Wound Healing Acceleration of *Spatholobus littoralis* Extract on Rat Incision Wound Infected by *Staphylococcus aureus*: A Preliminary Study

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Abstract. The efficacy of Spatholobus littoralis extract for incision wound is not fully understood. Here, we report the wound healing activities of Spatholobus littoralis extracts in incision wound models in rat. The aim of the study was to measure the wound length on rat's incision wound infected by Staphylococcus aureus. Fifteen male Rattus norvegicus rats weighing 200-250 g were randomly divided into four groups: (1) negative control (natural healing); (2) positive control (amoxicillin 10 mg); (3) 2.5 mg/kg/bw Spatholobus littoralis extract treatment; (4) 25 mg/kg/bw Spatholobus littoralis extract treatment. The treatment substances were applied topically daily. The length of the incision wound was measured every day after wound infliction. The results of the incision wound length measurement in treatment group showed better results; in average, the 2.5 mg Spatholobus littoralis extract treatment group showed that the wound was totally closed on day 13, and the 25 mg bajakah extract treatment group showed the wound closure on day 12. Meanwhile, the positive control group showed the wound closure on day 14 and negative control group wounds are not closed yet until day 14. The treatment with bajakah extract have an advantage on wound closure on male rats infected with Staphylococcus aureus.

Keywords: Spatholobus littoralis, Staphylococcus aureus, incision wound lenght.

1. Introduction

Skin wound management is the modification of wounds to establish a physiologic environment that is consistent with the organism's original state [1]. Wound healing in response to injury is a natural process that involves the overlap of complex and interconnected cellular and metabolic systems [2][3]. The appearance of wounds varies based on the cause, there are open wounds and closed wounds. An incision wound is the example oh the open wound, it is occurs as a result of a cut by a sharp object, for example during surgery, which is characterized by an open wound, pain, the length of the wound is

G. Setya Ayu Putri et al. (eds.), *Proceedings of the 2nd Lawang Sewu International Symposium on Health Sciences: Medical Laboratory Technology (LSISHS-MLT 2023)*, Advances in Biological Sciences Research 40,
https://doi.org/10.2991/978-94-6463-457-0
https://doi.org/10.2991/978-94-6463-457-0_16

greater than its depth [4]. According to Wardani [5], et al if open wounds are not properly repaired cause severe damage, initiation of an infection,.

Bacteria are part of the skin microbiota and wounds. These bacteria can form a biofilm, which may impede wound healing [6]. The most common strains detected in patients with infected wounds are Staphylococcus aureus, methicillin-resistant S. aureus (MRSA), and Pseudomonas aeruginosa [7]. Antibiotics have improved human health by treating infections, but many pathogenic types continue to create significant problems due to antimicrobial resistance [6]. Natural products are currently being researched as a treatment for infected wounds.

Spatholobus littoratis is a plant native to Kalimantan (Borneo Island) which people use to treat diarrhoea, dysentery, pain, and heal wounds, and is even considered an anti-cancer agent [8][9]. This plant contains phenolic compounds, steroids, tannins, alkaloids, saponins, terpenoids and alkanoids [8]. In theory, all of these chemicals can increase collagen development and the production of new epithelium (re-epithelialization), hence accelerating wound closure [10]. Spatholobus littoratis has the potential to be explored and developed as a natural remedy for infected wounds. The purpose of this study was to determine the efficacy of Spatholobus littoratis ethanolic extract on the healing of incision wounds infected with Staphylococcus aureus.

2. Materials And Method

2.1. Animals

This research is a laboratory experimental research. This research used 16 male rats (Rattus norvegicus) weighing 200-250 grams and aged 2-3 months which were obtained from the Semarang College of Pharmaceutical Sciences (STIFAR) which were divided into 4 groups, namely: 1) negative control (natural healing); 2) positive control (amoxicillin 10 mg); 3) 2.5 mg/kg/bw Spatholobus littoralis extract treatment; 4) 25 mg/kg/bw Spatholobus littoralis extract treatment. Staphylococcus aureus was obtained from the Microbiology Laboratory of Universitas Muhammadiyah Semarang. The ethanol extract of Spatholobus littoratis was made in the toxicology laboratory at Universitas Muhammadiyah Semarang. Meanwhile, the animal wound treatment was carried out at the Animal Laboratory Universitas Muhammadiyah Semarang.

2.2. Wound and treatment

The rats were kept and fed for a week for acclimatization. On the wounding day, the rats were anesthetized (Ketamine-xylazine) and the dorsum was shaved and disinfected with 70% ethanol to make an incision with a length of 2 cm and a depth of 2 mm. S.aureus was suspended in 0.9% NaCl (MC Farland 0.5 standard) and then induced in 20 µL rat incision wounds. The Spatholobus littoralis extract for the wound treatment were using concentration of 2.5 mg/KgBW and 25 mg/KgBW orally.

2.3. Wound observation

The wounding day was designated as day 0. The Observation of wound closure length was monitored starts from day 1 to day 14 under anaesthesia and images of wounds were taken.

