

The Effect of Giving Gel Combination of Binahong Leaf Extract and Turmeric Rhizome Extract on Histopathological Epithelial Thickness in II B Degree Burn of *Rattus norvegicus*

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ABSTRACT

This aim of this research was to study the effect of giving a combination gel of binahong (*Anredera cordifolia*) leaf and turmeric (*Curcuma longa Linn*) rhizome extract on histopathology of epithelial thickness which has II B degree burns. Twenty five male white rats (*Rattus norvegicus*) were divided into five groups. K(-) group was normal skin, K(+) group was skin burns treated with 1% silver sulfadiazine, P1, P2, and P3 was skin burns treated with gel combination of binahong leaf extract and turmeric rhizome extract with increase concentration of binahong leaf extract i.e. 1.25%, 2.5%, and 5%, while the concentration of turmeric rhizome extract i.e. 2% for each treatment. The combination gel of binahong leaf extract 1.25% and turmeric rhizome extract 2% has potential to be an effective treatment for II B burns as indicated by increased of epithelial thickness. Therefore, this study concluded that 1.25% combination gel of binahong leaf extract and turmeric rhizome extract 2% is the effective dosage to increase epithelial thickness on II B healing degree burns wound healing.

Keywords: Binahong leaves, tumeric rhizome, epithelial thickness, healing burns

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INTRODUCTION

The prevalence of burns in Indonesia in 2018 was 1.3% and experienced an increase of 0.6% compared to 2013 of 0.7%. The highest proportion of burns was found in Papua, which reached 2.1% (RI Ministry of Health, 2018).

Burns are classified according to the depth of tissue injury into three, namely I degree (epidermal) burns, II degree (partial- thickness) burns, and III degree (full- thickness) burns. Clinically, II degree burns can be categorized into two, namely II A degree burns or superficial and II B degree burns or deep. II B degree burns are burns that extend to the lower dermis layer and will heal within 3-9 weeks and cause scarring (Kagan, et al., 2013).

Silver sulfadiazine 1% is a material that is commonly used as a topical ingredient for the treatment of burns. The use of silver sulfadiazine has negative effects such as inhibited and incomplete re-epithelialization processes, formation of black scars, hypersensitivity, neutropenia, toxicity to silver, thrombocytopenia, and is ineffective against some microorganisms (Saeidinia, et al., 2017).

The binahong plant (*Anredera cordifolia*) contains bioactive compounds such as flavonoids, saponins and tannins. Flavonoids in binahong leaves have anti-inflammatory effects. Saponins work as an antiseptic that can stop or prevent the growth of microorganisms in wounds to avoid infection, increase the number of

fibroblast cells and stimulate cell regeneration (Garmana, et al., 2014).

Traditional medicine with turmeric rhizome (*Curcuma longa* Linn) is used as an anti-inflammatory, antiseptic, anti-irritant, anorexia, wound medicine, and liver disorders. Turmeric rhizome contains curcumin compounds which can increase the speed of re-epithelialization, cell proliferation, and collagen synthesis (Wientarsih, et al., 2012).

METHODS

Time and Place of Research

The research was conducted at the Faculty of Veterinary Medicine, Airlangga University, Surabaya from October to December 2022.

Sample and Sample Size

This study used 25 white rats (*Rattus norvegicus*) from the wistar strain as experimental animals and the skins of white rats as samples. Samples will be taken on the 15th day after being treated and made into histopathological preparations.

Preparation of binahong leaf extract and turmeric rhizome

Binahong leaves and turmeric rhizome which have been obtained, washed, dried, cut into small pieces then dried again in aerated manner continued in the oven at 40°C until dry and ground separately until smooth.

Each sample that had become powder was soaked in 96% ethanol with a ratio of 1: 10 for five days in a measuring cup separately. After five days the debris and the first filtrate from the binahong leaves and turmeric rhizome were separated using filter paper. The first debris from each sample was then soaked again using 96% ethanol in a different vessel for two days. Then the debris and the second filtrate

from each sample were separated using filter paper.

The first and second filtrates of the binahong leaves are combined and filtered again to ensure that there is no debris left. The same thing was done with the first and second filtrate of turmeric rhizome. Each filtrate of the two samples was evaporated using a vacuum evaporator with a temperature of 60oC to obtain an almost thick extract and continued using a water bath at 60oC to obtain a thick extract (Paju, et al., 2013).

