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The Effect of Aloe Vera Extract to Extensive Lesion and Expression of Transforming Growth Factor (TGF-β) on Alkaline Chemical Trauma Cornea Model

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Abstract

Background: Chemical trauma to the cornea is an emergency condition of the eye that requires early diagnosis and good treatment. Alkaline have ability to saponify fatty acids in cells and cell membranes which can make penetration into the stroma and destroy proteoglycans and collagen in cells. Aloe vera (AV) contains several active substances that are reported to have anti-inflammatory, immunomodulatory, and wound healing effects. AV has been reported to accelerate the healing process of corneal epithelial defects by increasing fibroblast proliferation, collagen production and growth factor production. This study aims to determine the difference between the effect of aloe vera extract with a concentration of 10%, 20%, 40% and BSS on the healing of extensive corneal lesions in white wistar rats alkaline trauma models.

Method: This study was an experimental study with a pre and posttest only with control group design in vivo approach to 30 Wistar white rats which were divided into 5 treatment groups for 3 days. Comparative analysis of effectiveness using the ANNOVA test or the Kruskal Wallis test and continued by the post hoc test.

Results: Based on the one way ANOVA test there was a statistically significant difference in effectiveness between the five treatment groups on the percentage of corneal wound healing area and TGF-β expression with an assessment of p = 0,000 each. The administration of alloevera (AV) concentration of 20% had a significant difference in percentage of healing of corneal lesions and TGF-β expression compared with other treatment groups with p = 0,000 each. Large

differences in the area of corneal lesions in the 40% AV group were -0.45 in the BBS group, 0.146 in the 10% AV group, 0.493 in the 20% AV group. The difference in the AV group 10% was 0.30 in the BBS group, -064 in the AV group 20%, and -0.14 in the AV group 40%. However, TGFβ expression in the normal control group that did not receive treatment was 54.94 (53.21-56-12). TGFβ levels in the BSS group were 10.44, the 10% aloe vera group was 25.43, 47.99 for the 20% aloe vera group and 37.95 for the 40% aloe vera group.

Conclusion: There is a difference between the effect of aloe vera extract with concentrations of 10%, 20%, 40% and BSS on the extensive healing of corneal lesions in white wistar rats with alkaline chemical trauma models.

Keywords: Aloevera, Alkaline chemical trauma, Wistar white rat, expression of transforming growth factor (TGFβ)

Introduction

Chemical trauma to the cornea is an emergency condition of the eye that requires early diagnosis and prompt and prompt treatment. Chemical injuries to the eye can cause severe damage to the surface of the eyeball and anterior segment and cause visual impairment and disability. Chemical trauma due to substances that are basic (alkaline) is more destructive than acid trauma. WHO data in 2008 ocular trauma resulting in unilateral blindness occurred as many as 19 million people, 2.3 million decreased bilateral vision, and 1.6 million experienced bilateral blindness due to eye injury. Most (84%) are chemical trauma. The incidence of chemical trauma to the eye reaches 11.5% -22.1% of all cases of eye trauma. Nearly two-thirds occur in young adults and more often than acid chemical trauma. [1-4]

Bases have the ability to saponify fatty acids in cells and cell membranes so that penetration reaches the stroma and destroys proteoglycans and collagen in cells. When the cell membrane is damaged by a stimulus, the enzyme phospholipase is activated to convert phospholipids to arachidonic acid, then converted by the cyclooxygenase (COX) form COX 1 and COX 2. The cyclooxygenase (COX) enzyme will become prostaglandin G2 (PGG2), prostacyclin (COX) to form COX 1 and COX 2. The cyclooxygenase (COX) enzyme will become prostaglandin G2 (PGG2), prostacyclin (COX) to form COX 1 and COX 2. PGI2) and thromboxane (TXA2). Prostaglandin G2 undergoes a peroxidase reaction to a protaglandin H2

(PGH2) compound. [5]

