

***THE EFFECT OF CREAM-BASED APPLICATION OF MORINGA LEAF (MORINGA OLEIFERA) EXTRACT ON THE INCISION WOUND HEALING ON WHITE RAT***

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**ABSTRACT**

**Background:** High injury incidence is a consideration of wound prevention with the prevalence of iris/torn/puncture injuries 20.1% in 2018. Indonesian people have a high level of confidence in traditional medicine and one of them is Moringa. Moringa phytochemical content has the potential to speed up the healing process of incision wounds.

**Purpose:** This study aims to determine the effect of moringa leaf extract cream with concentrations of 5%, 15%, and 45% on the healing process of white rat incision wounds (*Rattus norvegicus*) by assessing the healing phase and using the Bates-Jensen Wound Assessment Tool (BWAT).

**Method:** This research is experimental with a post-test-only control group design. Sampling by simple random sampling method and calculated using Federer's formula with the number of samples of 30 white rats divided into five groups, namely two groups of control and three treatment groups with 6 white rats in each group for 21 days.

**Results:** In the analysis of BWAT score data with the MANNOVA test, there were insignificant results on day 1 ( $p=0.840$ ) and day 7 ( $p=0.888$ ), then there were significant results on day 14 ( $p=0.001$ ). Post-hoc results showed significant differences between the negative control group and positive control over the 45% moringa leaf extract creaming group. In the analysis of healing phase data with the Kruskal-Wallis's test, significant results were obtained in the coagulation phase ( $p=0.000$ ) and the proliferation phase ( $p=0.001$ ). The results of the Mann-Whitney test in the coagulation phase showed significant differences between the control and treatment groups while in the proliferation phase there were significant results between the control group and the treatment and there were no meaningful differences between the treatment groups.

**Conclusion:** There is no significant difference in effectiveness between the administration of moringa leaf extract cream to accelerate the healing process of incision wounds.

**Keywords:** Cream, moringa leaf extract, incision wound, BWAT, coagulation, proliferation.

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## INTRODUCTION

Skin is the human largest organ that functions as a barrier from external injuries and shields mechanical injuries and also functions as the body's thermoregulator<sup>1,2</sup>. Wounds are the result of trauma that caused morphological damage to the skin tissue or the underlying tissue. Based on the report of Basic Health Research (Riskesdas) 2013, the prevalence of laceration wound (23,2%)<sup>4</sup> whereas on Riskesdas 2018, the prevalence of laceration wound became 20,1%.<sup>5</sup> Wound healing is the regeneration of the skin's tissue and/or the underlying tissue. Normal wound healing consists of five phases which are coagulation and hemostasis, inflammation and debridement, proliferation, epithelialization, and remodeling<sup>1,6,7</sup>.

Pharmacological therapy can be given through several pathways, namely through enteral, parenteral, topical, inhalation, or transdermal pathways.<sup>8</sup> Topical or transdermal pathways have a high bioavailability rate of 8% to 100%.<sup>8</sup> The dermis layer has a high permeability rate of many substances, especially lipids, so absorption can be improved by using preparations suspended by oils such as creams.

According to Riskesdas 2010 that the percentage of Indonesians who have consumed herbal medicine as much as 59.12% in the age group over 15 years and 95.60% feel the benefits<sup>9</sup>. One of the herbal remedies that are widely used and researched is *Moringa oleifera*.

*Moringa* is a traditional medicine derived from the family Moringaceae that has been used by the people of Indonesia for a long time. Almost all parts of *moringa* have a good effect on health, especially the leaf has a rich content of vitamin A, vitamin B (B9, B6, B3), vitamin C, vitamin D, and vitamin E and has the potential as an anti-oxidant, anti-cancer, anti-inflammatory, antibiotic, and anti-diabetic. In addition, *moringa* leaves are also rich in calcium and iron. The phytochemical content of *moringa* has high levels such as alkaloids, steroids,

saponins, flavonoids, tannins, and triterpenoids. Flavonoids have an effect that prevents stiffness and pain as well as reduces inflammatory reactions during bleeding<sup>10-12</sup>.

