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The Effect of Binahong Leaf Gel (Anredera cordifolia) with the Addition of HPMC (Hydroxy Propyl Methyl Cellulose) on Wound Reduction and Angiogenesis in the Skin of Mice (Mus musculus) with Second-Degree Burns

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Abstract

Second-degree burn injury is a form of tissue damage to the skin and its structure that can cause the risk of infection and hypovolemic shock, so effective treatment is needed to accelerate healing and minimize long-term impact. One of the medicinal plants that can be used to treat burn injuries is the Binahong plant (Anredera cordifolia) which contains active compounds that can increase the work of Vascular endothelial growth Factor so as to increase angiogenesis. Therefore, this study aims to determine the effect of binahong leaves with the addition of HPMC on changes in wound area and angiogenesis. The type of research used in this study is true experimental, which consists of 5 treatments, namely negative control (K-, without ointment and binahong leaf extract gel), positive control (K+, treated with bioplacenton ointment), treatment 1 (P1, treated with binahong leaf gel with 6.7 grams of extract), treatment 2 (P2, treated with binahong leaf gel with 8 grams of extract), and treatment 3 (P3, treated with binahong leaf gel with 12 grams of extract). Mice treated with various treatments were observed every 2 days until day 14 to observe scab formation. Other parameters also observed in this study were wound area narrowing and angiogenesis observed after treatment. The data obtained were tested with ANOVA and continued with LSD test at α 0.05. The results showed that binahong (Anredera cordifolia) leaf gel with the addition of HPMC had an effect on wound area narrowing and angiogenesis, with the most optimal results found in P3 (binagong leaf gel with 12 grams of extract).

1. INTRODUCTION

Burn trauma cases in Indonesia show higher numbers compared to other types of traumas, approximately 1.3% [1]. One type of burn that causes trauma is second-degree burns. These burns cause tissue damage to the skin and its structures due to direct contact with heat sources such as electricity, fire, hot liquids, chemicals, or mechanical sources [2], resulting in damage to the epidermis and dermis [3]. The impacts include pain, infection, scar tissue formation, changes in skin pigmentation, treatment and handling costs [4], and hypovolemic shock [5].

Hypovolemic shock occurs when the body loses a significant amount of blood or fluid, resulting in serious issues with body functions [6]. Although the body has a natural capacity to heal burn wounds, the process is often slow and carries a high risk of infection and suboptimal healing [7][8].

In an effort to accelerate burn wound closure, synthetic drugs applied topically have become a common choice used by the public [9]. Synthetic drugs have been proven to accelerate healing but can cause negative side effects if used continuously [10]. Therefore, alternative methods are needed to accelerate the wound healing process while preventing negative side effects, using traditional methods with medicinal plants [11]. Binahong leaves are a medicinal plant that can be used as a traditional remedy due to their active compounds such as saponins, tannins, terpenoids, alkaloids, and flavonoids [12]. Binahong leaf extract is known as an effective source of natural antioxidants, with an IC50 value of 40.27 ppm [13].

To enhance the effectiveness of binahong leaf extract in wound healing, the addition of HPMC (*Hydroxypropyl Methylcellulose*) is a viable option. HPMC increases the stability and adhesion of the binahong leaf extract formulation, making it optimal for wound healing [14].

This study aims to explore the potential for increasing the effectiveness of burn wound healing, focusing on wound area reduction and the amount of angiogenesis. Angiogenesis is crucial as it ensures adequate blood and nutrient supply to the wound area [15]. By monitoring angiogenesis, the formation of new blood vessels can be measured to support the wound healing process [16].

2. MATERIALS AND METHODS

Preparation of Experimental Animals

The mice used were 25 mice of the BALB/c strain, male, 8-12 weeks old and weighing 20-30 g. Having characteristics of skin without wounds, clean fur and not standing. Mice were kept in wire cages with husk mats. Each cage was placed 4 mice. Acclimatized for a week.

Preparation of Binahong Leaf Extract

The extraction of binahong leaves is carried out using the maceration or soaking method. 500 grams of binahong leaf powder is placed in a glass vessel and 2500 ml of 96% ethanol is added, left for 1-2 days while occasionally stirring. Next, the macerate is separated, and the solvent is separated from the extract using a rotary evaporator at a temperature of 50°C until a thick extract is obtained [13; 20].

Preparation of Binahong Leaf Gel Dosage

In the initial stage, 3% HPMC is dispersed in 50 mL of distilled water at a temperature of 80-90°C until it expands (mixture A). Methyl paraben and propyl paraben are mixed in 10 mL of propylene glycol (mixture B). The mixture is then added to the dispersed HPMC (mixture A). The remaining distilled water is added and stirred until homogeneous. Next, the gel base is mixed with the desired amount of extract [14].

Burn Creation

Second-degree burns were created by heating a 2.4 cm diameter coin using a blue Bunsen flame for 5 minutes, then applying it to the shaved back of the mice for 5 seconds. The success of the burn wound was assessed by the formation of a white wound, sligh burning, mild edema, and no fur **[17]**.

