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The Activity of *Jatropha curcas* Cream on Day 5 of Skin Wound Healing in Mice Infected with *Staphylococcus aureus*

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ABSTRACT
This research aimed to determine *Jatropha* sap cream's activity on day 5 of the healing process of skin wounds of mice infected with *Staphylococcus aureus.* Twenty-seven male mice (*Mus musculus*) aged 2 months and weighed 30-40 g were used. All mice underwent a 20 mm incision in the dorsal area (back) and infected with 0.2 ml suspension of *S. aureus* ATCC 25923 PK/5. Treatment of open wounds was carried out twice per day. Group P1 (negative control) was smeared with cream base; group P2 (positive control) smeared with 0.1% sulfadiazine cream; and group P3 was smeared with 10% *Jatropha* sap cream. Macroscopic observations were carried out for 21 days, while histopathology were observed on 5th day of healing which performed with hematoxylin-eosin (HE) staining. On day 5, pathological anatomy showed hyperaemic (redness) of the skin wounds, swelling (oedema) of the wound edges and wetness due to exudate. Based on the length of the wound on day 5, it was clear that the effect of 10% *Jatropha* cream and sulfadiazine cream was better than the base cream. Based on HE staining, P1, P2 and P3 had a very significant effect on the number of inflammatory and neovascularization cell infiltration in the inflammatory phase ($p<0.01$). The mean of inflammatory cells in P3 was significantly different from P2 and P1 ($p<0.01$). It can be concluded that 10% *Jatropha* cream can be used as a topical medicine for infected wounds of mice's skin.

Keywords: *inflammation, jatropha sap cream, Staphylococcus aureus, wound healing*

1. INTRODUCTION

Infection is an important factor affecting wound healing. Infection is caused by various pathogenic microorganisms such as bacteria [1]. *Staphylococcus aureus* is a gram-positive extracellular bacterium that are responsible for skin infections, cellulitis, folliculitis, subcutaneous abscesses, ulcer infections and wounds [2]. *Staphylococcus aureus* is a major cause associated with infection with a wide range of clinical manifestations from minor skin surface, soft tissue infections to deep wounds and organs [3].

In wound care, antibiotics are usually used to prevent infection. The widespread and irrational use of antibiotics results in bacterial resistance [4]. One of the efforts to overcome infection and bacterial resistance in wounds is to find alternative antibacterial compounds. Jatropha sap contains antimicrobial chemical compounds, namely flavonoids, saponins, tannins, and polyphenols which play a role in killing *Escherichia coli, S. aureus, E. faecalis,* and *S. flexineri* [5]. *Jatropha* leaves and twigs have antioxidant activity [6], immunomodulators [7], and as a wound healing [8].

The flavonoid compound of *Jatropha* sap could be used as antifungal, antiseptic, anti-inflammatory and cell regeneration or repairing processes. Saponins can stimulate collagen growth in wound healing, relieve pain, and stimulate new cells' formation [9]. Flavonoid compounds play a role in the inflammatory phase of wound healing by increasing interleukin-2 (IL-2) activity and lymphocyte proliferation [10]. Lymphocyte proliferation will affect the cluster of differentiation 4 (CD4 +) cells, then cause T helper 1 (Th1) cells to be activated [11]. The Th1 cells affect Specific Macrophage Activating Factor (SMAF) and Interferon-gamma (IFN γ) which can activate macrophages [12]. The flavonoid, quercetin and routine compounds in *Jatropha* sap improve early-phase wound healing as shown by the regulation of vascular endothelial growth factor expression for new blood vessel growth [13].

In topical wound care, the activity of *Jatropha* sap needs to be formulated in a cream form in order to be easily applied. Creams also soothe the inflammation, relieve itching and pain [14]. Based on the explanation above, it is necessary to know the concentration and potential of *Jatropha* cream as a natural antibacterial that inhibits bacterial growth and how the macroscopic (anatomical pathology) and histopathological features on day 5th of healing process of the *S. aureus* infected wound.

