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Efficacy of *Sauropus androgynous* Leaves Extract Gel on Burn Wound Healing in Albino Rats

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Abstract

Burns not only damage skin locally but generally affect the body system and have been related as the secondary cause of death. Burns can be effectively treated with a topical drug to prevent chronic inflammation. Burn wound healing is really important for ensuring overall health and well-being. This study aimed to determine the effect of *Sauropus androgynous* leaves extract gel on the number of fibroblasts, fibrocytes, and collagen density on burn wound healing in albino rats. A total of 50 male albino rats were randomly divided into five groups i.e. (C-) was normal skin, (C+) was skin burn treated with placebo, (T1) was skin burn treated with 2.5% of *S. androgynous* leaves extract gel, (T2) was skin burn treated with 5% of *S. androgynous* leaves extract gel, and (T3) was skin burn treated with 10% of *S. androgynous* leaves extract the gel. The amount of 25 albino rats' skin samples were collected on the 8th day, and the remaining samples were collected on the 15th day. The results showed that 10% of *S. androgynous* leaves extract gel improved faster, as indicated by the increased number of fibroblasts, fibrocytes, and collagen density. This study can be concluded that 10% of *S. androgynous* leaves extract gel was the effective dosage to increase the number of fibroblasts, fibrocytes, and collagen density in burn wound healing.

Keywords: collagen, fibroblast, fibrocyte, health, *Sauropus androgynous*

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INTRODUCTION

Burn wound is one of the accidents that can happen to pets. Lam (2012) stated that domestic conditions such as boiling water or direct heat from a fireplace in the household, also devices used at veterinary clinics, or grooming devices such as hair dryers or heating pads are common sources of pet burns. Burns not only damage the skin locally but generally affect the body system and have been related as the secondary cause of death (Lam *et al*., 2012). Healing of burn wounds depends on the depth of the burns, which can be classified into four degrees (Markiewicz-Gospodarek *et al*., 2022). The concept of good wound healing occurs when injury tissue only undergoes acute inflammation (Rohl *et al*., 2015). Prolonged inflammation can promote chronic inflammation, which in turn may cause necrotic cells as a result of oxidative stress and invasive growth of keloids (Johar *et al*., 2004; Ogawa, 2017). Burns can be effectively treated with a topical drug to prevent chronic inflammation (Sucita *et al*., 2019). The gel has the best potential for topical drug preparation because the gel contains a lot of water and does not leave an oily layer on the skin pores, so it can reduce serious inflammation (Rani *et al*., 2022).

The gap of knowledge in this study emphasized the importance of herbal medicine. Herbal medicine is still developing because people believe herbal medicine is safer and more affordable, so it can be an alternative medicine in the future. *Sauropus androgynous* is known as a nutritious plant scattered throughout Indonesia (Fikri and Purnama, 2020). Traditionally, in

Indonesia, *S. androgynous* leaves have been used to increase the milk supply for nursing mothers (Asokawati *et al*., 2021). *S. androgynous* leaves extract contains a high amount of vitamin A and vitamin C, carotenoids, and flavonoids that have antioxidant, anti-inflammatory, and antibacterial potentials (Khoo *et al*., 2015).

Vitamin A and vitamin C can significantly promote wound healing activity because they can accelerate fibroblast proliferation and collagen synthesis (Arnold and Barbul, 2006). In a study by Mathew-Steiner *et al*. (2021), 5% of *S. androgynous* leaves extract ointment has been shown to increase the number of fibroblast and collagen bundles that have a key role in tissue repair during the wound healing process.

This study was conducted to determine the effect of *S. androgynous* leaves extract gel on the number of fibroblasts, fibrocytes, and collagen density on burn wound healing in albino rats.

MATERIALS AND METHODS

Ethical Approval

This study was approved by Universitas Airlangga Animal Care and Use Committee (ACUC) No 2.KE.026.02.2019.

