

The Effect of Aloe Vera Topical Gel Extraction on Tgf-B1 Expression in Thermal Burn Eyelid Grade II Rats Model

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Abstract

Grade II eyelid burns are burns that reach the epidermis and part of the dermis of the palpebrae and can have impaired wound healing. TGF β 1 has the broadest spectrum of action, affecting all cell types involved in all stages of wound healing. TGF β 1 increases rapidly after injury and reaches peak levels three days after injury, which coincides with the peak of the inflammatory phase of wound healing. Decreased expression of TGF β 1 is associated with impaired wound healing. Aloe Vera has long been known in Indonesia as a healing plant. Aloe Vera extract contains a variety of bioactive compounds that play a role in stimulating wound healing. The administration of Aloe Vera extract on thermal burns aims to reduce inflammation, increase collagen content, and increase the rate of re-epithelization. The administration of Aloe Vera gel extract allows faster healing of thermal burns and rebuilds the vascularity of the wound tissue. Objective of this study is to determine the difference between the expression of Transforming Growth Factor Beta 1 (TGF β 1) in a rat model of thermal burn injury grade II of the eyelids treated with topical Aloe Vera gel extract with the concentrations of 20%, 40%, Balanced Salt Sodium (BSS) and a control group that was not given burn intervention. An experimental study with the post-test-only approach with a control group design. 24 Rats have induced grade II eyelid thermal burns, divided into four groups: Normal (without treatment), negative control (BSS), given Aloe vera 20%, 40%. Then TGF β 1 expression was quantified using the Image J application. The Topical application of Aloe vera gel can increase the expression of TGF along with the increase in dose. The highest rise in TGF expression was found in the group with 40% Aloe vera gel, followed by 20% Aloe vera gel compared to the negative control (BSS Balanced Salt Solution). Aloe Vera can increase TGF expression in a rat model of grade II palpebral burn injury and is dose-dependent.

Keywords: Grade II Eyelid Thermal Burns, Aloe Vera Gel, Transforming Growth Factor Beta 1 (TGF β 1), Rats Model

1. Introduction

Thermal burns with eye involvement occur between 7.5%-27%. The part most commonly affected by thermal burns is the eyelid. The skin of the eyelids has thin contours without subcutaneous fat, thus causing deeper thermal burns compared to similar exposures elsewhere. In thermal burns that reach the dermis, there can be cicatricial changes that can lead to eyelid retraction, ectropion, lagophthalmus, and corneal exposure.¹⁻³

The first phase of healing thermal burns is the hemostasis phase which is directly followed

by the inflammatory phase where body cells secrete cytokines, one of which is Transforming Growth Factor Beta 1 (TGF- β 1). TGF- β 1 has the widest spectrum of action, affecting all cell types involved in all stages of wound healing. TGF- β 1 is important for initiating inflammation, granulation tissue formation, cell migration, and stimulating the production of extracellular matrix molecules. TGF- β 1 increases rapidly after injury and reaches peak levels three days after injury, which coincides with the peak of the inflammatory phase in the wound healing stage. Decreased expression of TGF- β 1 found in

humans and animals is associated with impaired wound healing.³⁻⁵

Aloe vera (AV) or aloe vera plant (*Aloe barbadensis* Miller) is widely found in Indonesia. This plant has become known as the "healing plant". Aloe vera extract contains a wide variety of bioactive compounds that play a role in stimulating wound healing. The administration of Aloe vera extract in thermal burns aims to reduce inflammation, increase collagen content, increase the rate of re-epithelialization, and is angiogenic. So the administration of Aloe vera gel extract allows faster healing of thermal burns and rebuilds vascularization of wound tissue.⁶

Based on research conducted by Fahcreza, the effect of Aloe vera Gel extract on lesion area and TGF- β expression in rat corneal base chemical trauma, it was found that Aloe vera extract with a concentration of 20% had the smallest corneal lesion area and the highest TGF- β value compared to the control group, Aloe vera 10%, and Aloe vera 40% at 72 hours after induction of alkaline chemical trauma.⁷

Taking into account the potential effects and wide availability of Aloe vera, this extract could be a possible candidate for treating thermal burns of the eyelids. To date, no studies have linked the role of the effect of Aloe vera gel extract on the healing of thermal burns on the eyelids with the expression of Transforming Growth Factor Beta 1 (TGF- β 1) in rats model of grade II thermal burn trauma.⁸

2. Materials and methods

White Rats Wistar strain with an age of 6-8 weeks and a body weight of 150-200 grams, moving actively, from external examination do not appear anatomical abnormalities, especially wounds on the palpebra.

