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The Effect of *Apium graveolens* (*Linn.*) Extract on the Number of Neutrophils and Angiogenesis in Wound Incision of *Sprague Dawley* Rat

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ABSTRACT

Wounds were a challenging clinical problem that often caused morbidity and mortality. *Apium graveolens* (*Linn.*) was an herbal plant that had anti-oxidant and anti-inflammatory effects which were considered to accelerate the wound healing process. The number of neutrophils and angiogenesis was an element that played an important role in the wound healing process. Thus, the administration of *Apium graveolens* (*Linn.*) extract was expected to help in the process of wound healing which could be viewed from the number of neutrophils and angiogenesis. To determine the effect of giving *Apium graveolens* (*Linn.*) extract on the number of neutrophils and angiogenesis in incisional wounds of Sprague Dawley rat. This study was an experimental study with "Randomized post test with control group" design in rats given incisional wounds on their backs, and randomly divided into 4 groups (@5 rat). The number of neutrophils and angiogenesis were seen microscopically and assessed by 2 certified pathologists. The data were analyzed using the One Way Anova - Post Hoc hypothesis test. The number of neutrophils and angiogenesis in the *Apium graveolens* (*Linn.*) extract group was significantly lower than the group given the cream without extract ($p < 0.05$) and no difference compared to the group given gentamicin 0.1% cream ($p > 0.05$). The group with *Apium graveolens* (*Linn.*) extract 70% had the lowest number of neutrophils and angiogenesis compared to other groups ($p < 0.05$). The application of the topical *Apium graveolens*

(*Linn.*) extract is effective in the incisional wound healing process by decreasing the number of neutrophils and angiogenesis. The application of the topical *Apium graveolens* (*Linn.*) extract 70% is more effective than the extract 50%.

1. Introduction

Wounds were a challenging problem, with initial and advanced complications that often cause morbidity and mortality [1]. According to the data of Riskesdas Indonesia (Indonesia Basic Health Research) in 2013, laceration wounds were in third place (23.2%) after bruises / abrasions (70.9%) and sprained wounds (27.5%) [2]. The wound would cause damage to the skin and would be the entrance of bacteria which then caused inflammation and infection locally or systemically, so the goal of wound management was to achieve healing as soon as possible and reduced the formation of scars produced [3]. Wound healing was divided into 3 phases (the inflammatory phase, the proliferation phase and the remodeling phase) [4]. Neutrophils and new blood vessel formation occurred in the inflammatory phase. Neutrophils had the role of securing signals that strengthen inflammation in the early stages of healing, and also acted as a signal to close the healing phase of inflammation [5]. The formation of new blood vessels was an important component in the healing process that carried oxygen and micronutrients to the growing tissue and removed catabolic waste products [6- 8].

Herbs were not on the main therapy list [9]. However, due to low toxicity and side effects, herbal therapy regained momentum and was accepted as a complementary and alternative therapy in combination with first-line therapy [10]. *Apium graveolens* (*Linn.*) was an herbal plant and had chemicals such as flavonoids, saponins, tannins, apiin, essential oils, apigenin, choline, vitamin A, B, C, asparagine. Flavonoids had antioxidant activity, anti-inflammatory, antihepatotoxic, antitumor, antimicrobial, antiviral and influenced on the central nervous system [11].

This study aimed to determine the effect of giving *Apium graveolens* (*Linn.*) extract to the number of neutrophils and angiogenesis in incision wounds of Sprague Dawley rats in the process of wound healing.

2. MATERIALS AND METHODS

2.1 Animals and Treatment

This study was an animal experimental study with "Randomized post-test with control group" design in Sprague Dawley male rats that were incised on their right back with 5cm wound length and 2mm wound depth. All subjects were treated equally in terms of postoperative and normal amounts of diet. Experimental animal subjects were divided randomly into four equal groups (2 control groups and 2 intervention groups), namely: K1 was given cream without *Apium graveolens (Linn.)* Extract, K2 was given gentamicin cream 0.1%, P1 was given cream with 50% *Apium graveolens (Linn.)* Extract, P2 was given cream with 70% *Apium graveolens (Linn.)* Extract. Giving each cream to each group topically to the incision wound twice a day for 7 days. The thickness of the cream is about 0.5cm.