Study Period and Location

This study was conducted from January–March 2019 in the Faculty of Veterinary Medicine, Universitas Airlangga. *S. androgynous* leaves extraction was conducted at the Nutritional Laboratory, the leaves extract gel preparation in the Department of Basic Medicine, and the histology sample preparation and scoring were performed in the Laboratory of Pathology.

Experimental Design

This study was an experimental design that used a factorial randomized controlled trial to reveal the interaction between two independent variables. This study used 50 three-month-old male albino rats strain Wistar, weighed 200–250 grams that were randomly divided into five treatments, and each treatment consisted of 10 replications. The treatments were (C-) was normal skin, (C+) was burn treated with placebo, (T1) was burn treated using 2.5% of *S. androgynous* leaves extract gel, (T2) was burn treated with 5% of *S. androgynous* leaves extract gel, and (T3) was burn treated with 10% of *S. androgynous* leaves extract gel.

S. androgynous Leaves Extract Preparation

A total of 760 grams of *S. androgynous* leaves powder was soaked in 4 liters of 96% ethanol for three days at 27ºC and must be stirred once a day. After three days, the mixture was filtered and squeezed to separate the debris and the filtrate. The filtrate was evaporated using a rotary evaporator bath at 60ºC for 90 minutes at 55 rpm speed to obtain a thick extract form. The extract obtained was 40 mL.

S. androgynous leaves extract gel was made based on Santoso *et al*. (2014). *S. androgynous* leaves extract with different dosages 2.5%, 5%, and 10% as medicine, CMC Na as a gelling agent, glycerin as an emollient, propylene glycol as a humectant, nipagin as a preservative agent, and aquadest as solvent. Each gel was made of 25 grams.

Burn Wound Procedure

The burn wound made in the right gluteal of the albino rats with thermostat tool, which burn wound was made in the right gluteus of the rats with a thermostat tool, which has a 1 cm diameter of the stainless steel plate and 85 ± 5ºC degree (Cai *et al*., 2014; Abdeldjelil *et al*., 2017). The thermostat tool was placed on the rats' skin for 5 seconds, so deep partial-degree burns were made (Porumb *et al*., 2017). The therapy was given twice a day for seven days and 14 days.

Histopathological Preparation

All samples were euthanized by decapitation. The skin sample was taken at a full thickness in a 2 x 2 cm^2 square shape and fixed in 10% formalin for histopathological preparation. The skin sample was then processed into HE staining.

Microscopic Assessment

The microscopic assessment was done under a Nikon H600L microscope, Optilab, and Image

Raster software with 100x magnification to focus on the healing center, then 400x magnification. Fibroblast and fibrocyte evaluation were done by manual counting, and collagen evaluation was done by scoring based on modification shown in Table 1.

Data Analysis

The data were analyzed using SPSS 20.0 for Windows. The number of fibroblasts and fibrocytes was analyzed using Two Way ANOVA followed by the Tukey Test. The assessment of collagen density was analyzed using Kruskal-Wallis followed by Mann-Whitney U with a significant level of $p < 0.05$.

RESULTS AND DISCUSSION

Fibroblast and Fibrocyte

The result showed an increase in the number of fibroblasts in *S. androgynous* leaves extract gel groups compared to the control group. The highest number of fibroblasts was shown by 10% of *S. androgynous* leaves extract gel which has increased from 7 days to 14 days. This result was increased along with an increasing dosage of *S. androgynous* leaves extract gels (Table 2). The histopathological appearance of fibroblast in 7 days and 14 days is shown in Figure 1.

The negative control group (C-) showed the lowest number of fibroblasts compared to all treatment groups, while the number of fibrocytes and collagen density showed the highest results compared to all treatment groups, and these results showed not significantly different in 7 days, and 14 days (Table 3). The negative control group was normal skin that had undamaged skin tissue. Fibroblasts play a key role in synthesizing extracellular matrix, such as collagen, in repairing tissue damage (Tracy *et al*., 2016), while normal tissue does not require extracellular matrix restoration (Xue and Jackson, 2015).