Burn Testing

Burn wound testing was conducted by applying gel formulationsadded with (6.7, 8, 12) grams of extrac as test material, bioplacenton gel as the positive control, and base gel as the negative control. Each treatment was applied as muach as 0,1 gram. The application was done once a day for 14 days.

Data retrieval

Wound area measurements were performed by documenting images of the wound area with a distance of 10 cm and processing the images using *ImageJ* applications. The obtained skin tissue was prepared for histological examination with *Hematoxylin* & *Eosin* staining. Histological slides were photographed using a microscope with a 40x objective lens. Data collection for angiogenesis variables was taken by counting the number of capillaries, which are channels lined with a single layer of endothelium containing red blood cells, in five different fields of view **[18]**.

3. RESULTS

The healing process of burn wounds involves several stages that indicate tissue repair and recovery. The study results show various effects caused by the treatments on wound area and angiogenesis. The data obtained can serve as an alternative for burn wound healing with low risk of side effects, affordability, and accessibility.

Visual observations of changes in second-degree burn wounds were recorded from day 1 to day 14. The results indicated that scab formation in the various treatments (P1, P2, P3, and K+) occurred on the second day. However, in the K(-) group, scab formation occurred on the fourth day, indicating a delay in scab formation for K(-) (Table 1).

Observation of burn wounds showed a healing process in all groups, indicated by the reduction in wound size from the beginning of the study. The average wound area measurements revealed differences in wound contraction across the groups (Figure 1). The P3 group had the greatest average contraction of 2.73 cm^2, followed by the P2 group with 2.32 cm^2. The P1 group showed a contraction of 1.85 cm^2, which was smaller than P2 and P3. Meanwhile, the K(+) group experienced a contraction of 1.70 cm^2, and the K(-) group had the smallest contraction of 1.34 cm^2 (Table 2).

Based on the research results, the application of binahong leaf gel (*Anredera cordifolia*) with HPMC on the healing of second-degree burn wounds increased angiogenesis in all treatments (P1, P2, P3, K+, and K-) (Figure 2). However, the P3 group showed a denser pattern compared to other groups, with three angiogenesis in one field of view, while other treatments only had one angiogenesis. The average calculations showed that P3 had the highest angiogenesis value among the treatment groups with an average of 5.0, followed by P2 with an average of 3.4, which was higher than P1's average of 2.4. The K(+) group had an average of 2.2, and the K(-) group had the lowest average of 1 angiogenesis (Table 3).

Table 1. Observations of scab formation

Group Treatment	Formation of scab on day…						
	2	4	6	8	10	12	14
P1	+	+	+	+	+	+	+
P2	+	+	+	+	+	+	+
P3	+	+	+	+	+	+	+
K+	+	+	+	+	+	+	+

K-	-	+	+	+	+	+	+

Information: Description: (-) scab has not formed, (+) scab formed

Table 2. Effect of binahong gel on the mean area of burn

Treatment	Different in Wound Area Narrowing ± Stdev
K(-)	1,34 ± 0,07[a]
K(+)	1,70 ± 0,13[b]
P1	1,85 ± 0,23[b]
P2	2,32 ± 0,26[c]
P3	2,73 ± 0,08[d]

Description: numbers followed by the same letter indicate not significantly different (0.05>α)

Table 3. Effect of binahong gel on mean increase in angiogenesis

Treatment	Mean Number of Angiogenesis ± Standart Deviation
K(-)	1 ± 0,70[a]
K(+)	2,2 ± 0,83[b]
P1	2,4 ± 0,54[b]
P2	3,4 ± 0,54[c]
P3	5,0 ± 1,00[d]

Description: numbers followed by the same letter indicate not significantly different (0.05>α)

A B C D E

Figure 1. Effect of binahong (*Anredera cordifolia*) leaf gel with HPMC addition on changes in wound area. (A) P1; (B) P2; (C) P3; (D) K+; (E) K-.