Gel combination preparation

The gel combination formulation of binahong leaf extract and turmeric rhizome was made with a concentration of 1.25% binahong leaf extract + 2% turmeric rhizome extract, 2.5% binahong leaf extract + 2% turmeric rhizome extract and 5% binahong leaf extract + 2% rhizome extract turmeric in 25 g of medicinal preparation. All ingredients used are weighed according to the formulation then mixed and stirred until homogeneous and a gel is formed. For the preparation of gel with a concentration of 1.25% binahong leaf extract + 2% turmeric rhizome extract, 2.5% binahong leaf extract + 2% turmeric rhizome extract and 5% binahong leaf extract + 2% turmeric rhizome extract is done in the same way (Kurnianto, 2017).

Procedure for making second degree burns

After the rats were acclimatized for seven days, they were first given intramuscular anesthesia in the form of a mixture of ketamine and xylazine. Shave the right gluteal part of the rat that will be burned and apply 70% alcohol using a cotton swab. A modified heated thermostat was applied to the rat's right gluteal area for five seconds to produce second degree B burns.

Treatment and treatment procedures for second degree burns

Mice were divided into five groups, namely K(-) were normal skin, K(+) were burns treated with 1% silver sulfadiazine, P1, P2, and P3 were burns treated with a gel combination of binahong leaf extract and turmeric rhizome with the addition of the concentration of binahong leaf extract was 1.25%, 2.5%, and 5%, while the concentration of turmeric rhizome extract was 2% for each treatment.

Observation and measurement of histopathological preparations

Observation and measurement of histopathological preparations is based on the thickness of the epithelium. Histopathological preparations were observed using a microscope with a 100x lens magnification and documented in photographs using the OptiLab Viewer. The results of the photos were then measured for the thickness of the epithelium in μm units using Image Raster 3 software. From five fields of view, one point had the thinnest epithelium and one point had the thickest epithelium was then added and averaged per treatment group per each repetition (Palumpun, et al., 2017).

Data analysis

Data were analyzed using the SPSS program and a One Way ANOVA statistical test was performed. If there is a significant difference between the treatment groups, then the Duncan's test will be continued.

RESULT AND DISCUSSION

First, the data normality test was carried out using the Kolmogorov-Smirnov (KS) and the results obtained were $p > 0.05$, which means that the research data were normally distributed. Second, a data homogeneity test was

carried out using Levene Statistics and the results obtained were $p > 0.05$, which means that the data came from populations with the same (homogeneous) variance.

The One Way ANOVA statistical test was carried out and the results obtained were $p < 0.05$, which means that there were significant differences for each treatment group. The final process is Duncan's test. The average in μm units and standard deviation (SD) of epithelial thickness of the K(-), K(+), P1, P2, and P3 treatment groups can be seen in Table 1.

Based on Table 1, the results for K(-) were significantly different from K(+), P1, P2, and P3 ($p < 0.05$). K(+) was significantly different from P1 ($p < 0.05$) and not significantly different from P2 and P3 ($p > 0.05$). P1 was not significantly different from P2 and P3 ($p > 0.05$). P1 showed no significant difference with P2 and P3, so to determine the right dose of gel combination of binahong leaf extract and turmeric rhizome it is necessary to compare the three treatment groups with K(-) and K(+). The results show that P1 is significantly different from K(-) and K(+). An illustration of the results of epithelial thickness measurements can be seen in Figure 1.

P1 epithelial thickness showed the highest average of $83.17 \pm 20.41 \mu\text{m}$. P2 was $69.24 \pm 19.60 \mu\text{m}$ which was not much different from P3 $63.89 \pm 21.75 \mu\text{m}$. K(+) $46.12 \pm 15.67 \mu\text{m}$ and finally K(-) $15.73 \pm 0.66 \mu\text{m}$. The results of observations of epithelial thickness showed that K(-) had the thinnest epithelium, followed by K(+). P2 and P3 have epithelial thickness that is not much different and P1 has the thickest epithelium. Histopathological picture of epithelial thickness can be seen in Figure 2.