3. Result

3.1. Wound length measurement

Data on the results of measuring the length of incision wounds on the rat's dorsum induced by Staphylococcus.aureus in all treatment groups can be seen in Table 1

Table 1. Wound length average from day 1 until day 14.

Group	Days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
negative control	20	20	19,3	19	17,3	17,3	16,3	15	12,6	8,3	8,3	6	6,5	2,3
positive control	20	20	19	19	17	15,3	13,6	11,3	7,6	3,6	3,6	1,6	1,6	0
2.5 mg/kg/bw *Spatholobus littoralis*	20	20	19,6	18,3	16	15	15	10,6	7,3	3	3	6	0	0
25 mg/kg/bw *Spatholobus littoralis*	20	20	19	17,6	15,3	15,3	12,6	10	7	3	3	0	0	0

Based on the results of the observations (table 1), it can be seen that the average days needed for the wound closure in the 25 mg/kg/bw Spatholobus littoralis group is faster compared to the other group, the data showed that at 12th day after wounding the wound had closed and fully covered by epithelium. the 2.5 mg/kg/bw Spatholobus littoralis group showed a complete wound closure on day 13. The positive control group showed the average days required for wound closure on day 14. In the other hand, the Negative control shows that the wound has not closed completely until the 14th day, the length of the wound is still more than 2 mm. Data analysis using Anova analysis showed $p=0.659$ ($p>0.05$), which mean that there is no statistically difference in wound closure length among group.

3.2. Macroscopic condition of the wound

The macroscopic conditions of the wound were taken by a camera (figure 1).

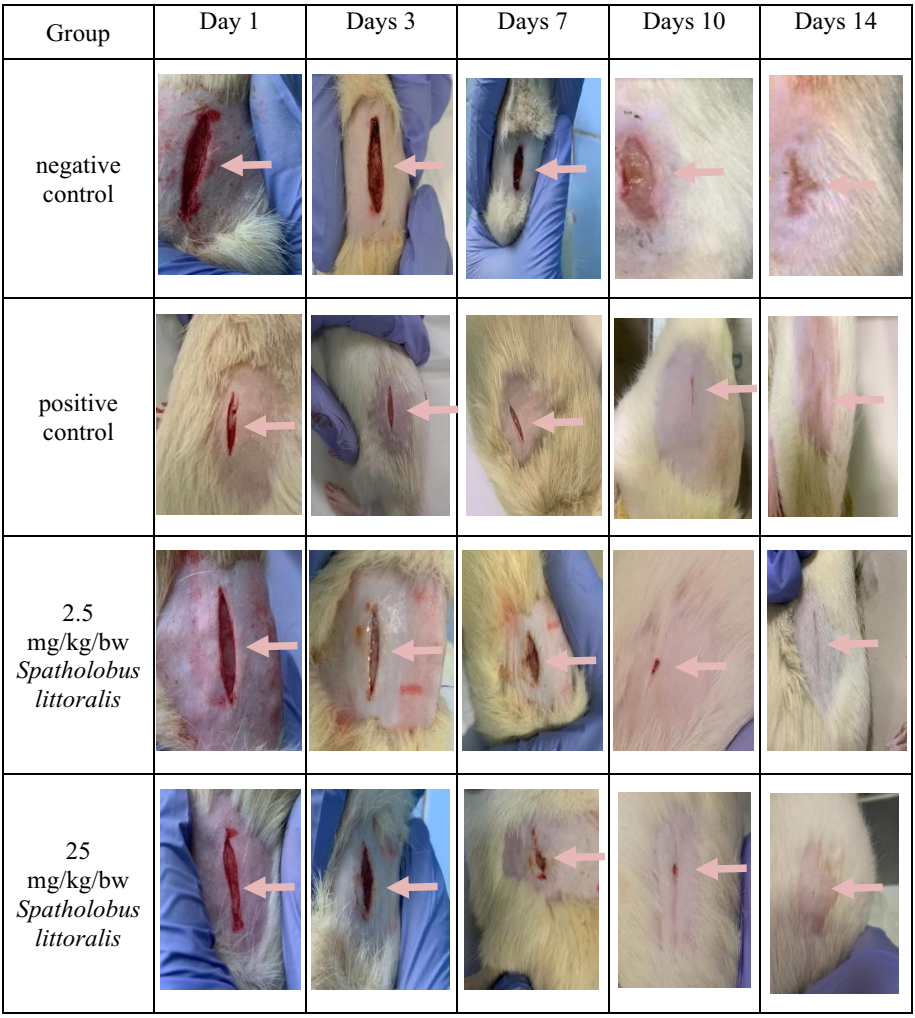

Fig. 1. The figure of the rat's dorsum for macroscopic observation of incision wound healing progression. Pink arrow indicates the wound.

Based on the figure 1, the observation of the incision wound on mice in all treatment groups were done until 14 days. Day 1 showed that the wound was red (erythema), wet, swollen and open. On the 3rd day, the wound was also still red (erythema), wet, swollen and open

in negative control group. In positive control, 2.5 mg/kg/bw and 25 mg/kg/bw group showed a better condition. On the 7th day, the wounds in group 2.5 mg/kg/bw and group 25 mg/kg/bw were already dried and had a little scab. It is different when compared to the negative control group, the wound was still open and swollen and the positive control group's wounds were starting to close. In day 14, the positive control group, 2.5 mg/kg/bw, and 25 mg/kg/bw showed the wound was completely closed, but in the other hand the negative control group showed the wound was still not closed completely, but the wound was dry.