2. MATERIALS AND METHODS

2.1 Jatropha sap Cream Preparations

The collection of *Jatropha curcas* L. sap was carried out purposively. The sap was obtained from Jatropha plant from the village around Universitas Syiah Kuala (USK), Banda Aceh. Plants were identified at Laboratory of Plant Biology, Study Program of Biology, Faculty of Mathematics and Natural Sciences, USK. The sap was collected at 08.00 a.m. by breaking the leaf stalk, and the sap was stored in a sterile bottle.

Jatropha latex cream was made on an oil-in-water (O/W) basis using excipients which include the oil phase (stearic acid, adeps lanae and liquid paraffin) and the water phase (aquadest, triethanolamine and nipagin). The cream was made by heating at 60-70 °C the oil and water phases, separately. The oil phase was heated on water bath while the water phase was heated on hotplate. Heating process was performed until all of components in the oil phase melted and the water phase dissolved, then the two phases were mixed by pouring the water phase into the oil phase while constantly stirring as the temperature decreased until cream was formed. The cream base was then gradually added with 10% Jatropha sap to a porcelain dish containing 100 g of cream and stirred until homogeneous at room temperature [15].

2.2 Experimental Animal

The experimental animals used were 27 male mice (*Mus musculus*) aged 2 months with a bodyweight of 30-40 g obtained from the Faculty of Veterinary Medicine USK. The mice were placed in a sealed cage. The cage was 30x40x30 cm^3 which was equipped with drinking water and feed containers. The food given to mice was the standard type of T79-4 pellet produced by PT. Central Proteina Prima Medan. This pellet was also used for feeding experimental animals for research purposes. The same pellet was used in both preparation and treatment periods.

During the adaptation period, each mouse was placed in one cage, fed with pellets and water *ad libitum*. Before being placed in a cage, according to the treatment group, the mice were weighed for initial status of body weight before treatment. Bodyweight data was used as a baseline in grouping mice for testing.

2.3 Wound Incision and Staphylococcus aureus Infection

In this study, an incision was made in the dorsal area (back) of all mice according to Salim *et al.* [16]. The procedure for wound incision was by shaving the hair with a diameter of 3 cm around the area of the skin, and disinfected with 70% alcohol. In order to reduce pain, local anesthesia was used on the skin area, then a skin incision is performed with a wound length of 20 mm to the subcutaneous area. Then, all mice were infected with 0.2 ml suspension of *S. aureus* ATCC 25923 PK/5 at the incision site.

2.4 Activity Test of Jatropha Cream on Wound Healing

In wound healing study, mice were divided into three groups withthree different periods of sample collection, and three replications.. In each group, open wounds were treated with the same intensity, twice per day at 08.00 a.m. and 06.00 p.m., with the following treatments: group P1 (negative control) was treated with cream base; group P2 (positive control) was treated with 0.1% sulfadiazine cream; and group P3 was treated with 10% *Jatropha* sap cream.

2.5 Macroscopic and Histopathological Observations

The parameters observed were the phase of inflammation, epithelialization, granulation, formation of crusts, and duration of incision healing in a matter of days marked by wound closure (length and width of the wound in mm). For histopathological observations, mice in each group were sacrificed by using ether solution. Skin tissue samples at the incision wound site were collected on 5th day with 3 skin tissue samples per period. Skin tissue was cut perpendicularly. Observation on the wound healing process, namely the inflammatory phase, was carried out by using HE staining.

2.6 Statistical Analysis

Data from the observation of anatomical pathology were analyzed descriptively. Histopathological data were analyzed by comparing treatment groups and the sampling period. The quantitative data obtained from the observations were analysed by ANOVA. Comparison of the effects between treatments was carried out by posthoc Duncan's multiple test. Quantitative data were analyzed by SPSS.

