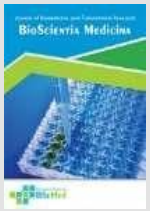
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

The Effect of Ethanolic Extract from *Moringa oleifera* Leaves in Collagen Density and Numbers of New Capillary Vessel Count on Wistar Rats Burn Wound

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ARTICLE INFO

Keywords:
Moringa oleifera
Silver sulfadiazine
Collagen density
Capillary vessel

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v6i6.536

A B S T R A C T

Background: Burns are a major cause of morbidity, including prolonged length of stay and disability, which requires no small amount of treatment costs. Currently, there are many studies to accelerate the healing of wounds, one of which is with Moringa oleifera (MO). This study aimed to prove the effect of ethanolic extract from MO leaves on collagen density and new capillary vessel count on Wistar rats' burn wounds. **Methods:** This research is randomized post-test only with a control group design. Twenty-four male Wistar rats induced burn wounds and were randomly divided into 4 groups which were given topical medicine once daily for 10 days; P1 (MO leaves extract 10%), P2 (Silver Sulfadiazine + MO leaves extract 10%), P3 (SSD), and P4 (negative control). Collagen density was assessed using the Kruskal-Wallis test and continued with Mann-Whitney Test. New capillary vessel count was assessed using Hematoxylin-Eosin staining and One Way ANOVA. The correlation test between two variables was tested using the Spearman test. **Results:** Collagen Density shows significant difference in group P1 compared to P4 (p=0,016), group P2 compared to P3 (p=0,047), group P2 compared to P4 (p=0,009). A significant increase in new capillary blood vessel counts was seen in group P1 compared to P3 and P4 (p=0.001; 0,000) and group P2 compared to P3 and P4 (p=0,000;0,000). A positive correlation was found between the new capillary blood vessel count and epithelization percentage (p=0,001), with a strong correlation (rho=0,682). **Conclusion:** Ethanolic extract from Moringa oleifera leaves proved to be effective in increasing collagen density and new capillary vessel count.

1. Introduction

Burns are a major cause of morbidity, including prolonged length of stay and disability. The World Health Organization (WHO) 2011 noted that burns are the seventh cause of injury in the world.[1]

There are 3 phases of wound healing in cases of burns, namely: the inflammatory phase, the proliferative phase, and the remodeling phase. In the proliferative phase of wound healing, it is characterized by angiogenesis, starting with the response of vascular endothelial growth factor (VEGF), the formation of new blood vessel capillaries, and the formation of new capillaries.[2,3]

several factors, one of the most common causes being the colonization of microorganisms. One choice of topical drugs is topical silver sulfadiazine because of its broad-spectrum antimicrobial properties and ease of use.[4,5]

Currently, there are many studies on *Moringa oleifera* which show therapeutic potential for wound healing by increasing the ability of tissue proliferation. In addition to the potential for wound healing, the use of *Moringa oleifera* leaf extract also showed a significant increase in tensile strength compared to controls.[6,7]

This study aims to prove the effectiveness of giving Moringa oleifera leaf ethanolic extract in increasing the response to burn wound healing in Wistar rats in terms of increasing collagen density and the number of new blood vessel capillaries.

2. Methods

This research is randomized post-test only with a control group design. Twenty-four male Wistar rats induced burn wounds and were randomly divided into 4 groups which were given topical medicine once daily for 10 days; P1 (MO leaves extract 10%), P2 (Silver Sulfadiazine + MO leaves extract 10%), P3 (SSD), and P4 (negative control). Inclusion criteria in this study: male rats aged 2 months, Wistar strain induced by partial-thickness burns, bodyweight ± 150-200 grams, no anatomical abnormalities were seen. Exclusion criteria: no partial-thickness burns, drop-out criteria: during induction and treatment, rats looked sick (inactive motion) or died.