2.2 Histopathological Examination

On the 7th day, termination was carried out by taking the incision wound tissue on the back of the rat by

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anesthetizing the mice with a mixture of Ketamine-Xylazine (Ketamine dose 80mg / kgBW; Xylazine dose 10mg / kgBW intraperitoneally and cutting the skin and subcutaneous tissue with a scalpel with a size of 6 cm x 1 cm x 1 cm. Then the tissue was stained with Hemato-eosin, and the number of neutrophils and angiogenesis was counted under a binocular microscope with 100x magnification.

2.3 Statistical Analysis

The data were analyzed using SPSS 25.0 for windows. Data analysis includes descriptive analysis and hypothesis testing. In descriptive analysis, the dependent variable is presented in the form of table mean, SD, median and box plot graph. Then performed a data normality test with the Saphiro-Wilk test.

The data in this study were normally distributed and homogeneous, then the data was continued with the One Way Anova hypothesis test followed by a Post-Hoc Test to determine the differences between groups. The limit of the degree of significance is if $p \leq 0.05$ with a 95% confidence interval.

3. RESULTS

The study was conducted on 20 rats, which were divided into 4 groups; namely the group of rats to be given topical cream without *Apium graveolens (Linn.)* extract (K1), topical gentamicin cream 0.1% (K2), topical cream with *Apium graveolens (Linn.)* extract 50% (P1), and topical cream with *Apium graveolens (Linn.)* extract 70% (P2), each group consisted of 5 rats, and was still in good health until the end of the study.

Data on rat body weight were normally distributed and homogeneous. The highest rat body weight was found in the group to be given topical cream with *Apium graveolens* (*Linn.*) extract 50%, which was 160.60 ± 12.74 grams.

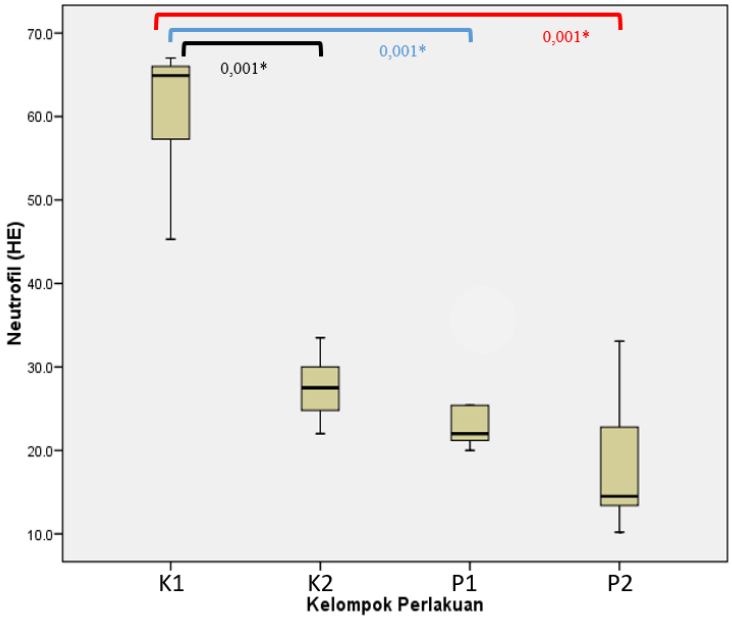

Figure 1. Boxplot graph of the number of neutrophils from each group. K1: given topical cream without *Apium graveolens (Linn.)* extract, K2: given topical gentamicin 0.1% cream, P1: given topical cream with *Apium graveolens (Linn.)* extract 50%, and P2: given topical cream with *Apium graveolens (Linn.)* extract 70%.

From the Fig. 1, it showed that the difference in the number of neutrophils from the group given topical

gentamicin cream 0.1%, the group given topical cream with *Apium graveolens (Linn.)* extract 50%, and the group given topical cream with *Apium graveolens (Linn.)* extract 70% had a significant value compared to the group given topical cream without *Apium graveolens (Linn.)* extract ($p < 0.05$). Whereas the difference in the number of neutrophils from the group given topical gentamicin cream 0.1% did not significantly different when compared to the group that gave topical cream with *Apium graveolens (Linn.)* extract 50% ($p = 0.584$), and the group given topical cream with *Apium graveolens (Linn.)* 70% extract ($p = 0.086$).