4. Discussion

The study's findings demonstrated the capability of Spatholobus littoralis on the Staphylococcus aureus-induced incision wound healing process on the dorsum of rats. After receiving therapy for 14 days, the results demonstrated the healing process of the wound. The observations of wound size demonstrated the clear benefits of utilizing Spatholobus littoralis for wound healing. The incision wounds in the negative control groups remained red (erythema) and wet from exudates on day three, with the wound still being widely open. Comparing this condition to the other groups, which displayed superior conditions and reveals differences. This was a very good sign of wound healing during the inflammatory stage. The positive control group at 2.5 mg/kg/bw and the 25 mg/kg/bw group experienced a shorter inflammatory phase, suggesting a quicker transition from this phase to the proliferative phase. A number of reasons, such as elevated levels of inflammatory mediators, wound infection, hypoxia, and inadequate nutrition, can generally be blamed for the prolonged healing time [11].

According to a prior study, exudate production peaked during the inflammatory phase and then decreased as the healing process advanced. Day 2 or Day 3 is when the generation of exudate peaks. The formation of exudate during the inflammatory phase of a wound is ascribed to the blood capillaries' permeability. The lining of capillaries, which is porous and rich in carbohydrates, the tight spaces between cells and capillary walls, and the disruption of endothelial cells by inflammatory mediators all play significant roles in controlling the flow of fluid into the surrounding tissues, which causes the wound to become wet [2].

The stem of Spatholobus littoralis contains flavonoid, saponin, and tannin chemicals as secondary metabolites [12]. According to Liu et al [13], the majority of the secondary metabolites in the other Spatholobus species were flavones, isoflavones, and anthocyanins. Aglycone flavonoid molecules have strong anti-inflammatory properties that counteract oxygen radicals (ROS). It is believed that the neutralization of free radicals by flavonoid compounds modulates anti-inflammatory cytokines and can raise the growth factor of tissues that are undergoing inflammation [14].

Skin infections and the rate at which wounds heal are both influenced by the skin microbiota and the dry, wet, and sebaceous skin microenvironment that surrounds it [15]. When the

skin becomes compromised, foreign microorganisms and the regular skin flora can quickly penetrate to the underlying tissues, providing the ideal conditions for their growth and multiplication [16]. On the other hand, the wound's natural microbiota changes into more aggressive microbial kinds when healing is delayed. Consequently, the ideal environment for microbial colonization and multiplication may be an open wound. When a chronic wound is first being constructed, Gram-positive bacteria, primarily Staphylococcus aureus, are the most common [17].

As was previously indicated, tannin, saponin, and flavonoids are present in Spatholobus littoralis secondary metabolites. The carbonyl group-containing phenolic flavonoid from Spatholobus littoralis contributes to the extract's antibacterial qualities. The way that flavonoids have antibacterial activities is by forming complex flavonoids with extracellular proteins that have the ability to break down bacterial cell walls. The ability of flavonoids to form soluble complexes with extracellular proteins is another way that they have antibacterial properties. These complexes can damage bacteria's cell membranes and cause the release of internal chemicals that may cause the bacterium to die [18].

Then, tannin compounds have the ability to impede the creation of cell proteins by forming irreversible complexes with proteins that serve as an antibacterial agent [19]. Additionally, from the antibacterial saponin mechanism to pushing the surface with increased cell permeability or intracellular cell leakage [20].

In the proliferative-remodelling stage of wound healing, the 2.5 mg/kg/bw and 25 mg/kg/bw groups similarly displayed improved conditions. It was demonstrated that in those groups, the wound healing process moved more quickly, closing entirely by day 14. These findings suggest that Spatholobus littoralis may aid in hastening the healing of wounds. In addition to their antibacterial properties, flavonoids also function as antioxidants by scavenging free radicals (ROS), which prevent oxidative damage [21]. Due to the fact that this disease slowed down the healing process, flavonoids derived from Spatholobus littoralis may be able to counteract oxidative stress. In order to stop ROS from stealing electrons from molecules and forming tissues, flavonoids provide their electrons to ROS [22]. Additionally, Spatholobus littoralis saponin may hasten the wound's cell epithelization and boost collagen synthesis [23]. Tannins' ability to repair injured tissue and speed up the contraction of fibrous tissue during wound healing may also be crucial to the healing process. [24].

5. Conclusion

Based on the length of the wound, the findings of observations made from day 1 to day 14 indicate that the best chance of hastening the healing of incision wounds is to inject Spatholobus littoralis extract at a concentration of 25 mg/Kg/BW.

Authors' Contributions. All authors contributed equally to this work.

Acknowledgments. The author would like to thank the Department of Biology Education, Faculty of MIPA and Tecnology Education, Institut Keguruan dan Ilmu Pendidikan PGRI Pontianak, Pontianak, West Kalimantan, Indonesia for providing the Spatholobus littoralis stem.

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