Some studies show that the content of flavonoids, tannins, and saponins in *moringa* has anti-inflammatory, antibiotic, and anti-fungal effects proven to speed up the wound healing process in mice. One study mentioned that using *moringa* extract gel preparations at a concentration of 15% effectively accelerates the healing of incision wounds on guinea pigs. In another study, it was mentioned that<sup>13</sup> the use of hydrogels of 5% and 10% was also effective in accelerating the healing of incision wounds in white mice in the cell proliferation of mitogenic activity. In addition, the use of gel preparations and creams also has a good influence on the process of healing burns.<sup>14</sup>

Bates-Jensen Wound Assessment Tool (BWAT) has a sensitivity of 61% and specificity of 52% which is quite high, has a high application value in the assessment of the wound healing process. Quantitative assessments at BWAT include wound size (using wound surface area), wound depth, wound end, edge of the wound, number and type of necrosis, type and number of exudates, skin color around the wound, peripheral tissue induration and edema, granulation tissue, and epithelialization.<sup>16,17</sup>

Based on the data above, previous research has proven that *moringa* can accelerate the healing process of incision wounds in mice, so this study was conducted to compare the difference in the effectiveness of the use of *Moringa oleifera* in cream preparations with concentrations of 5%, 15% and 45% in the wound healing process topically through macroscopic assessment and acceleration of the healing process of wounds. In white rats (*Rattus norvegicus*) using the Bates-Jensen Wound Assessment Tool (BWAT). Concentrations of 5%, 15%, and 45%.

## 1. METHODS

The research was conducted experimentally using the posttest only control group design method. The study used white mice (*Rattus norvegicus*) male Wistar strains. The sample is collected by randomization. The sampling method in this study is simple random sampling.

The subjects of the study were 30 white rats (*Rattus norvegicus*) divided into 5 groups. The negative control group was not given treatment, the positive control group was given 10% povidone-iodine, treatment group 1 was given moringa leaf extract cream 5%, treatment group 2 was given cream moringa leaf extract 15%, treatment group 3 was given cream moringa leaf extract 45%.

The test animals were adapted for 7 days to observe the physical condition, behavior of mice, and maintain the weight of mice to remain within the inclusion criteria. After adaptation for 7 days, the test animals were then given the effect of sedation first using a mixture of 1 to 1 xylazine and ketamine with doses of 10mg xylazine and 50 mg of ketamine. (0.1 cc/g of rat's weight). After that, the fur on the back of the rat will be shaved 3x3 cm on the area to be done incision and then disinfect using 10% povidone-iodine in the area. Incision wound making is done using a scalpel with a wound length of two centimeters with a wound depth of 2 mm by a veterinarian. After the incision process, the mice were treated based on the treatment group 2 times a day in the morning and evening for 21 days. During treatment, the coagulation and hemostasis phase of the wound will be assessed by pressing using sterile gauze periodically and the wound healing process is measured using micrometers and assessed. Macroscopic uses the Bates-Jensen Wound Assessment Tool (BWAT).

## 2. RESEARCH RESULTS

### BWAT Score Assessment Results

Observations were made daily for 21 days using the Bates-Jensen Wound Assessment Tool (BWAT) which consists of the size of the wound (using the surface area of the wound), the depth of the wound, the end of the wound, the edge of the wound, the edge of the wound, the number, and type of necrosis, the type and number of exudates, the color of the skin around the wound, the duration and edema of peripheral tissues, granulation tissue, and epithelialization. It's in Table 1.

After 21 days of observation, the observations were analyzed with Shapiro Wilk's normality test at  $p=0.000$  ( $p<0.05$ ), and the homogeneity test was obtained at  $p=0.548$  ( $p>0.05$ ). Then the data analysis continued using the MANNOVA test to find out if there are significant differences in the administration of moringa leaf extract cream.

