

RESEARCH ARTICLE

Topical Administration of Aloe vera Extract Gel Increased the Number of Macrophages and Epithelialization in UVB-Induced Sunburn

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ABSTRACT

Introduction: The UVB exposure may cause sunburn (second degree burn). Aloe vera (AV) is widely used in wound healing, including sunburn.

Objective: To evaluate the effect of topical Aloe vera extract gel on the number of macrophages and epithelialization in UVB induced sunburn.

Methods: This posttest only-control group design used twenty female BALB/c mice with UVB-induced sunburn randomly divided into 4 groups. Control groups (Cntrl-G) were given a base gel. Group AV-25, AV-50, and AV-75 were topically treated with Aloe vera gel at the concentration of 25%, 50% and 75% respectively for 5 days. On day 5, after terminated, the samples of skin tissue were prepared for histological study using hematoxylin eosin (HE) staining. Data were analyzed using one way ANOVA, Post-hoc LSD and Pearson correlation.

Results: ANOVA analysis showed that the number of macrophages and epithelialization among groups was a significantly difference ($p < 0.05$). Post hoc analysis demonstrated that the mean number of macrophage in AV-75 (6.50), AV-50 (5.13), and AV-25 (3.93) were significantly higher compared to that of Cntrl-G (2.93). The mean of epithelialization AV-75% (25.35), AV-50 (18.15), and AV-25 (15.38), were significantly ($p < 0.05$) higher compared to that of Cntrl-G (9.30). The Pearson correlation test indicated that there is a strong positive correlation (0.88) between the number of macrophages and epithelialization ($p < 0.05$).

Conclusion: The topical administration of Aloe vera gel extract for five days increased the number of macrophages and epithelialization in sunburned UVB induced sun burn.

Keywords: sunburn, Aloe vera, macrophages, epithelialization.

ABSTRAK

Pendahuluan: Kulit yang terpapar sinar UVB dapat mengalami sunburn (luka bakar derajat II). Lidah buaya (Aloe vera; AV) banyak dipakai untuk penyembuhan berbagai luka. **Tujuan:** untuk mengevaluasi apakah gel ekstrak AV topikal dapat meningkatkan jumlah makrofag dan epitelisasi kulit yang mengalami sunburn akibat paparan sinar UVB.

Metode: Dalam post testonly control group design, 24 ekor mencit betina galur balb/c dengan sunburn akibat paparan UVB dibagi 4 kelompok secara acak. Kelompok kontrol (Cntrl-G), diberi base gel. Kelompok AV-25, AV-50, dan AV-75, masing-masing mendapatkan gel AV topikal konsentrasi 25%, 50% dan 75% selama 5 hari. Hari kelima mencit dimatikan, diambil kulit sunburn, dibuat preparat histologi dengan pengecatan HE. Data dianalisis dengan uji one way ANOVA, post-hoc LSD dan korelasi pearson.

Hasil: Analisis statistik anova menunjukkan bahwa terjadi perbedaan bermakna di antara kelompok pada jumlah makrofag dan epitelisasi, $p < 0.05$. Analisis Post hoc menunjukkan bahwa rerata jumlah makrofag pada AV-75 (6,50), AV-50 (5,13), dan AV-25 (3,93) lebih tinggi bermakna dibanding kontrol (2,93), $p < 0,05$. Jumlah epitelisasi pada AV-75 (25,35), AV-50 (18,15), dan AV-25 (15,38) lebih tinggi bermakna dibanding kontrol (9,30), $p < 0,05$. $p = 0,01$. Uji korelasi pearson menunjukkan jumlah makrofag dan epitelisasi mempunyai korelasi positif kuat (0,88), $p < 0,05$.

Kesimpulan: Pemberian ekstrak gel Aloe vera topikal selama lima hari berpengaruh terhadap peningkatan jumlah makrofag dan epitelisasi kulit sunburn akibat paparan sinar UVB.

Kata kunci: sunburn, Aloe vera, makrofag, epitelisasi.