The results of this study were that the gel combination of binahong leaf

extract and turmeric rhizome could increase the thickness of the epithelium in second degree B burns. The highest epithelial thickness was $83.17 \pm 20.41 \mu\text{m}$ in the P1 treatment group where the burns were treated with the combination binahong leaf extract gel 1 25% and 2% turmeric rhizome. P1 epithelial thickness was higher than P2 with 2.5% binahong leaf extract and 2% turmeric rhizome of $69.24 \pm 19.60 \mu\text{m}$ and P3 with 5% binahong leaf extract and 2% turmeric rhizome of $63.89 \pm 21.75 \mu\text{m}$.

The increase in P1 epithelial thickness was better than P2 and P3 proving that P1 has the right composition so that it shows optimal results even though it has a smaller concentration and contains fewer active substances. This is due to the synergistic interaction between binahong leaf extract and turmeric rhizome (Kurnijasanti, et al., 2014). In accordance with research conducted by Indriani, et al. (2019) that the addition of turmeric extract can increase the analgesic power of binahong leaf extract by more than 50% compared to a single dose. Evidenced by the dose of binahong leaf extract which is smaller than the effective dose (5%) can show better results with the addition of 2% turmeric rhizome extract.

The results of P2 are not optimal in increasing epithelial thickness, but according to research conducted by Suryaningtyas (2019) a gel combination of 2.5% binahong leaves and 2% turmeric rhizome is an effective concentration in increasing collagen density and maturity. This could be due to the concentration of binahong leaf extract contained in the gel combination which was too little so that the compound content in the extract was also small. Concentrations of plant extracts that are too low contain only small amounts of saponins which result in the healing process of burns not being

optimal because of a lack of saponins in stimulating collagen.

The results of P3 were also not better than P1 and P2 in increasing epithelial thickness, but according to research conducted by Theresia (2019) a concentration of 5% binahong leaf extract and 2% turmeric rhizome was an effective concentration in increasing the number of fibroblasts in burns. This can be caused because P1 has too much water content and too high humidity so that the number of fibroblasts does not increase.

Data on epithelial thickness P2 and P3 are not better than P1 because in the healing process of burns, certain humidity conditions are needed. High humidity causes oxygen in the wound tissue to also be high so that the proliferation process increases (Paramita, 2016). Another influencing factor is that the levels of flavonoids will decrease at high concentrations. This is because the increase in the consistency of the solution results in a decrease in antioxidant activity (Nijveldt, et al., 2001). According to Indraswary (2014) a high level of solution consistency can inhibit saponins from penetrating the mucous membrane and causing membrane permeability to increase resulting in cell death.

The K(+) treatment group which was given 1% silver sulfadiazine treatment with an average epithelial thickness of $46.12 \pm 15.67 \mu\text{m}$ did not give a better increase in epithelial thickness than P1, P2, and P3. This is because silver sulfadiazine does not have an active substance that can affect the healing process of burns, especially re-epithelialization. According to Lee and Moon (2003) silver sulfadiazine can damage keratinocytes which play a role in the wound re-epithelialization process and cause fibroblast damage which interferes with the maturation process of the collagen matrix.

The K(-) as a negative control which is normal skin has the lowest epithelial thickness of $15.73 \pm 0.66 \mu\text{m}$. This can be caused because in normal skin there

is no inflammatory and proliferative process caused by reactions to the healing process of burns. Re-epithelialization is known to occur in the

Table 1. Average and standard deviation of epithelial thickness in II B degree burns

Treatment Group	Mean \pm SD of Epithelial Thickness (μm)
K(-)	$15.73^a \pm 0.66$
K(+)	$46.12^b \pm 15.67$
P1	$83.17^c \pm 20.41$
P2	$69.24^{bc} \pm 19.60$
P3	$63.89^{bc} \pm 21.75$

different superscripts in the same column show significant differences ($p < 0.05$).

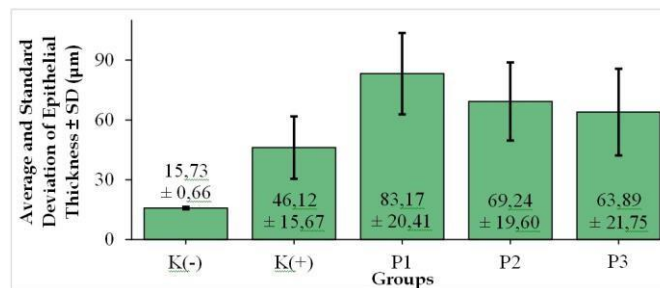


Figure 1. Graph of mean and standard deviation of epithelial thickness in second degree B burns of *Rattus norvegicus*.

