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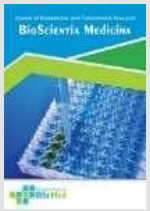

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Effects of Ozonized Aloe Vera Oil on Full-Thickness Excision Wound Healing: In Vivo Study

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A B S T R A C T

Background. Disruption to the healing process of these wounds will consume large resources and will often require long-term medical management. Aloe vera oil is rich in anthraquinone compounds, 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. The ability of ozone to trigger mild oxidative stress plays an important role in triggering a cascade of cytokines and chemokines, including the initiation of vascular endothelial growth factor (VEGF) proteins that play a role in the initiation of angiogenesis. **Methods:** This study is an in vivo experimental study. A total of 60 rats were divided into 10 treatment and control groups. The treatment group was given ozonized aloe vera oil 600 mg/mL, 1200 mg/mL and 1800 mg/mL. Furthermore, the assessment of VEGF and new blood vessel formation was carried out. Data analysis was performed using SPSS version 25 software with univariate and bivariate tests. **Results:** The administration of ozonized aloe vera oil was able to increase the expression of VEGF, and the number of new blood vessels in the excision wound tissue. **Conclusion:** Ozonized aloe vera oil is effective in promoting excision wound tissue repair in vivo.

1. Introduction

The skin plays an important role in the protection of the internal environment of the body and is the largest organ in the human body, so serious damage to this organ can cause several problems in its survival.[1] The skin consists of two layers of epidermis and dermis, which are placed over the subcutaneous adipose. The epidermis consists mainly of a layer of keratinocytes into which several other cell types have spread, including melanocytes and Langerhans cells. The epidermis is separated from the dermis by a basement membrane. The dermis is composed of papillary and reticular cells consisting of an

extracellular matrix or a basal substance consisting of collagen, fibrous tissue, elastin, and glycosaminoglycans.[2,3] Disruption to the healing process of these wounds will consume large resources and will often require long-term medical management. Serious and extensive damage to the skin, such as burns, threatens the survival of the organism and impairs the regenerating capacity of the skin. In addition, with the increasing prevalence of diseases such as diabetes, vascular disease, and obesity, chronic wounds are becoming a major global problem with a limited choice of treatment strategies, unsatisfactory therapeutic effects, and high medical costs.[4,5]

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. Aloe vera is one of the most common plants in Indonesia and is a plant with extraordinary medicinal potential. This plant has been used for generations to treat various health problems such as constipation, diarrhea, and skin health. Aloe vera is rich in primary metabolites, secondary metabolites, and various vitamins and minerals. The content that is rich in various metabolites, vitamins, and minerals makes Aloe vera rich in benefits. Aloe vera oil is rich in anthraquinone compounds, 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.[6-10]

Ozone is one of the most widely researched and developed therapeutic modalities today. Ozone has the potential for disinfection and is capable of triggering mild oxidative stress in tissues. The ability of ozone to trigger mild oxidative stress plays an important role in triggering a cascade of cytokines and chemokines, including the initiation of vascular endothelial growth factor (VEGF) proteins that play a role in the initiation of angiogenesis.[11-13] This study is the first study that seeks to explore the potential of the combination of Aloe vera oil and ozonation in the wound healing process by exploring the ability of the combination of Aloe vera oil and ozone in trigger the angiogenesis process in vivo.

2. Methods

This study is an experimental study with a post-test-only approach with a control group design. A total of 60 rats (*Sprague dawley*) strain was included in this study and met the inclusion criteria in the form of the male sex, weight between 150-200 grams, and age 8-10 weeks. First, the rats were acclimatized for 7 days, then divided into 10 groups (K1, K2, K3, K4, P1, P2, P3, P4, P5, and P6) randomly, where each group consisted of 6 rats. Groups K1 and K3: were controlled and were given aloe vera oil, where K1 was terminated on the third day, and K3 was terminated on the seventh day. Groups K2 and K4: controls negative induced by excision wound, negative induced excision wound and given 0.1% gentamicin ointment, where K2 was terminated on the third day, and K4 was terminated on day seventh Groups P1, P2, and P3 each received Ozonized aloe vera oil 600 mg/mL, 1200 mg/mL and 1800 mg/mL, respectively, where groups P1-P3 were terminated on the third day and groups P4-P6 were treated as follows. P1-P3 with termination on the seventh day. This study has been approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Diponegoro, with the number No. 66/EC/H/FK-UNDIP/VII 2020.

Induction of the excision wound was carried out by the first anesthetic, the rat using ketamine (dose of 0.015 mg/g BW) intramuscularly and chlorate (0.0025 mg/g BW) subcutaneously. Shave clean the hair in the area of the dorsum of the rat, and mark the area where the wound will be made (in this case, on the back of the rat) with a diameter of 1 centimeter.