INTRODUCTION

Sunburn is a common in countries with four seasons like America. Every year, one third of adults and children in America have sunburn, especially during the summer. Therefore, sunscreen is an option

to protect the skin from exposure to ultraviolet B (UVB) (Ambizas and Maniara, 2016). Sunscreens are classified into two categories, physical and chemical sunscreen. Physical sunscreens contain inorganic compounds that function to refract and scatter UVB light. The

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chemical sunscreens contain organic compounds that can absorb UVB light and prevent cell damage. Although rare, various side effects may occur, especially burning, stinging, and contact dermatitis (Ambizas and Maniara, 2016). Aloe vera (AV) has been a common alternative for the treatment of burns because it is easy to obtain and cheap. Some studies have shown that the administration of AV shorten the time needed for epithelialization compared with silver sulphadiazine (SSD) 1% in second degree burns (Khorasani et al., 2009). In addition, AV was shown to increase the number of macrophages in third degree thermal burns (Hidayat, 2013) and increasing epithelialization of the wound in mice (Rahayu et al., 2013). However, the effect of AV on the number of macrophages and epithelialization in sunburn is unknown.

Ultra violet B (UVB) exposure on the skin has a number of biological effects, some of which are harmful including sunburn. The most common sunburn reaction is first degree burn, even though on some cases it may cause second degree burn (Rhodes et al., 2009). In response to injuries, the skin repairs or healing by involving some cell including platelets, macrophage, fibroblasts, endothelial cell, and keratinocytes (Barrientos et al., 2008). Macrophages are cells that play an important role in the wound healing. Therefore, the lack of macrophage in the wound disturb healing caused by deterioration of debridement, proliferation and maturation of fibroblasts, slowed down angiogenesis, and re-epithelialization (Krzyszczyk et al., 2018; Velnar et al., 2009).

Macrophage reaches the site of injury 48-72 hours after sunburn and produces cytokines pro-inflammatory such as interleukin-1 (IL-1), interleukin-6 (IL - 6) and tumor necrosis factor- α (TNF- α). In addition, macrophages also produce various growth factors such as platelet-derived growth factor (PDGF), transforming growth factor - β (TGF- β), epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and Insulin-like growth factor (IGF) playing an important role in re-epithelialization. Epithelialization is an essential component in wound healing, so it is used as a parameter for wound healing. Epithelialization is the process of restoring the epidermis after trauma modulated by keratinocytes (Pastar et al., 2014). Epithelialization is characterized by the migration of epithelial cells from the edge of the wound to create a single layer of cells above the wound, followed by increased mitosis of cells at the edge of the wound. Cell migration penetrates the layer to temporarily attach to the lower matrix. After the cells that migrate from the

edge of the wound meet, the cell then stops migrating and begins the formation of the basement membrane (Velnar et al., 2009).

Aloe vera contains 75 active ingredients playing a role in wound healing, including acemannan, glycine, sterol, auxin, giberelins, vitamins A and E. Acemannan has immunomodulatory effects through macrophage activation to secrete IL-1 cytokines, IL-6, TNF- α , interferon- γ (INF- γ) (Hamman, 2008). Furthermore, with various growth factors, such as PDGF, TGF- β , EGF, FGF, VEGF, and IGF, macrophage can trigger proliferation of fibroblasts, and collagen metabolism, epithelial cell proliferation and granulation tissue, matrix deposition and wound contraction, angiogenesis, increases the thickness of the epidermis and dermis (Hashemi et al., 2015). In addition, acemannan has also been shown to stimulate the healing and epithelial cell growth (Hashemi et al., 2015). Sterol (β - esterol), vitamins A and E contained in AV, have been shown to be able to stimulate angiogenesis through increased VEGF and its receptors and increase epithelialization (Cowser, 2010). Other factors that also play a role in modulating keratinocytes and increasing epithelialization are IL-1, IL-6, TNF- α , FGF, and TGF- β which are produced by macrophages due to AV administration (Pastar et al., 2014).

The aim of this study was to evaluate the effect of the administration Aloe vera extract gel on sunburn (second degree burns) in UVB-induced sunburn.

METHODS

This study was designed as a post - test only controls group study. A total of 24 female BALB/c mice with sunburn (second degree burns) were randomly divided into 4 groups. The control group (Cntrl-G) was given base gel. AV-25, AV-50, and AV-75 group received a topical Aloe vera extract gel at the concentration of 25%, 50% and 75% respectively three times a day for 5 days. On day five, all mice were terminated. Histopathological preparations were made from skin tissue. The study was conducted between August and September 2015 at the Laboratory of Biology, Laboratory of Pharmacology, and Chemistry Laboratory Sultan Agung Islamic University, Semarang. The study was conducted after obtaining approval from the Bioethics Commission of medical/Health research, Faculty of Medicine, Sultan Agung Islamic University, and Semarang.