2.1. Trauma Model Thermal burns

A total of 24 rats were divided into 4 groups, 1 control group and 3 case groups, in 3 control groups made II degree thermal burn trauma. The eyelid thermal burn trauma model was made by giving an aluminum plate heated for 1 minute in boiling water with a temperature of 1000 Celsius then attached for 2 seconds in the superior palpebra medial part in one eye of experimental animals then irrigated with RL for 1 minute. Assessment of thermal burn grade based on macroscopic picture.

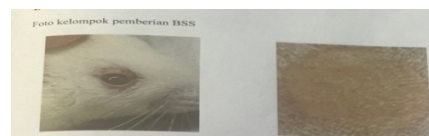


Figure 1. Macroscopic results after BSS administration

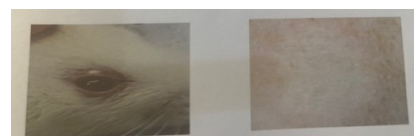


Figure 2. Macroscopic yield after administration of Aloe Vera 20%



Figure 3. Macroscopic Results After Administration of Aloe Vera 40%

2.2. Research Methods and Drug Administration

This study is experimental in vivo with a post-test only approach with control group design to determine the difference in expression of Transforming Growth Factor Beta 1 (TGF- β 1) in mice model of trauma palpebral thermal burn grade II given topical gel extract treatment Aloe vera with concentrations of 20%

and 40% compared to those not given therapy,

2.3. Making Aloe vera Gel Extract

Aloe vera simpliations were obtained from BP2OOT Tawangmangu Karang Anyar, Central Java (Ministry of Health) as many as 2 pieces were prepared to be stored in the Biotechnology Laboratory at the Faculty of Medicine UNSRI, then simpliations were made aloe vera gel preparations with 20% and 40%.

2.4. TGFβ1 Expression Assessment

On the third day all palpebral burn model mice were decapitated under general anesthesia and petal evacuation

Eyes. Next, an eyelid sample was taken and put into NBF (normal formaldehyde buffer) solution 10x the volume of the organ. Furthermore, the organ is paraffinized and cut using microtomes, and histological preparations are made.

Histological preparations are parafinized processed, then non-specific antigen blocking process and retrival antigens are carried out so that immunohistochemical preparations (CPI) are obtained. To see how the expression of TGFβ1 in the eyelid tissue of grade II burn rats

2.5. TGFβ1 Expression Analysis

CPI preparations are assessed using image software, so that the percentage of TGFβ1 expression will be obtained.

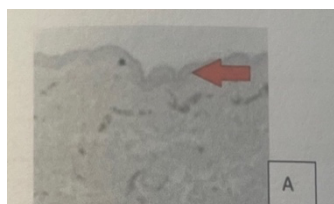


Figure 4. Uninduced Wistar Rat Eyelid Tissue Grade II burns are still intake between epidermis and Dermis (Red Arrow)

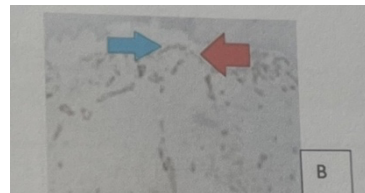


Figure 5. Eyelid Tissue of Wistar Rats Induced Grade II burns BSS Blue arrow shows TGF β1 protein expression, red arrow indicates no intake between epidermis and dermis

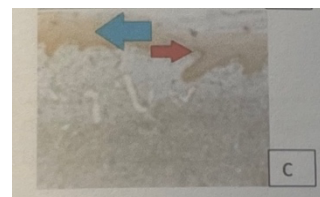


Figure 6. Eyelid Tissue of Wistar Rats Induced Grade II burns. Burns treated with 20% aloe vera gel Blue arrows show TGF β1 protein expression (brown color), red arrows indicate intakes between epidermis and dermis

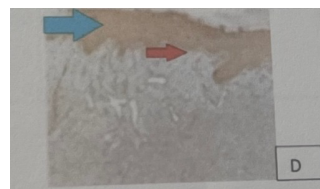


Figure 7. Eyelid Tissue of Wistar Rats Induced II Degree burns 40% aloe vera gel Blue arrow shows TGF β1 protein expression (brown color), red arrow indicates intake between epidermis and dermis.