Protaglandins can trigger oxidative stress. Inflammation is a redox sensitive mechanism, oxidative stress can activate transcription factors such as NF-κB, which regulate the release of proinflammatory cytokines such as TNF-α, IL-1, IL-10, IL-12. Production of reactive oxygen species (ROS), also triggers the production of enzymatic antioxidants such as catalase (CAT), hydroperosidase (HPx), superoxide dismutase (SOD). The amount of ROS formed will be a disruption to homeostasis or stimulation of growth, survival, and cell signaling, depending on how much ROS is produced. If the production of high ROS levels exceeds the capacity, it can damage cells through lipid peroxidase, DNA modification, protein destruction, and mitochondrial damage. Oxidative stress causes various damage to cell components and contributes to the pathogenesis of various diseases. Tumor necrosis factor alpha is a major mediator in the acute inflammatory response. Tumor necrosis factor alpha is proven to be a modulator strong general response that mediates the induction of adhesion molecules, other cytokines and neutrophil activation. Tumor necrosis factor alpha functions as a fibroblast growth factor (FGF) which results in the formation of connective tissue. If TNF-α production continues, these tissues can become new lymphoid tissue where B and T lymphocytes accumulate.

The body's cells secrete anti-inflammatory cytokines such as IL-10 and TGF-β, which bind to cell surface receptors and initiate a double signal transduction cascade. So that the provision of preparations or materials that can increase TGF-β can stimulate the formation of extracellular matrix (ECM), ie increase cell movement, increase production of macrophages, proteoglycans, fibronectin, fibroblasts, collagen and collagenase and reduce the production of protease enzymes that damage the matrix. This process as a whole will cause deposition of new tissue formation known as the proliferation phase and after all epithelialization, granulation, and neovascularization processes are completed it will be followed by a remodeling process for the formation of new structures approaching the initial conditions.[7] The current difficulty in the management of basic chemical trauma is prolonged inflammation so that it continues to corneal lesions and corneal perforation can occur. This collagenase begins to form 9 hours after trauma and its peak is on the 12-21 hour day. The process of corneal defects stops only when complete epithelialization or vascularization has closed the corneal defect. [5.6]

Aloe vera (AV) contains several pharmacologically active substances including

polysaccharides, antraquinones, lectins, superoxide dismutase, glycoproteins, vitamins A, C, and E, as well as minerals which are reported to provide anti-inflammatory, immunomodulatory and wound healing effects. AV has been reported to accelerate the healing process of corneal epithelial defects by increasing fibroblast proliferation, collagen production, and growth factor production. Recently, an in vitro study showed that AV solution is useful in healing superficial defects by helping to reduce fibrosis and accelerate re-epithelialization.

Nakamura et al have revealed that the topical application of substances P and IGF-1 accelerates the healing process of corneal epithelial wounds. In a previous in vivo study, we reported that AV accelerates wound healing and increases epithelialization through increased expression of TGFβ1 and bFGF.10 Mechanical studies conducted by Atiba et al. On healing corneal epithelial defects due to alkaline chemical trauma in diabetic rats have clearly demonstrated that AV can inhibit the inflammatory process, not only by reducing the level of proinflammatory cytokines, but also by reducing leukocytes, adhesion and infiltration at the site of the wound, and decreasing edema. [18-20]

Therefore, this study was designed to evaluate the effect of the effects of aloe vera on the expression of transforming growth factor (TGF-β) and healing of corneal epithelial wounds in white rat models of chemical trauma.

Methods

This research is an experimental study of pre and post test only with control group design in vivo. This research was conducted at the Animal House Laboratory and Biotechnology Laboratory at the Faculty of Medicine, UNSRI. The overall implementation time was 6 months. The target population of the study is the White Rat Wistar strain. The sample size is calculated by the Federer formula. The inclusion criteria is healthy white wistar rats (active moves) and 6-8 weeks old with 150-200 gram body weight. The inclusion criteria is rats that get sick or die before getting treatment, injuries to the eyeball / cornea due to trauma or bite. For drop out criteria is rat appear sick (inactive movements, do not want to eat, dull or fall hair).

Experimental animals were obtained from Animal House Faculty of Medicine UNSRI, housed in stainless steel cages, with the minimum required volume of cages size 500 cm2 for two rats and a minimum height of 20 cm cage, fed with pellets and given enough drinking water. Then,

placed in a room temperature of 20-240C, the cycle of dark-light for 12 hours. Before treatment, the rats were acclimatized for seven days.