The positive control group (C+) showed an increase in the number of fibroblasts in 7 days and 14 days compared to the negative control group (C-). It can be assumed that a placebo or gel base can increase the number of fibroblasts. According to Gun'ko *et al*. (2017), the gel has a high content of water, so it is called hydrogel. Hydrogels can absorb and resist the presence of wound exudates. Thus, they can promote fibroblast proliferation (Al-Anshori *et al*., 2023). The number of fibrocytes and collagen density also increased in 7 days and 14 days, but these results were the lowest compared to all treatment groups.

The *S. androgynous* leaves extract gel groups (T1, T2, T3) showed an increase in the number of fibroblasts and fibrocytes in 7 days and 14 days. These results were increased along with the increasing dosage of *S. androgynous* leaves extract gels, which were 2.5%, 5%, and 10%.

Fibroblast proliferation started during the inflammatory phase and increased until the proliferation phase. Macrophages play a key role in stimulating fibroblast proliferation (Witte and Barbul, 2002). A high amount of fibrocytes is an indication that previously, fibroblasts had actively synthesized extracellular matrix, and Reilkoff *et al*. (2011) mentioned that fibrocyte is known as mature and inactive fibroblasts.

Collagen Density

The analysis of collagen density using Kruskal-Wallis showed significant differences in all treatment groups ($p < 0.05$). The difference in each treatment continued with the Mann-Whitney U test, as shown in Table 4.

The result showed that the 10% of *S. androgynous* leaves extract gel group had the highest result compared to the other *S. androgynous* leaves extract gel groups and the positive control group, where this result was closest to the negative control group. The histopathological appearance of collagen density in 7 days and 14 days is shown in Figure 2. The characteristic of mature collagen showed clear boundaries and dark red colors (Verhaegen *et al*., 2012). Increasing collagen density helps to form new tissue tensile strength (Nayeem and Karvekar, 2011). Tensile strength by collagen type I produced by fibroblast will cause injured tissue not to be easily reopened (Ireton *et al*., 2013).

Burn wound healing in 7 days still showed scab and unclosed epithelium. The scab is a necrosis tissue due to the inflammation process.

Table 1. Collagen density assessment

Description	Score
The width of collagen fibers is smaller than the width of the distance between collagen fibers, collagen looks very thin or few, does not spread, and there is a scab	1 (very loose)
The width of collagen fibers is smaller than the width of the distance between collagen fibers, collagen looks very thin or few, spreads, and there is a scab	2 (loose)
The width of collagen fibers is the same as the width of the distance between collagen fibers, collagen looks a lot, does not spread, and there is a scab	3 (moderate)
The width of collagen fibers is greater than the width of the distance between collagen fibers, collagen looks a lot, spreads, and there is a scab	4 (few dense)
The width of collagen fibers is greater than the width of the distance between collagen fibers, collagen spreads a lot, is bound, and there is a scab	5 (dense)
The width of collagen fibers is greater than the width of the distance between collagen fibers, collagen spread diffusely, perfectly bound, no scab, and epithelium closed	6 (very dense)

Table 2. Number of fibroblasts in all treatment groups in 7 and 14 days

Treatment	Number of fibroblast (x ± SD)	
	7 days	14 days
C-	16.0^{a} ± 1.0	17.0^{ab} ± 0.7
C+	20.2^{bc} ± 1.4	21.2^{cd} ± 1.9
T1	21.8^{cd} ± 1.3	24.8^{cd} ± 2.0
T2	23.6^{d} ± 1.4	30.0^{e} ± 2.5
T3	29.8^{e} ± 2.3	34.4^{f} ± 1.8

Different superscripts in the same columns indicate significant differences ($p < 0.05$).

