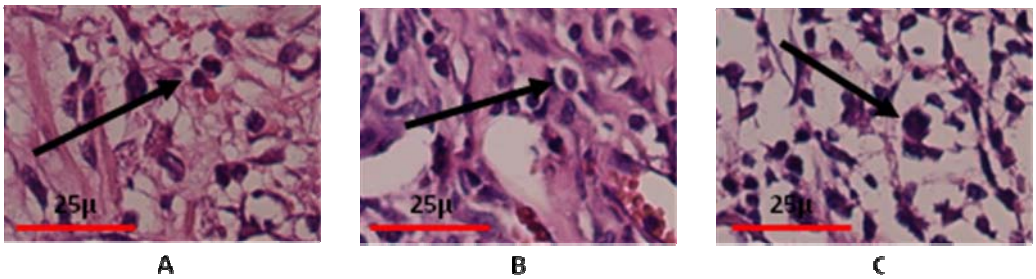

Figure 3. Photomicroscopic Images of Skin Tissues Demonstrating Wound Healing of Burn Injuries between the Group Treated with the Plantain of *M. paradisiaca* var *sapientum* Lamb and the Control Group on the 7th Day Smeared with HE Staining (400 x Magnification). (A) The Group Treated with *M. paradisiaca* var *sapientum* Lamb; (B) The Positive Control Group (Treated with Bioplacenton); (C) The Negative Control Group (Untreated), (L) PMN Leukocytes (Arrow Sign)

On the 7th day, the highest number of PMN leukocytes was found in the negative control among the other groups (Figure 3); while on the 14th day, the highest number of fibroblast was found in the group treated with ambonese plantain banana, followed by the group treated with bioplacenton gel and the untreated group (Figure 4).

The healing effect of the plantain of *M. paradisiaca* var *sapientum* on burn injuries may be due to the presence of saponin, tannin, lectin, ascorbic acid, quinone and anthraquinone. Moreover, the stalk of *M. paradisiaca* var *sapientum* also contains serotonine, norepinephrine, dopamine, vitamin A, B, and C [14,16].

Saponin has both antibacterial and antibiotic effect causing decreased number of inflammatory cells, including reduced number of PMN leukocytes. Saponin enhances angiogenesis and induces growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF). Ascorbic acid may contribute in epithelialization, fibroblast proliferation and collagen formation [18-19].

Saponin may induce growth factors such as VEGF, EGF and FGF. The function of VEGF includes enhancing angiogenesis in wound healing, as well as the EGF.

Simultaneously, EGF may also act as mitogenic factors in various epithelial cells and keratinocytes as well as in formation of tissue granulation. In the wound healing, it is produced as mitogenic factor in various epithelial cells, keratinocytes, and fibroblast. It also stimulates migration of keratinocytes, macrophage, and inflammatory cells which migrate toward the wounded area. FGF may have role in angiogenesis, fibroblast proliferation, and migration of ephitelium to form new epidermis. Epithelization is affected by *matrix metalloproteinase*-1 (MMP-1), produced by fibroblast epithelial cells and myofibroblast, condrocytes, osteoclast, endothelial cells and leukocytes [18,20].

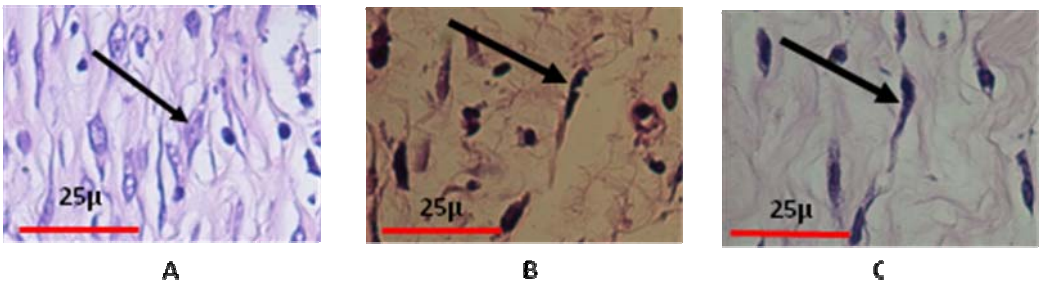

Figure 4. Photomicroscopic Images of Fibroblast Cell Numbers in the Group Treated with the Plantain of *M. paradisiaca* var *sapientum* Lamb and the Control Group during the Burn Wound Healling Process on the 14th Day with HE Staining (400 x magnification). (A) *M. paradisiaca* var *sapientum* Lamb Group, (B) Positive Controled Group (which was Given Bioplacenton) (C) Negative Controled Group (with no Medication), Fibroblast Cell (Arrow Sign)

Table 5. The Results of LSD Test on the Number of Fibroblasts during Wound Healing of Burn Injuries Among the Group Treated with the Plantain of *M. paradisiaca* var *sapientum*, the Positive Control Group (Treated with Bioplacenton), and the Negative Control Group (Untreated Group) on the 14th Day

Group	Mean ± Standard Deviation
Untreated group	15.00^{a}± 3.32
Group of wound model treated with bioplacenton gel	34.80^{b}± 1.30
Group of wound model treated with 20 microliters of ambonese plaintain banana	44.00^{c}± 1.59

Note : ^{a,b}, different letter shows significant difference

Table 6. The LSD Test Result on the Number of Fibroblast in Wound Healing of Burn Injuries among the Group Treated with *M. paradisiaca* var *sapientum* Lamb, the Positive Control Group (Treated with Bioplacenton), and the Negative Control (Untreated Group) on the 21st Day

Group	Mean ± Standard Deviation
Untreated group	23.60^{a} ± 3.85
Group of wound model treated with bioplacenton gel	34.80^{b} ± 4.20
Group of wound model treated with 20 microliters of ambonese plaintain banana	34.60^{b} ± 3.36

Note: a,b, different letter shows significant difference

In the inflammatory phase, tannin may have role in inhibiting the growth of free radicals to retain further excessive tissue damage. In the maturation phase, it has a role in limiting overgrowth of granulation tissues. Tannin has antiseptic properties, which may help to exert immunity against plants. Moreover, it also has antibacterial properties [16]. The growth of epithelial cells in the group treated with the plantain of *M. paradisiaca* var *sapientum* Lamb may be due to the presence of lectins that stimulates the skin growth [19].

4. Conclusions

The wound healing of burn injuries on the back skin of white rat treated with the plantain of *M. parasidiaca* var *sapientum* Lamb showed a better result compared to the untreated burn injuries. There was a lower number of leukocytes in the inflammatory cells, followed by increasing number of fibroblasts in the proliferation phase.

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