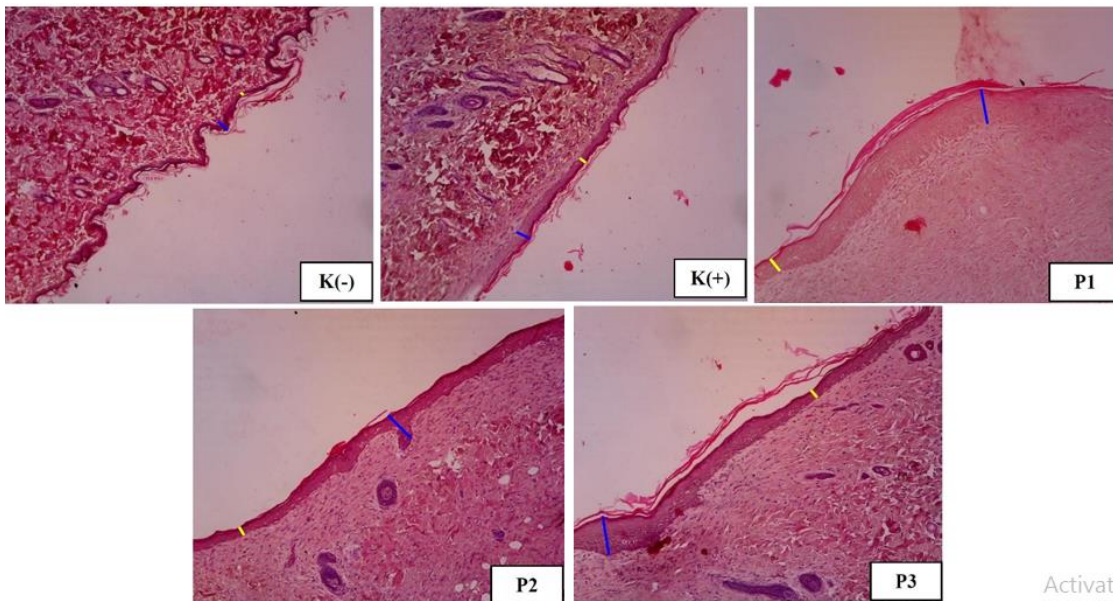


Figure 2. Thickest epithelium histopathology (\rightarrow) and the thinnest (\rightarrow) in second degree B burns with a magnification of 100x.

proliferative phase and will stop when the burn is completely closed then the

maturation phase will begin which lasts from the third week to one year. After re-

epithelialization is completely formed, the new epithelium must be restored so

that connective tissue growth does not occur (Azaria, et al., 2017).

The ability of flavonoids as antioxidants is needed in the healing process because free radicals can inhibit cell proliferation, inflammatory reactions and contraction of the formed collagen tissue, causing delays in the healing process of burns (Paju, et al., 2013).

Saponins are proven to induce blood platelet aggregation so that berberan in hemostasis. The content of frutesaponin B in saponins is known to have benefits as an anti-inflammatory which can suppress the inflammatory reaction in the early stages of injury and increase in the later stages. Saponins can also increase epidermal cell proliferation and migration of keratinocyte cells in the re-epithelialization process (Kim, et al., 2011).

The tannin content in binahong leaves has the benefit of being an astringent which can shrink skin pores, stop exudation and light bleeding so that they can close wounds and prevent bleeding in wounds. Tannins play a role in increasing the migration and proliferation of fibroblasts in the wound so that wound contraction will be faster (Paramita, 2016).

In this study also used 2% turmeric rhizome extract which is an effective concentration according to Mehrabani, et al. (2015). Turmeric has benefits in the wound healing process by accelerating the inflammatory phase and preventing microorganism infection. This is due to the content of curcumin compounds in turmeric which can inhibit the formation of prostaglandins and inhibit the activity of cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) enzymes which are

important enzymes in the inflammatory process. Curcumin compounds can also increase the speed of re-epithelialization, cell proliferation and collagen synthesis (Thangapazham, et al., 2007).

CONCLUSION

Based on the results of the study it can be concluded that the gel combination of binahong leaf extract and turmeric rhizome extract can increase the thickness of the epithelium in the healing process of second degree B burns of *Rattus norvegicus*.

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