Table 3. Number of fibrocytes in all treatment groups in 7 and 14 days

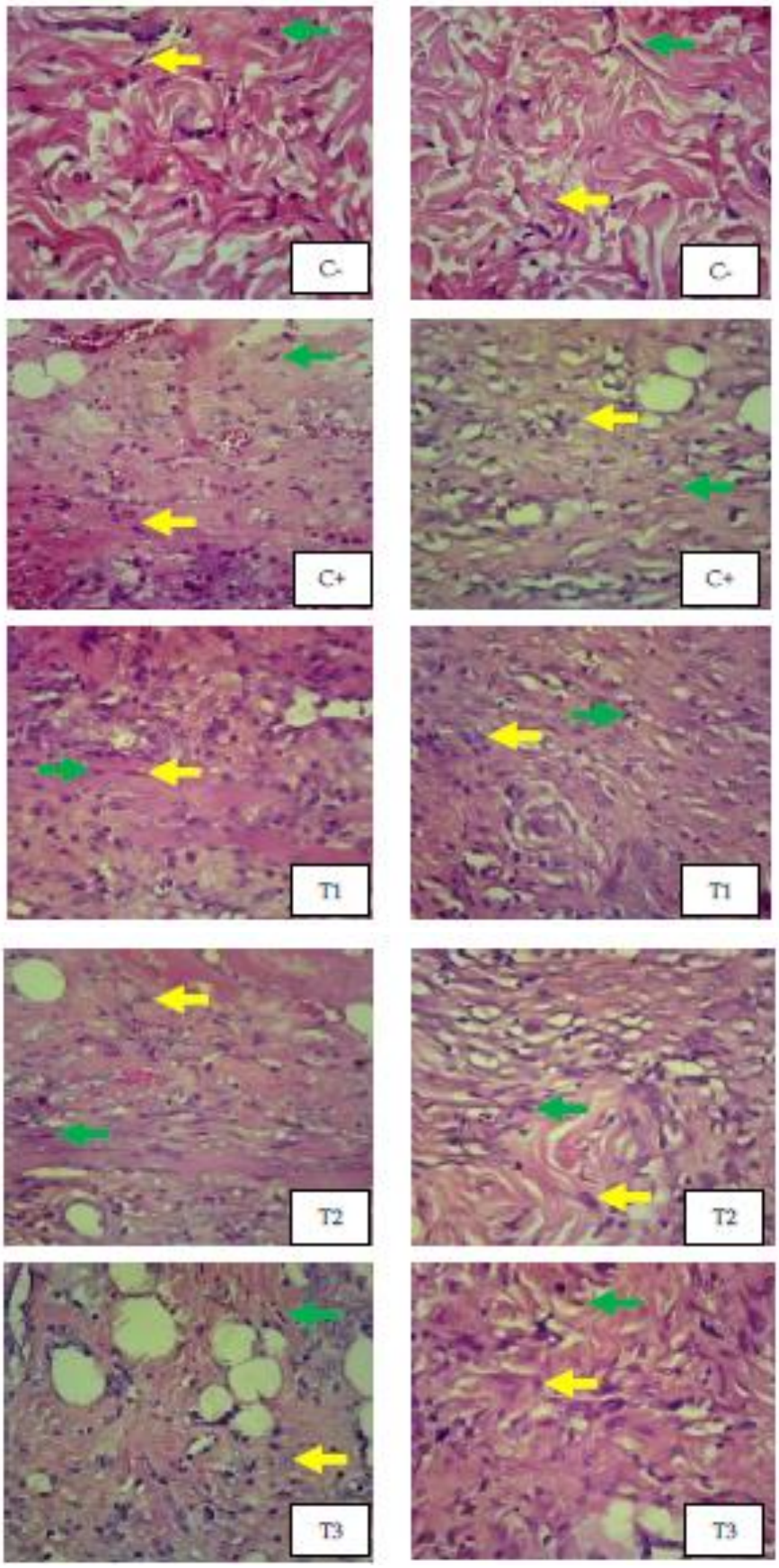
Treatment	Number of fibrocyte (x ± SD)	
	7 days	14 days
C-	12.8^{f} ± 1.9	13.0^{f} ± 1.5
C+	3.4^{a} ± 0.2	4.8^{abc} ± 0.9
T1	4.1^{ab} ± 1.2	6.9^{cd} ± 1.4
T2	6.7^{bcd} ± 0.7	8.1^{d} ± 1.1
T3	9.4^{de} ± 1.5	11.6^{ef} ± 1.5