Table 1. Bates-Jensen Wound Assessment Tool (BWAT)<sup>16</sup>

	Date	Date	Date
	Score	Score	Score
Size			
Depth			
Edge			
Undermining			
Necrotic tissue Type			
Necrotic tissue amount			
Exudate type			
Exudate amount			
Skin color surrounding wound			
Peripheral tissue edema			
Induration			
Granulation tissue			
Epithelialization			

The results of the data analysis in Table 2 showed that there was no significant difference between the control group and the treatment group on day 1 ( $p=0.840$ ) and day 7 ( $p=0.888$ ), while there were significant results between the groups on day 14 ( $p=0.001$ ). After that, a post hoc test is conducted to find out the meaningful differences between groups. Since the above data is homogeneous, it will see the Bonferroni test value.

Table 2. Univariate ANOVA results in test

	Day	Sig.	Result
Group	1	0.84	Insignificant
	7	0.888	Insignificant
	14	0.001	Significant
	21	-	

MANNOVA BWAT Score

In the results of post hoc tests using MANNOVA obtained there is a significant result on the 14th day, between the KN group against the P3 group ( $p=0.000$ ) and the KP group against the P3 group ( $p=0.006$ ). So, it can be concluded that the administration of moringa leaf extract cream concentration of 45% has a meaningful influence on the decrease in BWAT score against the KN group and KP group on the 14th day.

### Results of Healing Phase Assessment

Shapiro Wilks' normality test results showed a  $p$ -value of  $<0.05$  and a homogeneity test  $p >0.05$  so it will use Wallis Kruskal test analysis to determine the difference in treatment results. In the Kruskal Wallis  $p$  test obtained in the results of the analysis of the coagulation phase which is  $p = 0.000$  ( $p <0.05$ ), it can be concluded that the administration of moringa leaf extract cream has a significant influence on the acceleration of coagulation and hemostasis.

It can also be seen that in the proliferation phase of Asymp value. Sig. showing the value  $p = 0.001$  (the critical limit value of the study  $p <0.05$ ) which means that there is a meaningful influence

in the administration of moringa leaf extract cream on wounds.

Table 3. Kruskal-Wallis Analysis Results

	Healing Phase	
	Coagulation	Proliferation
Asym. Sig ( $p <0.05$ )	0	0.001
Result	Significant	Significant

Next, continue with Mann Whitney's posthoc analysis test to find out the differences of each group. In the analysis of coagulation and hemostasis phases, Mann Whitney test results showed meaningful differences between the control group and the treatment group with significance  $p <0.05$ .

Table 4. Mann-Whitney Coagulation Phase Results

Group	Sig.	Result
KN v KP	0.004	Significant
KN v P1	0.004	Significant
KN v P2	0.004	Significant
KN v P3	0.004	Significant
KP v P1	0.004	Significant
KP v P2	0.004	Significant
KP v P3	0.004	Significant
P1 v P2	0.006	Significant
P1 v P3	0.004	Significant
P2 v P3	0.013	Significant

The proliferation phase analysis showed a meaningful difference between the control group and the treatment group. While between treatment groups, there is no significant difference. This meaningless difference is thought to be due to an anomaly in a mouse suspected of hematological abnormalities that led to 88 slower healing processes than in his group. In addition, it is also suspected that the activity of rats who clean the wound area after being given a dab of moringa leaf extract cream so that the cream does not work effectively in the mice.

Table 5. Mann-Whitney Proliferation Phase Results

Group	Sig.	Result
KN v KP	0.092	Insignificant
KN v P1	0.003	Significant
KN v P2	0.018	Significant
KN v P3	0.005	Significant
KP v P1	0.012	Significant
KP v P2	0.059	Insignificant
KP v P3	0.005	Significant
P1 v P2	0.742	Insignificant
P1 v P3	0.055	Insignificant
P2 v P3	0.130	Insignificant

### 3. DISCUSSION

#### BWAT Score Assessment Results

The results of research and data analysis based on BWAT Score assessment showed that the average BWAT score of the KN group was 16.38, the KP group was 16.07, then in The Treatment Group, 1 was 15.89. The Treatment Group) was 15.90, and the Treatment Group 3 15.69. This showed that Treatment Group 3 had the lowest average score indicating that the healing process was the fastest. In the MANNOVA test, it was found that the administration of moringa leaf extract cream did not have a meaningful effect on day 1 and day 7 but had a meaningful difference on the 14th day. It was also supported in *post-hoc* tests of comparison between control groups and between treatment groups did not give significant results on day 1 and day 7, and significant results were obtained between the KN group against the P3 group ( $p=0,000$ ) and also the KP group against the P3 group ( $p=0.006$ ). The MANNOVA test obtained insignificant results in this study because from the first day the score was already at a low level (21-30) and within 2 days entered the minimum category (13-20). The low value was obtained because the wounds in these mice included acute wounds with partial-thickness loss as well as a size that from the beginning was small ( $<4 \text{ cm}^2$ ).