The research was conducted in 4 places: the process of making an ethanolic extract of *Moringa oleifera* in Science Laboratory, Universitas Diponegoro, Semarang; *Moringa oleifera* leaf extract cream was made at the Pharmacology laboratory Laboratory, Semarang; the process of burn induction, treatment (including tissue collection) on experimental animals was carried out at LPPT IV UGM, Yogyakarta; the process of making preparations, Masson's Trichrome staining, Hematoxylin-eosin staining and readings of preparations by 2 observers were carried out at the PA UNS Laboratory, Solo, with research period between September and October 2018.

The independent variable of this study was the administration of 10% *Moringa oleifera* leaf ethanolic extract. The dependent variables in this study were the expression of collagen density and the number of new blood vessel capillaries.

Data analysis includes descriptive analysis and hypothesis testing. The data is presented in the form of tables and graphs. Prior to the analysis, the normality test was carried out with the Shapiro-Wilk test because the number of data was <50. The hypothesis test used on collagen density was the Kruskal-Wallis test because the data distribution was not normal, followed by the Mann-Whitney test to determine the differences between groups. The hypothesis test used on the number of new blood vessel capillaries is the One Way ANOVA test because the data is normally distributed and homogeneous, followed by the Bonferroni Post-Hoc test to determine the differences between groups. The relationship between variables was assessed by Spearman's test. All analyzes were performed by computer using the SPSS 25.0 statistical program. The difference is stated to be significant if the p-value = 0.05.

3. Results

The normality test of collagen density using the Shapiro-Wilk test showed that the data distribution was not normal, so the hypothesis test was continued with the Kruskal-Wallis test. The box plot in Figure 1 shows that the collagen density of the P4 group was lower than that of the P1, P2, P3, and P4 groups. The minimum value was obtained in the P4 group, and the maximum value was obtained in the p2 group.

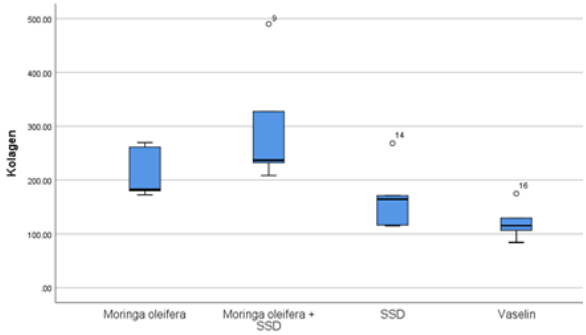

Figure 1. Collagen data box plot

It can be concluded that there was a significant difference in collagen density in the four groups, then the Mann-Whitney test was used to determine the differences between groups (p=0.009) (Table 1). From the results of the Mann-Whitney test, there were significant differences between P4 and P1 (p = 0.016), P2 and P3 (0.047), and P4 and P2 (0.009). with P3 (p = 0.076), and P4 with P3 (0.251).

Table 1. Kruskal-Wallis test for density collagen (*significant p<0,05).

Groups	Collagen density (%) (Mean ± SD)	P
P1	213,2654 ± 47,932	0,009
P2	298,9947 ± 115,9968	
P3	167,1346 ± 62,31881	
P4	122,1453 ± 33,79379	

Table 2. Mann-Whitney test for density collagen between groups (*significant p<0,05).

Groups	P1	P2	P3	P4
P1	-	0,175	0,076	0,016*
P2		-	0,047*	0,009*
P3			-	0,251

Figure 2 shows data on the number of new blood vessel capillaries, which is at P4, which is lower than P1, P2, and P3. The data normality test with the Shapiro-Wilk test showed a normal data distribution, and then the data homogeneity test was carried out. The homogeneity test of the data showed p = 0.139, so it was concluded that the data was homogeneous, and the hypothesis test was continued with the One Way ANOVA difference test.

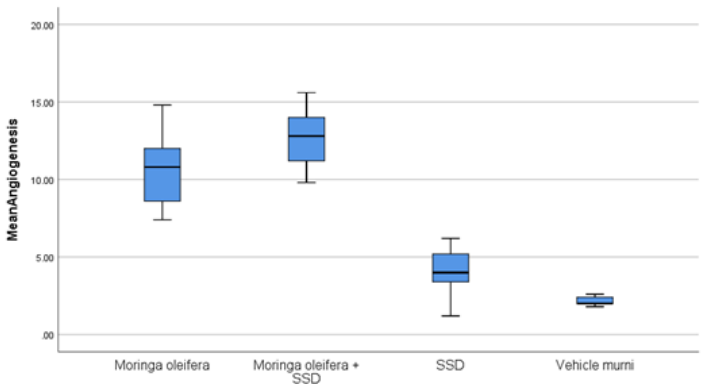

Figure 2. Box plot graph of the number of new blood vessel capillaries.