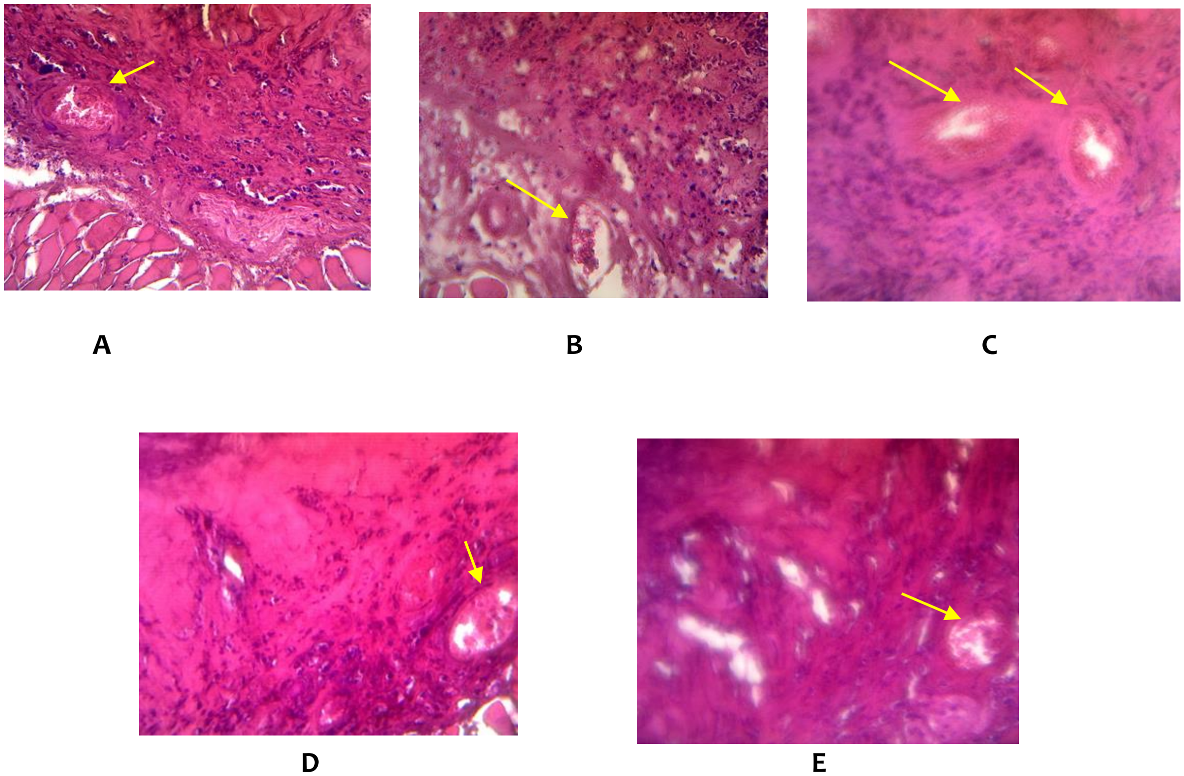

Figure 1. Effect of binahong (*Anredera cordifolia*) leaf gel with the addition of HPMC on increasing angiogenesis. (A) P1; (B) P2; (C) P3; (D) K+; (E) K-. Arrows indicate areas of angiogenesis

4. Discussion

Important indicators of burn wound healing are scab formation and wound area narrowing, which indicate progress in the healing process. Scab formation is a sign that the wound has entered the hemostatic phase, where red blood cells and plasma clump together as part of the body's natural mechanism to repair damage. **[19; 20]**. Additionally, angiogenesis serves as an indicator in the wound healing process because it positively affects maintaining cell metabolism and supplying oxygen and nutrients necessary to accelerate skin tissue regeneration **[21]**. Thus, angiogenesis is used as an indicator in burn wound healing processes.

Scab formation is influenced by gel formulation, where gels containing higher active compounds can accelerate the scab formation process. Binahong leaf gel with HPMC (*Hydroxypropyl Methylcellulose*) has the potential to accelerate scab formation, making it effective for treating second-degree burn wounds. The combination of plant extract with HPMC accelerates scab formation and enhances healing effectiveness. HPMC aids in the absorption of bioactive compounds, maintains wound moisture, and facilitates gradual drug release **[22]**. The addition of HPMC in the base provides optimal viscosity and ensures that the active ingredients of binahong leaves migrate to the absorption site, thereby enhancing the effectiveness of burn wound healing **[23]**.

The 12 grams of binahong leaf extract with 3% HPMC (P3) is most effective in accelerating scab formation. Active compounds in binahong leaves such as flavonoids promote blood flow, while tannins aid in blood clotting for wound closure. It's noted that these active compounds support fibrin formation, which forms the scab over the wound surface. Subsequently, tissue repair continues until new skin tissue forms, naturally causing the scab to detach **[24]**.

The quantity of active compounds in the gel plays a crucial role in enhancing wound healing effects beneficial for burn tissue regeneration, as evidenced in P3. Gels containing higher levels of bioactive compounds can expedite the inflammatory phase and stimulate rapid regeneration. Conversely, gels with fewer bioactive compounds result in slower effects during the burn wound healing process **[25]**.

The increased angiogenesis in P3 is attributed to higher levels of tannin in the gel formulation, which influences angiogenesis. Tannins can accelerate wound healing by promoting the formation of new blood vessels, reducing inflammation, and ensuring well-controlled inflammation for effective angiogenesis **[26]**. The formation of new blood vessels also signifies an enhanced supply of nutrients and oxygen to the injured tissue **[27]**. This process supports tissue regeneration and contributes to the overall healing of the wound **[21].**

5. CONCLUSION

It is concluded that binahong leaf gel (*Anredera cordifolia*) with the addition of HPMC has an effect in narrowing the wound area and increasing angiogenesis in mice (*Mus musculus*) with second-degree burns. The most optimal result was obtained in the P3 treatment, namely with 12 grams of Binahong leaf extract.

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