Aloe vera Extract Gel

Aloe vera extract was extracted using 96% ethanol and precipitated for 10 hours at the temperature of 10

Table 1. Mean (+ SD) number of macrophages and Increased Epithelialization

Variables	Cntrl -G	AV-25	AV-50	AV-75	p Anova
Macrophage (Σ)	2.93 ± 0.16	3.93 ± 0.24	5.13 ± 0.21	6.50 ± 0.20	0.000
Epithelialization (Σ)	9.30 ± 0.67	15.22 ± 2.39	18.15 ± 4.65	25.35 ± 3.01	0.000

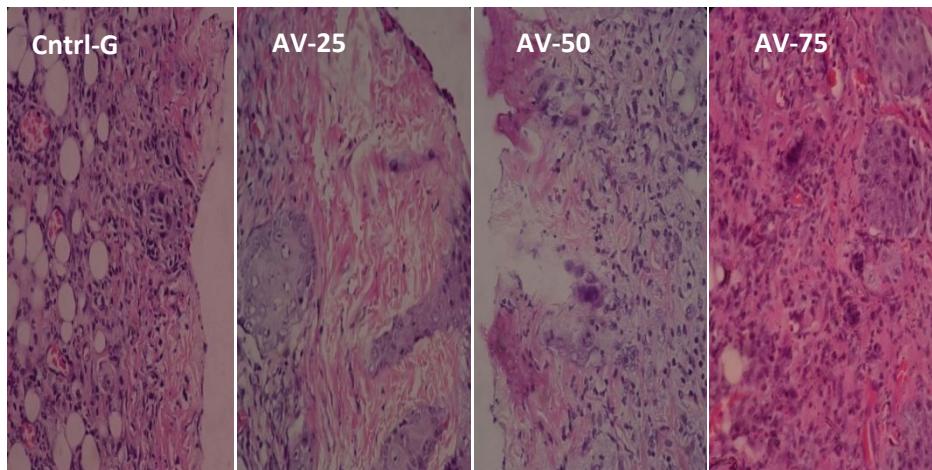


Figure 1. Number of Macrophage cells after UVB radiation and Aloe vera Topical treatment in each group identified with HE staining Method.

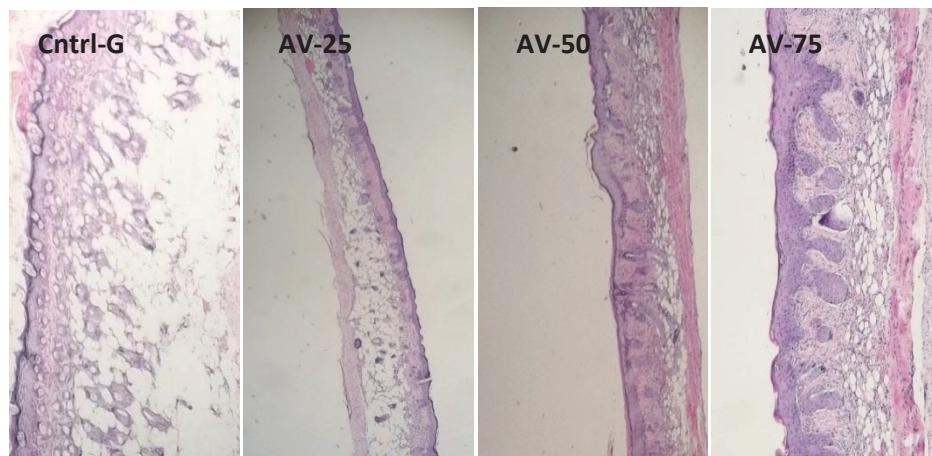


Figure 2. Epithelialization cells following UVB radiation and Aloe vera treatment in each group, were identified with HE stained Method