3. Results

3.1. TGF β1 Expression in Experimental Animals

Table.1 shows the mean TGF β1 expression of all treatment groups. In the group that was used as a TGF β1 expression value without treatment, experimental animals were not induced by burns and were not given any treatment, obtained an average TGF β1 expression of 4.843 ± 0.207 . In the group with BSS, aloe vera 20%, and aloe vera 40%, respectively, the average TGF β1 expression was 15.462 ± 0.549 ; 29.250 ± 0.515 and 46.005 ± 0.406 .

Table 1. TGF β1 Expression of Experimental Animals

TGF Expression Mean	Group β1
Normal	4.843 ± 0.207
BSS	15.462 ± 0.549
Aloe Vera 20%	29,250 ± 0,515
Aloe Vera 40%	46,005 ± 0,406

Table 2. TGF β1 Expression Normality Test (p>0.05)

Group	P Value
Normal	0.859
Untreated	0.077
Aloe Vera 20%	0.311
Aloe Vera 40%	0.721

Saphiro-Wilk, p = 0.05

Table 3. Effectiveness of Aloe Vera Gel Administration on TGF β1 Expression

Group	TGF β1 Expression Untreated	TGF β1 expression With treatment	Difference	P
BSS		15,462 ± 0,549	-10,618 ± 0,4930,000	
Aloe Vera 20%		29,250 ± 0,515	-24,407 ± 0,4090,000	
Aloe Vera 40%		46,005 ± 0,406	-41,162 ± 0.4110,000	
Group	TGF β1 Expression Untreated	TGF β1 expression With treatment	Difference	P
BSS		15,462 ± 0,549	-10,618 ± 0,4930,000	
Aloe Vera 20%		29,250 ± 0,515	-24,407 ± 0,4090,000	
Aloe Vera 40%		46,005 ± 0,406	-41,162 ± 0.4110,000	

Paired-Sample T Test, p < 0.05

Table 4. Comparison of Effectiveness of Aloe Vera Gel Administration to TGF β1 Expression

Group	Average ± SD	Group	Average ± SD	p value
BSS	15,462 ± 0,549	Aloe Vera 20%	29,250 ± 0,515	0,000
		Aloe Vera 40%	46,005 ± 0,406	0,000
Aloe Vera 20%	29,250 ± 0,515	Aloe Vera 40%	46,005 ± 0,406	0,000

Table 5. Conformity Test (Multivariate) Administration of Aloe Vera Gel

Group	BSS	Aloe Vera 20%	Aloe Vera 40%
BSS		0.000	0.000
Aloe Vera 20%	0.000		0.000
Aloe Vera 40%	0.000	0.000	

3.2. TGF β 1 Expression Normality Test

Normality tests were performed on TGF β 1 expression of each group. It was obtained that the results of TGF β 1 expression of all groups had a p value of > 0.05 , so to compare the expression of TGF β 1 between untreated groups with treatment used Paired T Test, while to see the comparison of TGF β 1 expression between the two treatment groups used Independent T Test

3.3. Effectiveness of Aloe Vera Gel Administration on TGF β 1 Expression

TGF β 1 expression in the untreated and treated treated group was then averaged and tabulated. Furthermore, the TGF β 1 expression of each group was compared to see the effectiveness of aloe vera gel administration. TGF β 1 expression in each group without treatment and with treatment then analyzed with Paired-Sample T Test, obtained a probability value of all groups < 0.05 . This showed that there was a significant difference in the average TGF β 1 expression of all groups after treatment. In this study, there was an increase in TGF β 1 expression after treatment, the largest increase was found in the aloe vera group of 40% by 41.162 ± 0.411

3.4. Comparison of the Effectiveness of Aloe Vera Gel Administration to TGF β 1 Expression

TGF β 1 expression after treatment of all groups was measured and then averaged and tabulated. Furthermore, TGF β 1 expression was compared to each group. With statistical analysis, the results are found to be differences in TGF β 1 expression between groups with BSS administration with 20% aloe vera group and 40% aloe vera and between 20% aloe vera group with 40% aloe vera group ($p < 0.05$).