3. RESULTS AND DISCUSSION

3.1 Anatomical Pathology

In the activity test of 10% *Jatropha* sap cream (P1) on day 5 (inflammatory phase), the skin wounds of mice showed hyperaemic (redness), swelling (oedema) of wound edges and wetness due to exudate. The length of the wound on day 5 of 10% *Jatropha* cream and sulfadiazine cream (positive control) was better than the base cream (negative control) (Table 1). The image of skin wounds of the mice receiving cream-based treatment, sulfadiazine cream and 10% *Jatropha* latex cream on the 5th day of healing is presented in Figure 1.

Table 1. Length of mice skin wounds infected with *S. aureus* on day 5 after topical application of cream base, sulfadiazine cream and *Jatropha* cream

Group	Length of wounds (cm)
Cream base (P1)	1.20
0.1% Sulfadiazine (P2)	0.96
10% *Jatropha* sap (P3)	0.96

The redness and oedema of the wound was the result of an inflammation process. This reaction was formed by vasoconstriction of blood vessels which was immediately followed by vasodilation. The presence of a blood clot was a reaction of activated platelets and fibrinogen protein which was released by blood vessels. Fibrinogen thenformed fibrin threads [17].

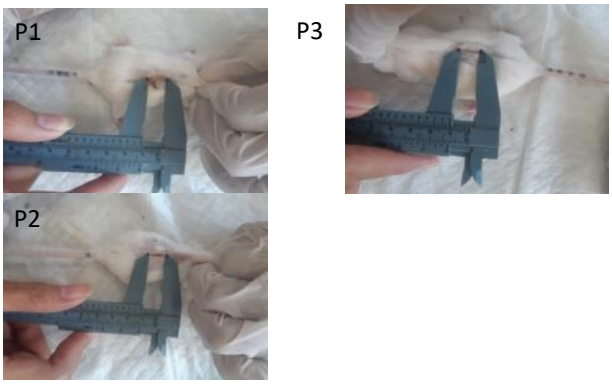

Figure 1 Overview of skin wounds infected by *Staphylococcus aureus* and treated with cream base (negative control, P1), Sulfadiazine cream (positive control, P2) and 10% *Jatropha* cream (P3) on day 5 of wound healing process.

3.2 Histopathology

The incision wound on day 5 after administration of cream base (P1) and sulfadiazine 0.1% (P2) was dominated by dense inflammatory cells. The administration of 10% *Jatropha* sap cream (P3) at the wound site showed spreaded inflammatory cells with moderate density (Figure 2). Based on one-way ANOVA test, the treatment with cream base(P1), 0.1% sulfadiazine (P2) and 10% *Jatropha* sap cream (P3) had a significant effect ($p < 0.01$) on the amount of inflammatory cell infiltration during the inflammatory phase of wound healing in mice skin. The Duncan test (Table 2) showed that the number of inflammatory cells after the administration of 10% *Jatropha* cream (P3) was significantly different ($p < 0.01$) from the number of inflammatory cells after the administration of 0.1% sulfadiazine (P2) and cream-base (P1).

Table 2. Average number (± SD) of inflammatory cells in the inflammatory phase of skin wound healing in *Staphylococcus aureus* infected mice

Groups	Means of inflammatory cells
Cream base (P1)	302.00 ± 7.20^{c}
0.1% Sulfadiazine (P2)	243.00 ± 8.80^{b}
10% *Jatropha* sap (P3)	148.67 ± 1.50^{a}

$^{a,\ b}$Different superscripts in the same column means significant differences ($P < 0.01$)

From our result, it can be seen that the number of inflammatory cells after the administration of 10% *Jatropha* cream was effectively reducing the number of inflammatory cells. It showed that the administration of 10% *Jatropha* cream accelerated the inflammatory phase of the wound healing process of *S. aureus* infection on micecskin. The involvement of inflammatory cells that dominate the wound area indicateed that the inflammatory process was occuring . The inflammatory phase is a very important stage in wound healing process, if the inflammatory process lasts for a long time, the wound healing will be hampered [18].