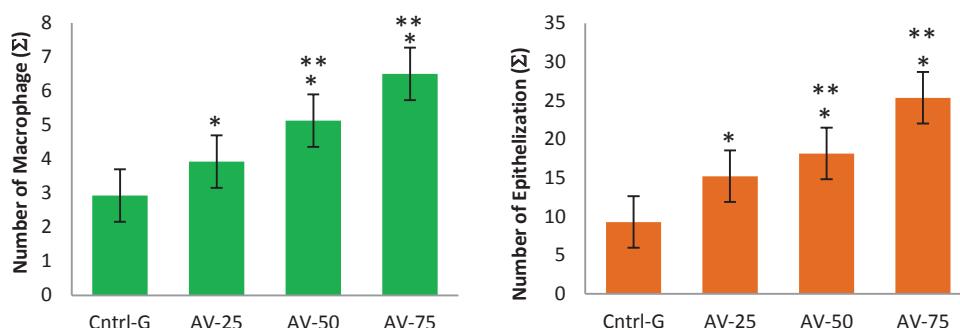


Figure 3. Number of Macrophage in Each Group following AV Treatment. Post Hoc analysis: * P<0.01 Cntrl-G as control; ** P<0.01 AV-25 as Control

Table 2. Correlation Data

		epitelisasi	Makrofag
Epitelisasi	Pearson Correlation	1	,88**
	Sig. (2-tailed)		,00
	N	24	24
Makrofag	Pearson Correlation	,88**	1
	Sig. (2-tailed)	,00	
	N	24	24

** Correlation is significant at the 0.01 level (2-tailed).

oC. The precipitation formed was separated from the solution is by using a suction strainer, then the dried with a vacuum at 37oC. Aloe vera was prepared in 3 different concentrations: 25%, 50% and 75%.

UVB Rays Exposure

The backs of mice were shaved 2x3 centimeters, and then exposed to UVB rays for 15 minutes/day in three days. One dose of UVB was one-half the minimal erythema dose (MED) that resulted in the second-degree burns.

Histopathological Preparation

The skin tissue sample was taken for histopathological preparations using standard procedure of HE staining. The number of macrophages was counted using microscope at 400X magnification. Skin epithelialization was measured using ocular micrometer 1: 1000 scale and 400X magnification. Histopathological examination was carried out at the Anatomical Pathology Laboratory of Sultan Agung Islamic Hospital, Semarang.

Statistical Analysis

Data were analyzed using one way ANOVA test followed by LSD with significance set at < 0.05.

RESULTS

After 5 days of treatment for female BAB/c mice with sunburn, the results of the number of macrophages and an increase in skin epithelialization as shown in Figures 1 and 2 were obtained. Mean number of macrophages and epithelialized skin in each group is presented in table 1.

The results of AV extract gel showed that the highest number of macrophages was found in AV-75% group, followed by the AV-50% group, AV-25, and Cntrl-G. Similarly, the number of epithelialization increases after treatment. The highest increase in epithelialization was found in the AV-75 group, followed by AV-50 group, AV-25%, and Cntrl-G. The results of analysis of ANOVA on both the number of macrophages and epithelialization of skin cells showed significant

differences between groups ($p < 0.01$). To determine the group with the significant different, the LSD Post Hoc test was carried out as described at Figure 3.

The number of Macrophage

The results of the Post Hoc LSD statistical analysis showed that the number of macrophages at AV-25, AV-50, and AV, 75 was significantly higher than that of C n trl-G, $p < 0.01$. The number of macrophages at AV-75 was significantly higher than AV-25, and AV-50 ($p < 0.01$). Similarly, the number of macrophages of AV-50 was significantly higher than AV-25, $p < 0.01$ (figure 1).

Epithelialization of Skin Cells

Post Hoc LSD analysis showed that the number of epithelialized skin cells in AV-25, AV-50, and AV-50 was significantly higher than that of Cntrl-G ($p < 0.01$). The number of epithelialized skin at AV-75 was significantly higher than AV-25 and AV-50, $p < 0.01$. Similarly, the number of epithelialized skin cells at AV-50 was significantly higher than that of AV-25 ($p < 0.01$) (figure 3).

DISCUSSION

This study showed that the administration of AV extract gel at the concentration of 25%, 50%, and 75% increases the number of macrophage and epithelialization of skin. The results of this study supports the findings of a study conducted by Khorasani et al. showing that the administration of AV fasten wound healing than silver sulfadiazine (Khorasani et al., 2009). Some studies showed that topical administration of AV not only accelerate wound healing and tissue remodeling (Oryan et al., 2016).