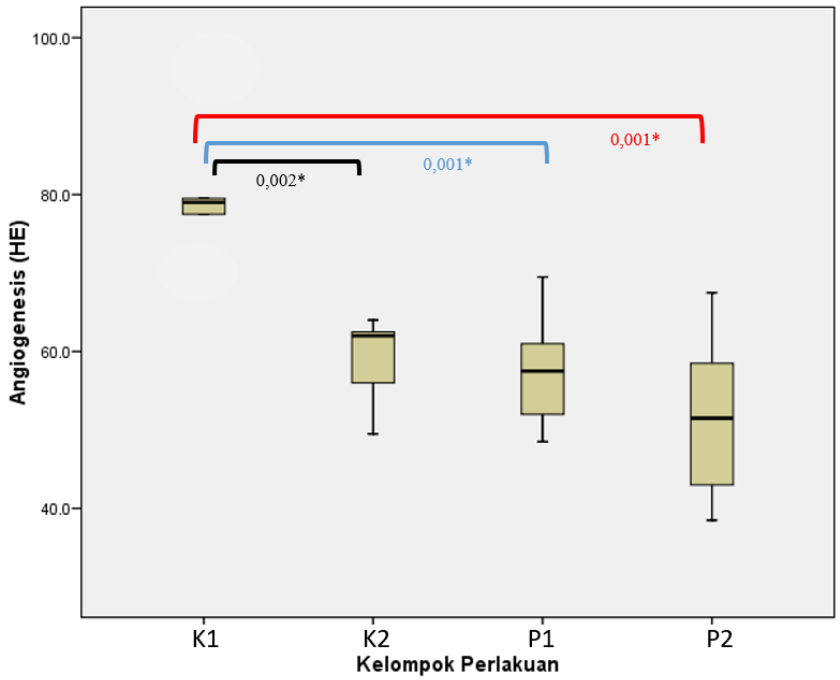

Figure 2. Boxplot graph of the number of angiogenesis from each group. K1: given topical cream without *Apium graveolens (Linn.)* extract, K2: given topical gentamicin 0.1% cream, P1: given topical cream with *Apium graveolens (Linn.)* extract 50%, and P2: given topical cream with *Apium graveolens (Linn.)* extract 70%.

From the Fig. 2, it showed that the difference in the number of angiogenesis from the group given topical gentamicin cream 0.1%, the group given topical cream with *Apium graveolens (Linn.)* extract 50%, and the group given topical cream with *Apium graveolens (Linn.)* extract 70% had a significant value compared to the group given topical cream without *Apium graveolens (Linn.)* extract ($p < 0.05$). Whereas the difference in the number of angiogenesis from the group given topical gentamicin cream 0.1% did not significantly different when compared to the group that gave topical cream with *Apium graveolens (Linn.)* extract 50% ($p=0.581$), and the group given topical cream with *Apium graveolens (Linn.)* 70% extract ($p=0.243$).

4. DISCUSSION

This study aimed to see the effect of *Apium graveolens (Linn.)* extract on the number of neutrophils and angiogenesis in the healing process of incision wounds performed on the backs of *Sprague Dawley* rat which met the inclusion criteria and were treated for 7 days. Then seen the number of neutrophils and angiogenesis through HE staining.

In this study, *Apium graveolens (Linn.)* was used because the phytochemical contents of *Apium graveolens (Linn.)* consisted of carbohydrates, phenols (flavonoids), alkaloids, steroids, limonene, selinen, prokoumarin

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glycosides, flavonoids, Vitamins A and C which could be as an antioxidant, anticancer, antibacterial, cardio protective, anti-inflammatory agent, immune system, and promotes and protects the skin from UV radiation [12], [13]. The role of antioxidants and anti-inflammation from *Apium graveolens (Linn.)* could help in the process of wound healing.