Table 3. One Way ANOVA test the number of new blood vessel capillaries

Groups	Mean ± SD	P
P1	10,72 ± 2,91	0,000*
P2	12,68 ± 2,28	
P3	4,00 ± 1,90	
P4	2,16 ± 0,33	

Post-hoc test (table 4) showed significant results in P1 with P3 and P4 (p=0.001;0.000) and P2 with P3 and P4 (p=0.000;0.000).

Table 4. Post-hoc test (Bonferroni) between groups number of capillaries blood vessels

Variable	Groups	P1	P2	P3	P4
Capillary vessel	P1	-	0,939	0,001*	0,000*
	P2	0,939	-	0,000*	0,000*
	P3	0,001*	0,000*	-	1,000
	P4	0,000*	0,000*	1,000	-

The correlation test was used to determine the relationship between the collagen density variable and the number of capillaries in all groups to determine whether there was a relationship between the two variables.

The normality test on the variable density of collagen and the number of new capillaries showed that the data distribution was not normal (p = 0.012; 0.041), so the correlation analysis used the *Spearman* correlation test. *Spearman's* test showed that there was a significant relationship between the variable collagen density and the number of capillaries (p=0.001). The number r=0.682 indicates that there is a positive correlation between collagen density and the number of new blood vessel capillaries with a strong correlation strength.

4. Discussion

The results of the test on the variable density of collagen and the number of capillaries of new blood vessels show a significant difference between the treatment groups using both *Moringa oleifera* leaf extract and the combination with SSD and the control group, namely the group receiving SSD treatment and the pure vehicle group. This is in accordance with previous research that the administration of Moringa oleifera leaf extract can improve vascularity conditions and collagen density.[8,9]

The results of research conducted by Gothai S, Aruselvan P, et al. (2016) showed the ethyl acetate fraction of Moringa oleifera leaf extract showed proliferative and migratory effects from normal human fibroblasts.[6] In the thesis, research conducted by Lasmadasari N, Hakimi M, et al. (2013) also showed that oral and topical administration of Moringa oleifera leaf extract accelerated wound healing in cuts.[8]

This increase in vascularity and collagen density is probably due to phytochemical compounds such as alkaloids, triterpenoids, tannins, and flavonoids. These compounds play an active role through antioxidant activity and protect tissues from oxidative damage. Furthermore, Moringa oleifera leaf extract also contains bioactive compounds such as kaempferol, quercetin, and vicenin-2. These

compounds are thought to increase healing ability.[9,11,12]

The low number of new blood capillaries in group III who received therapy with topical SSD was probably caused by SSD can inhibit wound healing. There are studies that state that SSD can inhibit wound healing in mice by interfering with the expression of IL-1 cytokines. It is suspected that the disruption of the IL-1 cytokine can cause a decrease in the number and activation of macrophages. Histological analysis revealed the depletion of macrophages and impaired collagen deposition and re-epithelialization.[13]

Limitation of this study includes; 1. The study was only carried out within 10 days, so the tissue healing effect was only partially observed 2. The cream of Moringa Oleifera leaf ethanolic extract used has not been through the toxicity test and irritation test, so the factors that might hinder wound healing from the cream have not been removed.

5. Conclusion

Moringa oleifera leaf ethanolic extract is proven to be effective in increasing collagen density in burns of Wistar rats in terms of increasing collagen density and the number of new blood vessel capillaries.

Further research is needed; to find the most effective dose of *Moringa oleifera* ethanolic extract in healing burns, to determine other factors that may be contributing to the healing factors of *Moringa oleifera,* and a toxicity study must be conducted so that factors that might inhibit wound healing from the extract can be ruled out.

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