Different superscripts in the same columns indicate significant differences ($p < 0.05$).

Table 4. Collagen density average in all treatment groups in 7 and 14 days

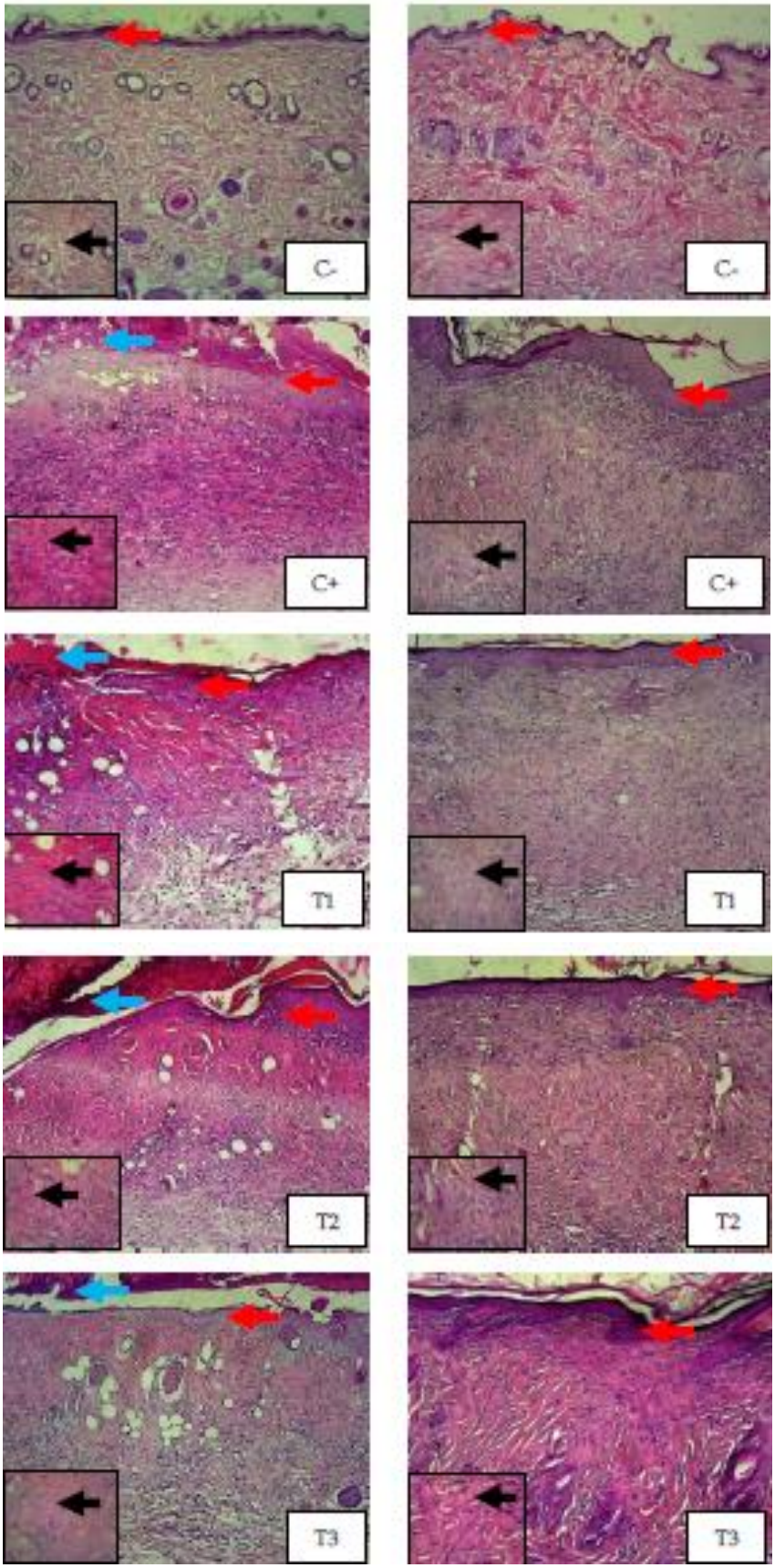
Treatment	Collagen density (x ± SD)	
	7 days	14 days
C-	6.0^{gh} ± 0.0	6.0^{h} ± 0.0
C+	1.4^{a} ± 0.5	2.6^{c} ± 0.5
T1	2.6^{b} ± 0.5	3.8^{e} ± 0.4
T2	3.4^{c} ± 0.5	4.4^{f} ± 0.5
T3	3.6^{cd} ± 0.8	4.6^{g} ± 0.5

