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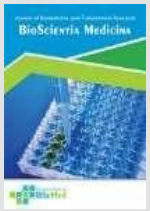

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The Effectiveness of *Moringa oleifera* Burn Healing: In Vivo Study

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A B S T R A C T

Background: Burns is a type of wound that requires more intensive treatment considering the damage from these wounds is quite massive and very often causes infection. Topical antimicrobials used today have few side effects and are only partially effective in wound healing. The aim of the study was to explore the potential of *Moringa oleifera* on burn wound healing in vivo. **Methods:** This study is an experimental study using 24 rats. Rats were grouped into 3 treatment groups and 1 control group. Furthermore, the number of fibroblasts and PMN cells was assessed in the wound tissue. Data analysis was done by univariate and bivariate. **Results:** The combination of sulfur sulfadiazine and *Moringa oleifera* was able to significantly increase the number of fibroblasts and reduce PMN cells in burn tissue. **Conclusion:** *Moringa oleifera* extract has the potential to accelerate burn healing by inhibiting inflammation and fibroblast activation.

1. Introduction

Wounds are injuries to parts of the body that mainly occur in the skin area.[1,2] There are various types of wounds, including cuts, lacerations, abrasions, bruises, ulcers, and burns. Wound care generally aims to prevent infection, considering the function of the skin as a barrier against infection.[3] Burns is a type of wound that requires more intensive care, considering that the damage from these wounds is quite massive and often causes infection.[4] Treatment of minor burns is done with ointments and topical, in contrast to the treatment of severe burns, which require intensive care and hospitalization.[5] Improper wound care can delay healing, cause the area to become infected, and lead to chronic wounds.[6] Antimicrobial ointments such as silver sulfadiazine, mafenide, silver nitrate, povidone-iodine, mupirocin, and bacitracin, are used to reduce the risk of infection in minor wounds and burns.[7] However, these topical antimicrobials have few side effects and are only partially effective in wound healing. Therefore, new drugs are needed to optimize burn healing.[8,9]

Indonesia is a country with the second largest biological wealth in the world after Brazil. The potential of this biological wealth is great for Indonesia

in the development of new modality therapy for wound management. *Moringa oleifera* is one of the plants that are often found in Indonesia and is a plant with extraordinary medicinal potential.[10] This plant has been used for generations to treat various health problems such as constipation, diarrhea, and skin health. *Moringa oleifera* (MO) is rich in primary metabolites, secondary metabolites, and various vitamins and minerals. The rich content of various metabolites, vitamins, and minerals makes *Moringa oleifera* rich in benefits. *Moringa oleifera* is rich in anthraquinones, especially emodin and chrysophanol. Anthraquinones have potent anti-inflammatory effects, which have the potential to activate various growth factors and chemokines and initiate angiogenesis processes that play a major role in wound healing.[11,12]

This study is one of the first studies to explore the potential of *Moringa oleifera* on burn wound healing in vivo. Wound healing was evaluated based on the number of fibroblasts and polymorphonuclear inflammatory cell infiltration.

2. Methods

This study is an experimental study with a post-test-only approach with a control group design. A total of 24 rats (*Rattus norvegicus*) strains were included in this study and met the inclusion criteria in the form of the male, weighing between 150-200 grams, and after 8-10 weeks. First, the rats were acclimatized for 7 days, then divided into 4 groups (K1, K2, K3, and K4) randomly, where each group consisted of 6 rats. Group K1: the group that induced burns and received 10% *Moringa oleifera* extract ointment. K2 group: burn-induced group and received 1% Silver sulfadiazine (SSD) topically and 10% *Moringa oleifera* topically. Group K3: the group that induced burns and received 1% Silver sulfadiazine topically, and K4: the group that induced burns and was only given a vehicle. This study has been approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Diponegoro, with the number No.110/EC/H/FK-RSDK/XI/2018.

Leaf extract of *Moringa oleifera* was obtained from the chemical laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Diponegoro. The material used was *Moringa oleifera*. Leaves *Moringa oleifera* obtained were collected and sorted wet, then washed with running water until clean and separated from the stems. Leaves of *Moringa oleifera* that have been cleaned are dried in a cabinet dryer with a max temperature of 40^{0}C. After drying, *Moringa oleifera* is sorted dry and reduced in size. Simplicia leaves were macerated with 96% ethanol solvent, then filtered. After filtering, the ethanol extract obtained was evaporated using a rotary evaporator, then the ethanol extract was weighed. The thick extract obtained was mixed with pure vaseline to obtain 10% *Moringa oleifera*.

Induction of burns was performed by anesthetic first in rats using ketamine (dose of 0.015 mg/gBW) intramuscularly and chlorate (dose of 0.0025 mg/gBW) subcutaneously. Induction of burns using a hot iron measuring 2 x 2 cm which has been soaked in hot water at 100^{o}C for 2 minutes, is done by attaching it to the body of the shaved rat. Rats were monitored daily for signs of distress and signs of infection. On the 10th day, the burn tissue was terminated at the same time: The rats were anesthetized with a mixture of Ketamine-Xylazine (Ketamine dose 80 mg/kgBW; Xylazine dose 10 mg/kgBW) intraperitoneally, and the skin and subcutaneous tissue were cut with a scalpel with a size of 1 x 1 x 1 cm. Pieces of skin tissue were put into a buffered formalin solution (10% formalin solution in sodium acetate buffer until it reached a pH of 7.0). Then do fixation for 18-24 hours. After fixation, the tissue was then put into aquadest for 1 hour so that the fixation solution was lost. The tissue pieces were then put in graded concentration alcohol, alcohol-xylol solution for 1 hour, and then pure xylol solution for 2 x 2 hours. The tissue was then put into a liquid paraffin solution for 2 x 2 hours, after which the tissue was embedded in solid paraffin, having a melting point of 56-58^{0}C. The tissue was cut about 6 m and glued onto a glass object that had been previously smeared

with polylysine. Glass object was then heated in an incubator at a temperature of 56-58^{0}C until the paraffin melted.