The decrease in the number of inflammatory cells after the administration of 10% Jatropha sap cream was due to the presence of saponins and flavonoids in Jatropha sap which had anti-inflammatory potency [7, 19, 20]. The bark and roots of *J. curcas* contain compounds with antibacterial activity [21]. *J. curcas* leaf extract showed very strong antibacterial and antifungal activity and relatively moderate antioxidants [22]. Methanol and hexane extracts of *J. curcas* leaves showed antimicrobial, antiviral, antioxidant, anticancer, analgesic, anti-inflammatory, anti-bleeding and wound healing properties [23]. Methanol extract of *J. curcas* showed systemic anti-inflammatory activity in rats induced by acute carrageenan [24]. The ethanol extract of *J. curcas* leaves had peripheral analgesic and antibacterial activity [25]. The crude extract of *J. curcas* stems had the ability to inhibit the growth of bacteria and fungi, thereforeit had the potency as broad-spectrum

antimicrobial substances and can be used in the management of microbial infections [9].

Observations of the incision on day 5 after applying cream base (P1), the wound area was haemorrhagic, hyperaemic, and neovascular was formed slightly. After the application of 0.1% sulfadiazine (P2) and 10% Jatropha cream (P3), the wound showed an increase in neovascular volume (Figure 2). Based on one-way ANOVA test, the treatment of cream base (P1), 0.1% sulfadiazine (P2), and 10% Jatropha sap cream (P3) had significant effect ($p < 0.01$) in the inflammatory phase of the wound healing on mice skin infected with *S. aureus* (Table 3).

Table 3. Mean number (± SD) of neovascular in the inflammatory phase of skin wound healing of *Staphylococcus aureus* infected mice

Groups	Means of neovascular number
Cream base (P1)	12.00 ± 5.50[a]
0.1% Sulfadiazine (P2)	24.00 ± 5.00[b]
10% *Jatropha* sap (P3)	26.60 ± 7.50[b]

[a, b] Different superscripts in the same column means significant differences ($P < 0.01$)

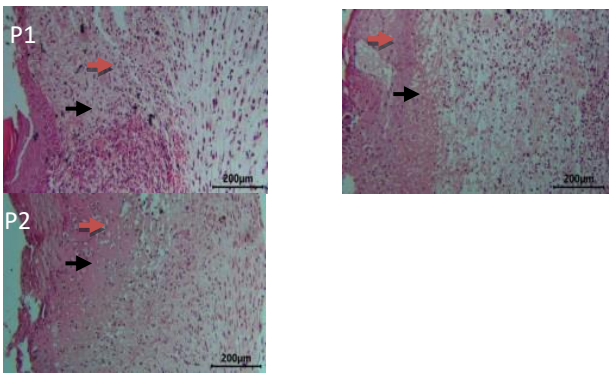

Figure 2. *Staphylococcus aureus* infected wounds of mice skin after treatment with cream base (negative control, P1), sulfadiazine cream (positive control, P2) and 10% Jatropha sap cream (P3) on day 5. Inflammatory cells (black arrows); neovascular (red arrow) (HE, 10x).

The Duncan test showed that the amount of neovascular after the administration of 10% Jatropha sap cream (P3) was significantly different ($p<0.01$) compared to the number of neovascular after cream base administration (P1). The amount of neovascular in 10% *Jatropha* cream administration(P3) compared to 0.1% sulfadiazine (P2) was not significantly different ($p>0.01$). The administration of 10% *Jatropha* cream (P3) increased the number of neovascularization in the wound area [16, 26]. The formation of new blood vessels in the wound area accelerated wound healing [26]. The saponin content in *Jatropha* sap plays a role in stimulating the growth of new cells and angiogenesis which is a process of forming neovascularization in wounds. The increased blood supply to the tissues carries nutrients necessary for the healing process [27].

4. CONCLUSIONS

The research confirmed that 10% Jatropha cream can be used as a topical treatment, which had antibacterial activity, anti-inflammatory, and neovascular stimulator in mice skin wound infected with *S. aureus*. It is necessary to study the bioactive compound or phytochemical substances contained in Jatropha cream responsible for antibacterial, anti-inflammatory and collagen tissue synthesis.

AUTHORS' CONTRIBUTIONS

MNS and DM designed, performed the research and prepared the manuscript.

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