Different superscripts in the same columns indicate significant differences ($p < 0.05$).



7 days 14 days

Figure 1. Histopathological evaluation of fibroblasts and fibrocytes in all treatment groups in 7 and 14 days (HE, 400x). (→) fibroblast, (→) fibrocyte.



7 days 14 days

Figure 2. Histopathological evaluation of collagen density evaluation (HE, 100x, 400x). (→) collagen fiber, (→) reepithelialization, (→) scab.

In the 10% of *S. androgynous* leaves extract gel group (T3) in 7 days, the scab had already detached, and the epithelium began to migrate from the edge of the wound to the middle of the wound. Keratinocytes in the epidermis initially proliferate from the edges of the wound and then migrate toward the middle of the wound until the wound closes (Li *et al*., 2007; Prastika *et al*., 2020). The skin epithelium in all treatment groups in 14 days had completely closed.

A study showed that *S. androgynous* leaves extract contains flavonoids, alkaloids, steroids, terpenoids, and tannins which have anti-inflammatory, antioxidant, and antibacterial effects (Khoo *et al*., 2015). This study explained that *S. androgynous* leaves extract may have the potential to accelerate burn wound healing. *S. androgynous* leaves also contain a high amount of vitamin A and vitamin C (Khoo *et al*., 2015). Vitamin A and vitamin C play a key role in increasing fibroblast and collagen synthesis, which can promote wound healing (Arnold and Barbul, 2006). The beginning of collagen synthesis occurs via intracellular, where the genes in the nucleus are activated and a single polypeptide mRNA changes, then converted into a triple polypeptide chain in the ribosome and then exits to extracellular as procollagen (Cahya *et al*., 2020; Lesmana *et al*., 2023). *S. androgynous* leaves extract also contains vitamin B, which acts as a co-factor in the synthesis and cross-linking of collagen to determine tensile strength in injury tissue (Bhaskar *et al*., 2009). This study has some limitations, such as a lack the observation points, and a lack of knowledge of the immune response caused by *S. androgynous* leaves extract in the burn wound healing process. Further study might be needed to investigate the immune response caused by this leaves extract.

CONCLUSION

Based on this study, it could be concluded that *S. androgynous* leaves extract gel can increase fibroblast and fibrocyte number. Meanwhile, collagen density on burn wound healing in albino rats with the effective dosage of extract was 10%.

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AUTHORS' CONTRIBUTIONS

RK: Conceptualization and drafted the manuscript. RTP, SAS, AM, and TVW: Treated the animal laboratory. ISY: Validation, supervision, and formal analysis. ARK: Performed the statistical analysis and the preparation of tables and figures. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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