The next step is to stain the tissue with hematoxylin-eosin. The tissue was put into xylol I and II preparations for 5 minutes each. Rehydrate using absolute alcohol for 3 x 2 minutes. The preparation was washed with running water for 2 minutes. The preparation was put in hematoxylin-eosin (Lilie-Mayer) for 5 minutes and rinsed with running water for 2 minutes. The preparation was differentiated with 0.6% HCl for 1-2 dips and rinsed with running water for 2 minutes. The preparation is immersed in a saturated lithium carbonate solution about 2-3 times and rinsed with running water for 2 minutes. If the color is not blue enough, the preparation can be put back into the H&E solution for 2 minutes, then rinsed with running water. The preparation was immersed in eosin for 3 minutes. Dehydrated using 70% alcohol for 3x3 minutes for each concentration. Clearing with xylol I and II, dripped 1-2 drops of entelan. The preparation is closed with a cover slip. Furthermore, histopathological assessment of fibroblasts and polymorphonuclear (PMN) was carried out by Anatomical Pathologists with the help of ImageJ software.

After the data is collected, data cleaning, coding, and tabulation are carried out. All results were assessed by means ± standard deviation accompanied by a normality test (Shapiro Wilk) and data homogeneity test (Levene Statistic). The test used in this study was one-way ANOVA, followed by a post-hoc test to assess differences between groups. The results are said to be meaningful if $p \leq 0.05$. Data analysis was performed using SPSS version 25 for Windows.

3. Results

Table 1 shows the effect of MO extract on fibroblast cells in burn tissue. Administration of MO (group 1) was able to increase the number of fibroblast cells significantly compared to group 4, which was not given treatment. The combination of SSD and MO (group 2) was significantly able to increase fibroblast cells much higher than those who received MO alone (group 1) and SSD only (group 3).

Table 1. Comparison of the number of fibroblasts between treatments

Group	Mean ± SD	p-value*
1	13.32 ± 3.82	0.002
2	15.76 ± 3.24	0.001
3	10.12 ± 4.42	0.03
4	11.24 ± 3.76	-

*post hoc test vs group 4, p=0.05

Table 2 shows the effect of MO extract on PMN cells in burn tissue. Administration of MO (group 1) was able to significantly reduce the number of PMN cells compared to group 4, which was not treated. Administration of a combination of SSD and MO (group 2) was able to significantly reduce PMN cells much lower than those who only received MO alone (group 1) and SSD only (group 3).

Table 2. Comparison of PMN cells between treatments

Group	Mean ± SD	p-value*
1	263.00 ± 28.97	0.002
2	175.28 ± 18.67	0.002
3	222.70 ± 34.83	0.003
4	315.52 ± 24.97	-

*post hoc test vs group 4, p=0.05

4. Discussion

Wound healing is an essential process to restore continuity of damaged tissue and restore normal function of affected skin. Wound healing involves ongoing interactions between body cells, which go through 4 overlapping phases, including inflammation, coagulation, migration, and remodeling.[13] The proliferative phase begins as an acute response to hemostasis and the initiation of the inflammatory process, a period in which wound repair begins with angiogenesis, fibroplasia, and epithelialization. After the inflammatory phase is complete, neovascularization will occur in the wound area, which will reach its peak formation on the 3-5th post-injury day and begin to decrease on the 7th day.[14] During the wound healing process, the number of fibroblasts will increase but will decrease in the final phase of the healing process. Fibroblasts will migrate immediately to the wound site, proliferate and synthesize large amounts of collagen matrix in order to help isolate and repair damaged tissue.[15] Extracts from *Moringa oleifera* are known to contain antioxidant properties derived from their flavonoid content. Flavonoids themselves have been shown to increase the migration and proliferation of epithelial cells as well as the activity of myofibroblasts. Studies show that these flavonoids significantly accelerate wound healing by increasing wound contraction, shortening the epithelialization period, increasing collagen deposition, and causing tissue granulation.[16]

Systemic response to burns is initially proinflammatory but later becomes predominantly anti-inflammatory in an attempt to maintain homeostasis and restore normal physiology. Elevated levels of proinflammatory cytokines are characteristic of the systemic response to burns.[17] IL-1β and TNFα are produced by various cells in this inflammatory response. These two cytokines induce fever, acute phase proteins, and catabolism. These two cytokines also induce the production of prostaglandin E_2, IL-6, and platelet-activating factor. IL-6 also induces fever and the production of acute phase reactants that activate T cells. IFNγ has an important role in macrophage activation and differentiation of CD_4 T cells into Th1. Inhibition from leukocyte migration is an important parameter that needs to be evaluated in the inflammatory response, where neutrophils are known to have a high concentration of ROS and can be associated with damage to the host. One of the properties of Moringa oleifera is the anti-inflammatory property, which is found to have an inhibitory effect against inflammatory cells in various diseases. Studies found that administering *Moringa oleifera* extract reduced the influx from PMN cells towards inflammatory tissue.[18,19]

5. Conclusion

Moringa oleifera extract has the potential to accelerate burn healing by inhibiting inflammation and fibroblast activation.

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