Group of rats divide into 4 group, group 1 given 10% Aloe Vera eye drops in the eye that was given alkaline chemical trauma 5 times a day one drop for 3 days, group 2 given 20% Aloe Vera eye drops on the eye that was given alkaline chemical trauma 5 times a day one drop for 3 days, group 3 given 40% Aloe Vera eye drops on the eye that was given alkaline chemical trauma 5 times a day one drop for 3 days, group 4 6iven 0.6 ml of BSS eye drops (Balanced salt Solution) to the eye given alkaline chemical trauma 5 times a day one drop for 3 days.

Simulations of Aloe vera were obtained from BP2OT Tawangmangu Karang Anyar, Central Java (MOH) as many as 3 pieces were prepared stored in the Biotechnology Laboratory at the Faculty of Medicine, UNSRI. Extraction making are by three parts of 15 g fresh plants, each crushed in an electric mill. Each was placed in a 250 ml bottom-bottom flask, and 70 ml of 1.1% heptane, respectively, added 2.1% ethyl acetate and 3.96% ethanol. Then it is transferred to an ultrasonic bath and complete extraction tripled at 45 ° C is carried out, each time using the same volume of fresh solvent.

The filtered solution from each solvent is combined (volume 210 ml) and the solvent is distilled in a rotary vacuum evaporator IKA RV 05-ST 1. The dry residue obtained is used in biological tests. Dry residue samples were analyzed in gas chromatography-mass spectrometry to check for the presence of solvents (Variant 3400 gas chromatography in combination with the Finnigan MAT ITS 40 ion trap detector used), Restt Rtx-5 capillary chromatography column (0.18 mm id × 20 m, film 0.25 μm; Restek Corp. Bellefonte, PA). Negative test: no solvent used was found in the material analyzed. After the solvent is removed, part of the dry mass is dissolved in DMSO, obtaining a stock solution (100 mg / ml). Next, the working solution (8-125 ug / ml) was prepared with extracts which were soluble in the culture media. In a 100mg / ml solution it will be divided into 10% (10mg / ml), 20% (20 mg / ml) and 40% (40mg / ml).

Assessment of corneal epithelial wound area of rat cornea was tested with 0.5% fluorescein. Then by using a loop and calipers the lesions (mm) were measured at 0, 24 hours, 48 hours and 72 hours. And for assessment of TGFβ Expression in rats (Immunohistochemistry) tests presented here detect either the active and secreted form of TGF-β, the phosphorylated form of Smad, which correlates with the intensity of the signal originating from the TGF-β receptor, or the

amount of GFP induced by TGF-βin mouse tissue. The first test detects active TGF-β regardless of cell competency to respond to TGF-β, because all cells do not have receptors for proper signal transduction. Detection of pSmad and GFP will identify a subset of cells that have receptors and successfully transfer signals to the nucleus. All tests have in common and there is flexibility in their implementation depending on the type and source of tissue, the fixative used, and the source of the antibodies.

The rats by divided into 4 groups named according to their treatment (Aloe vera 10%, 20%, 40% and BSS) were coded by entering the characteristics data of the rat sample before action, corneal lesions at 0 hours of action, and corneal lesions at 72 hours of action. Data analysis was performed with SPSS 25 and MedCal 2012 by perform data distribution analysis using the normality test (saphiro wilk, because the sample <50), the variance homogeneity test (test levene, because the variations are the same) and compare the effectiveness of each group of rats and their treatment using a comparison test (one-way anova) to see changes in the size of the corneal lesion and post-hoc test to see differences in the mean difference.

Result

In the group treated with normal saline and the group treated with aloe vera 10%, the size of the corneal lesion was significantly increased. the change in size from 0 hours to 72 hours between these treatment groups showed a significant difference in terms of changes in size. For the saline (BSS) group, the size increase was 0.32 mm or 25.66%, while for the aloe vera group 10% the size increase was only 0.02 mm (1.60%). The most noticeable changes were seen in the 20% aloe vera group where there was a reduction in size of 0.62 mm or a reduction of 50.2% compared to the initial size of the corneal lesion area. In the aloe vera group 40% there was also a significant reduction in the size of the corneal ulcer of 0.13 mm (10.52%).

Homogeneity test results indicate that changes in the size of the corneal lesions are homogeneous and normally distributed. In the post hoc test results it was seen that the greatest difference was found in the aloe vera group by 20%. -0,945 with the BBS group, -0.64 in the 10% AV group, and -0.49 in the 40% AV group. The smaller size of the lesion indicates a cure, so it was concluded that the AV group was 20% more effective in curing corneal base chemical trauma in rats. The difference in the 40% AV group was -0.45 in the BBS group, 0.146 in the 10% AV group, 0.493 in the 20% AV group. Large difference in the AV group 10% was 0.30 in the BBS

group, -064 in the AV group of 20%, and -0.14 in the AV40% group.