3.5. Conformity Test (Multivariate) Administration of Aloe Vera Gel

After the bivariate test, the analysis continued with the multivariate test. With the One-Way ANOVA test, it was found that there was a difference in TGF β 1 expression after treatment between the three groups in this study ($p = 0.000$). Before conducting a Post Hoc confirmation test, a homogeneity test is carried out first to see which test which we will use in Post Hoc analysis, using Lavene's test. In the lavene test, a probability value of 0.409 ($p > 0.05$) was obtained, which means that the TGF β 1 expression of all groups is homogeneous (the same variant) so that continued with the Post Hoc confirmation test using the Tukey Test, the following results were obtained.

From the results of the Tukey test, the probability value between the BSS group was obtained with the aloe vera group 20% and aloe vera 40% Tukey test, $p < 0.05$ and between the aloe vera group 20% with the aloe vera group 40% < 0.05 so that it can be concluded that there are differences in the average expression of TGF β 1 both between groups with BSS administration with aloe vera 20%, between groups with BSS administration with aloe vera group 40% and between groups aloe vera 20% with aloe vera group 40%.

4. Discussion

The expression level of Transforming Growth Factor Beta 1 (TGF- β 1) was the parameter investigated in this study. Measurement of TGF- β 1 expression level was performed on the 3rd day after treatment. From the results of measuring TGF- β 1 expression levels, it was found that there was a significant difference in the average TGF β 1 expression of all groups after treatment where there was an increase in TGF β 1 expression, the largest increase in TGF β 1 expression was found

in the aloe vera group of 40%. In addition, there were differences in TGF β 1 expression between groups without treatment with the aloe vera group 20%, between treatment groups with the aloe vera group 40% and between aloe vera groups 20% with aloe vera group 40%. From these results it can be seen that the higher the dose of aloe vera, the greater the increase in TGF β 1 that occurs.

The increase in TGF β 1 that occurred after administration of aloe vera was in line with research conducted by Hormozi et al in 2017 which reported the level of TGF β 1 expression in the study increased over time and increased dose.⁹ The dose and time with the largest TGF β 1 expression level were 150 μ g/ml and 72 hours (3 days).⁹ Another study conducted by Atik et al in 2021 showed the results of TGF β 1 immunohistochemical expression levels of rats with corneal lacerations on the first day in the strong positive category showed results of 10% without gel and 50% with gel ($p = 0.013$).¹⁰ Then on the 3rd day on In the group without administration of aloe vera gel, TGF β 1 expression showed 10%, while in the group with administration of aloe vera gel, strong positive showed 90% result ($p = 0.003$).

Fahreza et al's research in 2018 which examined the effect of Aloe vera gel extract on TGF- β expression in chemical trauma of mouse corneal bases found that Aloe vera extract with a concentration of 20% had the highest TGF- β value compared to the control group, Aloe vera 10%, and Aloe vera 40% at 72 hours after induction of alkaline chemical trauma.⁷ The difference between this study and Fahreza's study is that in this study the dose increase showed an increase in TGF β 1.

5. Conclusion

There were differences in the expression of Transforming Growth Factor Beta 1 (TGF- β 1)

in grade II palpebral thermal burn trauma mice given topical gel extract treatment Aloe vera with a concentration of 20% and Aloe vera with a concentration of 40% compared to those not given therapy. Topical gel extract treatment of Aloe vera with a concentration of 40% had the highest expression of Transforming Growth Factor Beta 1 (TGF- β 1) compared to other groups in mice model of grade II palpebral thermal burn trauma.

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