Rats were monitored daily for signs of distress and signs of infection. On the third and seventh days, the fistula tissue was terminated at the same time: The rats were anesthetized with a mixture of ketamine-xylazine (Ketamine dose 80 mg/kg BW; Xylazine dose 10mg/kg BW) intraperitoneally and the skin and

subcutaneous tissue were cut with a scalpel. size 1cm x 1cm x 1cm. Pieces of fistula tissue were placed in 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 2x2 hours. The tissue was then put into a liquid paraffin solution for 2x2 hours, after which the tissue was embedded in solid paraffin, having a melting point of 56-58^{0}C. The tissue was cut to about 6 µm 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 3x2 minutes. The preparation was washed with running water for 2 minutes. The preparation was put in hematocycline-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 and 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, drip 1-2 drops of entelan. The sediaan is closed with a cover glass. Furthermore, histopathological assessment of angiogenesis and VEGF (Immunohistochemistry) was performed 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 a 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

The highest mean VEGF expression (Allred score) on wound healing on the third day was found in the P1 treatment group (wound treatment with topical administration of 600 mg/ml ozone), followed by the P3 treatment group (wound treatment with topical administration of oil). 1800 mg/ml ozone), treatment group P2 (wound treatment with topical administration of 1200 mg/ml ozone), and positive control group K1 (wound treatment by topical application of aloe vera oil). Meanwhile, the lowest mean VEGF expression on wound healing on the third day was found in the K2 negative control group (wound treatment with topical administration of 0.1% gentamicin ointment). The highest mean VEGF expression on wound healing on the seventh day was found in the P5 treatment group (wound treatment with topical administration of 1200 mg/ml ozone), followed by the P4 treatment group (wound treatment with topical administration of 600 mg/ml ozonated Aloe vera oil). P6 Treatment group (wound treatment with topical administration of 1800 mg/ml ozone), and positive control group K3 (wound treatment with topical application of aloe vera oil). While the lowest mean VEGF expression on wound healing on the seventh day was found in the K4 negative control group (wound treatment with topical administration of 0.1% gentamicin ointment).

Table 1. Comparison of VEGF expression between groups on the third and seventh days

Group	VEGF expression (Allerd Score) third day Mean±SD	Group	VEGF expression (Allerd Score) seventh day Mean±SD
K1	7.2±0.9	K3	7.1±0,9
K2	6.7±0.8	K4	6.5±0.9
P1	7.7±0.5*	P4	7.5±0.5*
P2	7.5±0.5*	P5	7.6± 0.5*
P3	7.6±0.5*	P6	7.5±0.5*

*Post hoc test Games Howell versus K2, p<0.05.

The highest mean number of new blood vessel capillaries on wound healing on the third day was obtained in the P2 treatment group (wound treatment with topical administration of 1200 mg/ml ozone) followed by the P1 treatment group (wound treatment with topical administration of 600 mg/ml ozone), the P3 treatment group (wound treatment with 600 mg/ml ozone treatment). topical ozone oil 1800 mg/ml ozone) and the positive control group K1 (wound treatment with topical administration of aloe vera oil). Meanwhile, the lowest mean number of new blood vessel capillaries on the third day of wound healing was found in the K2 negative control group (wound treatment with topical administration of 0.1% gentamicin ointment). The highest mean number of new capillaries on wound healing on the seventh day was found in the P6 treatment group (wound treatment with topical administration of 1800 mg/ml ozone), followed by the P5 treatment group (wound treatment with topical administration of 1200 ozonated aloe vera oil). mg/ml ozone), treatment group P4 (wound treatment with topical administration of 600 mg/ml ozone), and positive control group K3 (wound treatment with topical administration of aloe vera oil). Meanwhile, the lowest mean number of new capillaries on wound healing on the seventh day was found in the K4 negative control group (wound treatment with topical administration of 0.1% gentamicin ointment).

Table 2. Comparison of new blood vessel formation between groups on the third and seventh day

Group	Number of new vessel formations on the third day Mean±SD	Group	Number of new vessel formations on the seventh day Mean±SD
K1	6.2±1.6	K3	9.8±0,6
K2	4.8±0.8	K4	8.6±1.2
P1	7.6±1.7*	P4	10.8±0.5*
P2	7.9±1.0*	P5	11.6± 1.0*
P3	7.6±0.9*	P6	13.4±2.0*

*Post hoc test Games Howell versus K2, p<0.05.

4. Discussion

This study showed that the administration of ozonized aloe vera oil was able to increase the process of angiogenesis in order to accelerate wound healing excision. Aloe vera oil is rich in anthraquinone compounds which have the potential as anti-inflammatory agents. Anti-inflammatory agents play a role in the activation of anti-inflammatory cytokines, IL10 and TGF-beta1. TGF-beta1 is a growth factor cytokine that has the potential to trigger the activation of alpha-SMA and VEGF proteins. VEGF is a pro-angiogenesis cytokine that plays a role in the initiation of the formation of new blood vessels in wound tissue. The formation of new blood vessels is an important

part of the wound healing process.[14.15]

The addition of the ozonation process showed optimal potential in the wound healing process. The ozonation process has a disinfecting effect that is quite useful in preventing colonization, invasion, and infection of various bacteria and microorganisms in wounds. Apart from these effects, the ozonation process is also beneficial in triggering mild oxidative stress in wound tissue. Mild oxidative stress is a condition of mild oxidative stress triggered by the ozonation process to trigger mild inflammation. Activation of mild inflammation will cause activation of anti-inflammatory cytokines, IL10 and TGF-beta1. TGF-beta1 activation will trigger VEGF cytokine activation. VEGF is a pro-angiogenesis cytokine that plays a role in the initiation of the formation of new blood vessels in wound tissue. The formation of new blood vessels is an important part of the wound healing process.[16.17]

The combination of aloe vera oil administration and the ozonation process was able to optimize the activity of the TGF-beta1 cytokine and the activation of the VEGF cytokine in triggering a series of new blood vessel formation processes. Several studies have also shown the potential of Aloe vera in promoting wound tissue repair through VEGF activation and the formation of new blood vessels.[18-20]

5. Conclusion

The administration of ozonized aloe vera oil can trigger excision wound tissue repair through the activation of VEGF cytokines and the activation of new blood vessel formation.

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