The results of this study also showed that the highest number of macrophages was in the administration of AV with a concentration of 75%, then AV with a concentration of 50% followed by AV at the concentration of 25%, and the lowest number of macrophages was in the control group. The results of this study illustrate that the higher the concentration of AV extract gel, the higher the number of macrophages.

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Therefore, giving AV extract gel with a concentration of 75% is dose and pitch results in increasing the number of macrophages and epithelialization of skin compared to a concentration of 25% and 50%. The results of this study are in line with the study reported by Jettanacheawchankit et al which showed that the administration of AV littoralis extract gel de ngan concentration of 100% more effective than AV gel with a concentration of 12.5% towards healing burns in mice (Jettanacheawchankit S et al., 2009). Oral AV studies in mice with wounds and type II diabetes also show that AV accelerates wound healing (Daburkar et al., 2014). Similarly, the results of a study conducted by Abdul that the oral and topical administration of the AV extract with diabetic wounds fasten wound healing (Hamza et al., 2008).

An increase in the number of macrophages and epithelialization of the skin due to the administration of AV extract gel has been associated with the acemannan in A. Acemannan plays a role as immuno potentiator, anti-inflammatory, and increase monocyte activity, macrophages and cytotoxicity, and stimulate killer T-cells. The main study conducted by Shahzad et al. showed that the topical administration of AV in mice with third-degree burns increases the number of macrophages (Shahzad and Ahmed, 2013). Other studies have also shown that AV administration in mice with burns has been shown to be able to inhibit inflammation characterized by reduced leukocyte adhesion, proinflammatory cytokines such as TNF- α and IL-6. Furthermore, oral administration of AV in mice with burns and type II diabetes mellitus has been shown to increase the expression of VEGF and TGF- β leading to faster wound healing (Daburkar et al., 2014). Other study also showed that oral administration of AV in mice with acute radiation-induced injury, showed wound healing by stimulating TGF- β and FGF (Rodero and Khosrotehrani, 2010).

Wound healing requires epithelialization. The epithelialization starts several hours after the formation of wound. Epithelialization consists of differentiation, migration and proliferation of keratinocytes. Three main MAP kinases involved in the differentiation process activated by various stimuli includes the entry of calcium, EGF, and TNF α (Cargnello et al., 2011). EGF and TGF- α directly stimulate keratinocyte migration and proliferation. FGF stimulates epithelialization through paracrine effects. Other cytokines such as IL-1, IL-6, and TNF- α can also modulate keratinocyte migration. IL-1 increases FGF and secretion IL-6 goes through the dependent STAT3 pathway, which allows keratinocytes to respond to mitogenic factors

and stimulate migration. TGF- β stimulating MMPs facilitates keratinocyte migration through fibrin and synthesizes ECM. MMPs, ECM components, and integrins, work together to help growth factors in promoting keratinocyte proliferation. MMPs work through proteolytic activity to release growth factors from the wound matrix and can digest latent forms of growth factors, such as IGF -1, then convert active forms (Seeger and Paller, 2015). Further, proliferation and migration of epithelial cells depend on oxygen supply. AV improves blood supply to the wound area to make more oxygen, increasing reepithelialization and the healing. Activated macrophages will secrete various growth factors and cytokines, and stimulate fibroblasts which will stimulate the process of keratinocyte migration and proliferation, thereby accelerating epithelialization (Hosseinimehr SJ et al., 2010).

Macrophages are playing an important and predominant role in wound healing. Macrophages have many functions in wound healing, including for defense, promotion and resolution of inflammation, anti-inflammatory functions, removing apoptotic cells, helping cell proliferation and wound tissue restoration, encouraging angiogenesis, fibroblast proliferation and extra cellular matrix formation (Mahdavian et al., 2011). Therefore, during the wound healing, an increase in the number of macrophages should be followed by an increase in epithelialization of the skin. Results Pearson correlation test between data on the number of macrophage and epithelialization, showed a strong positive correlation ($r = 0.88$, $p=0.01$) between the number of macrophages and epithelialization of the skin (figure 4).

CONCLUSION

The administration of topical Aloe vera extract gel at the concentration of 25%, 50%, and 75% was shown to increase the number of macrophages and increase epithelialization in UVB induced sunburn. AV at the concentration of 75% was the most effective.

CONFLICT OF INTEREST

There is no conflict of interest

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