From this study, skin tissue was taken on the 7th day after being given treatment for each group and in the treatment group using topical cream with *Apium graveolens (Linn.)* extract 70%, had the lowest neutrophil count and had significant value compared to the group other. According to the theory which state that neutrophils were the part of the inflammatory response and could secrete signals that strengthen inflammation in the early stages of healing, and also acted as a signal to close the inflammatory phase and were the first inflammatory cells that moved to the wound site as a defense against infection [5]. In the healing process of normal skin wounds, the inflammatory phase usually lasted for 2-5 days and stopped once dangerous stimuli had disappeared [14]. Neutrophils then experienced apoptosis which was eventually ingested by macrophages, and uptake of apoptotic cells by macrophages provided a strong signal for inflammatory resolution that allowed the wound to continue into the next healing phase [5]. The study of [15] states that apiuman in *Apium graveolens (Linn.)* has an anti-inflammatory effect that caused a decrease in interleukin-1β, increased interleukin-10 and decreased migration from neutrophils.

This study also assessed the amount of angiogenesis in skin tissue, on the 7th day after being given treatment for each group, there were found that the lowest number of angiogenesis and significant value was found in the treatment group given topical cream with *Apium graveolens (Linn.)* extract 70%. The results of this study indicated that this decrease might be due to tissue extraction on the 7th day had entered the final phase of proliferation in which the number of angiogenesis decreases and would return to the number of normal skin blood vessels. This was confirmed by the macroscopic appearance of incision wounds on days 3, 5 and 7 in the group given topical cream with *Apium graveolens (Linn.)* extract 50% and 70% showed better results than the group given topical cream without *Apium graveolens (Linn.)*. In accordance with the theory that angiogenesis was an important factor for repairing itself and for removing debris, providing nutrients and oxygen to the wound layer [16]. During granulation tissue formation, new blood vessels developed from pre-existing vessels [17]. In the proliferation phase, vessels blood at the base of the wound was estimated to increase five times to meet the demands of cell metabolism that improved tissue. Progress towards the remodeling phase resulted in a significant reduction in new tissue metabolic requirements that cause neovascular returned to blood vessel density which similar to the condition before the wound [16].

This study uses gentamicin which was included as one of the antibiotics most often used for the treatment of wounds on the skin. Previous research had suggested that topical administration of gentamicin ointment could be used to treat various skin infections where it was the most preventable challenge in terms of wound healing [18], [19]. This study found no significant difference between groups given topical cream with *Apium graveolens (Linn.)* extract 50% and the group given topical cream with *Apium graveolens (Linn.)* extract 70% to the group given topical gentamicin cream 0.1%. This proved that administration of cream with *Apium graveolens (Linn.)* extract had an effect that was more or less the same as giving gentamicin 0.1% cream in terms of wound healing when viewed from the number of neutrophils and angiogenesis.

The research of [20], compared wound healing to skin and mucosa of Balb/c mice and found that on the seventh day the wound healing process in Balb/c rat skin, the number of neutrophils and angiogenesis has decreased. This might prove that on the seventh day it had entered the end of the proliferation phase which

was characterized by a decrease in the number of angiogenesis and various inflammatory factors.

[21] found that on *Ipomoea carnea L*. containing flavonoids significantly increased the activity of the wound healing process, both on incision wounds and excision wounds on the back of Wistar rats.

In the study of [22] concluded that the administration of ethanol extract from *Apium graveolens (Linn.)* had the activity of healing wounds in rabbits *Oryctolagus cuniculus L*.

This study also had several disadvantages such as unevenness in the level of hygiene of each rat, taking tissue samples of wounds that are not identical, and cannot be determined the phase of healing of the wounds that occurred on the seventh day of the sampling.

5. CONCLUSSION

Giving *Apium graveolens (Linn.)* extract has an influence on the decrease in the number of neutrophils and angiogenesis in incision wounds of *Sprague Dawley* rat, so that it can accelerate the wound healing process. The administration of *Apium graveolens (Linn.)* extract 70% had a more significant difference when compared with the administration of *Apium graveolens (Linn.)* extract 50%.

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