#### Results of Healing Phase Assessment

The study also looked at the healing process based on its phases of coagulation and hemostasis, inflammation, and

proliferation. The coagulation and hemostasis phase is done by pressing sterile gauze periodically on the wound area and seeing if there is still blood in the gauze. The results of the study found that the KN Group got an average coagulation time of 639.50 seconds, then the KP Group got an average coagulation time of 557.83 seconds. The P1 group got an average coagulation time of 500.67 seconds, then the P2 group got an average coagulation time of 445.83 seconds, and the P3 group got an average coagulation time of 405.5 seconds. It can be seen that group P3 has the fastest coagulation time, and the administration of moringa leaf extract cream speeds up the wound coagulation process.

Povidone which is a synthetic polymer 1- vinylpyrrolidone which is hygroscopic (can absorb water from environmental areas) and can dissolve in water and can work as a "thickening and dispersing agent" that helps in freezing, and iodine content It is corrosive to tissues due to its oxidation potential, thus aiding in chemical cauterization. In the treatment group, there was a shorter bleeding time compared to the control group, this is thought to be because the flavonoid content plays a large role in shortening the bleeding time<sup>18</sup> to maintain the permeability of blood vessels and increasing peripheral blood vessel resistance so that vasoconstriction occurs. Then tannins are also suspected to be consigned by precipitating the thrombin, accelerating in the cascade of coagulation, precipitated thrombin turns fibrinogen into fibrin fibers that will stop bleeding.<sup>19</sup>

The inflammatory phase cannot be observed because it cannot be seen clearly, allegedly because of the use of a razor that is less able to completely clean the entire hair so that inflammatory signs such as heat, rubor, and tumors cannot be observed.

The proliferation phase in this study was seen when the wound was clean, there was no redness, no crust, and the same skin tissue color as the surrounding skin color, showed there-epithelialization was

successful and finished and proceeded to the *remodeling stage*. Based on the results of the study, the KN group got an average proliferation of 19.83 days, then the KP group at 18.83 days. In the treatment group, the P1 group and the P2 group had an average of 17 days of proliferation, and the P3 group got an average of 15.6 days of proliferation. It can be concluded that the P3 group has the fastest rate of proliferation compared to the P1 group and the P2 group.

Research by Poernomo shows that giving moringa leaf gel a 15% concentration significantly increases collagen density levels, supporting speeding up the proliferation phase and<sup>13</sup> remodeling phase. Flavonoids act as antibacterial, antioxidant, and anti-inflammatory by inhibiting the metabolic pathways of arachidonic acid and the release of histamine in the inflammatory phase, as well as by inhibiting prostaglandin secretion. Alkaloid content also tends to play a role in the process of collagen fiber formation that prevents cell damage through DNA synthesis so that the growth of new tissue in the wound becomes denser, stronger, and faster. Vitamin C stimulates collagen synthesis and fibroblast proliferation<sup>20</sup> and Vitamin A increases fibroblast proliferation, modulates cell differentiation, increases collagen synthesis and hyaluronics<sup>21,22</sup>

#### 4. CONCLUSION

So, it can be concluded that the administration of moringa leaf extract cream does not affect the healing process of white rat incision wounds and there is no difference in effectiveness in giving moringa leaf extract cream with concentrations of 5%, 15%, and 45% against the healing process of white rat incision wounds.

#### 5. SUGGESTION

1. A wound assessment tool that is more suitable for acute wounds with partial-thickness loss is required to see the effect

of Moringa leaf extract cream more clearly.

2. A better shaving tool is required to assess the skin more clearly.
3. In further research, it is necessary to minimize errors that occur when conducting research.

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