Table 1. Difference in Lesion Size at 0 Hours, 72 Hours and Difference Between Corneal Area Width

Group	The size of the lesion is 0 hours	The size of the lesion is 72 hours	Difference in corneal lesions	* p
	X ± SD	X ± SD	X ± SD	
BSS (N = 6)	1.24 ± 0.03	1.57 ± 0.02	-0.32 ± 0.03	0,000
Aloe Vera 10% (N = 6)	1.23 ± 0.02	1.25 ± 0.01	-0.02 ± 0.01	0,000
Aloe Vera20% (N = 6)	1.25 ± 0.17	0.63 ± 0 .02	0.62 ± 0.04	0,000
Aaloe Vera 40% (N = 6)	1.25 ± 0.19	1.12 ± 0.01	0.13 ± 0.12	0,000

* One-Way Annova, significant if p <0.05, Normally Distributed Data is presented with Mean (± SD).

***** Post hoc test, p value is significant if p <0.05

The highest levels of TGFβ after 72 hours were seen in the normal control group that did not get the lesion, which was 54.94 (53.21-56-12). TGFβ levels in the BSS group were 10.44, the 10% aloe vera group was 25.43, 47.99 for the 20% aloe vera group and 37.95 for the 40% aloe vera group. Test results with the One-way Anova test showed a significant difference in TGFβ levels between these treatment groups (p = 0.000).

Table 2. Comparison of TGFβ at 72 hours between treatment groups

Group	n	TGFβ at 72 hours	* p
		X ± SD	
Normal	6	54.83 ± 1.07	0,000
BSS	6	10.53 ± 0.28	1,000
Aloe Vera 10%	6	25.37 ± 1.18	0,000
Aloe Vera 20%	6	47.84 ± 1.16	0,000
Aloe Vera 40%	6	37.87 ± 1.52	0,000

* Post hoc test VS BSS, significant if p <0.05

To test whether there is a correlation between the size of the area of the corneal lesion with TGFβ levels, a Spearman correlation test was performed. The results showed a very strong negative correlation between TGFβ levels and the size of the corneal lesion after 72 hours of treatment (r = -0,933, p = 0,000).

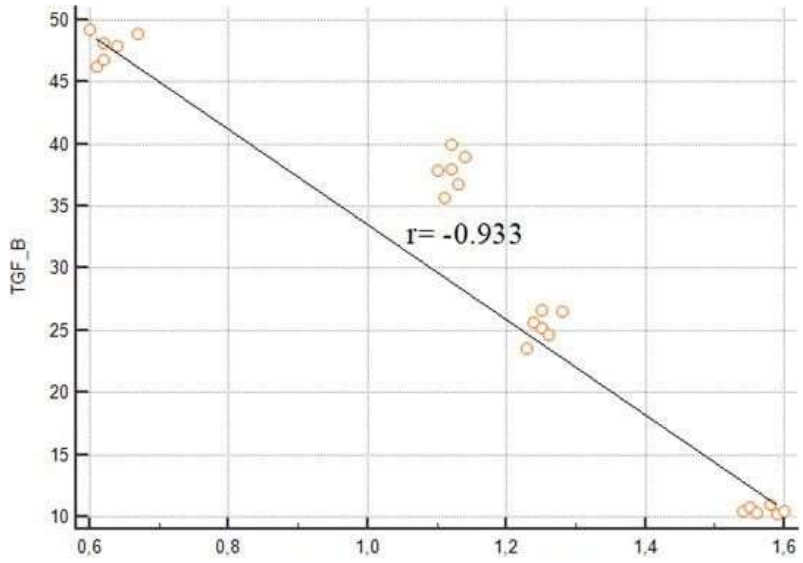

Figure 1. Correlation plot plot between corneal lesions after 72 hours with TGFβ

Figure 1. displays plot diagram data on the correlation between Corneal Ulcers after 72 hours and TGFβ. The results of correlation analysis show a negative value with a very strong correlation strength so it can be concluded that the higher the TGFβ value the smaller the size of the corneal lesion.

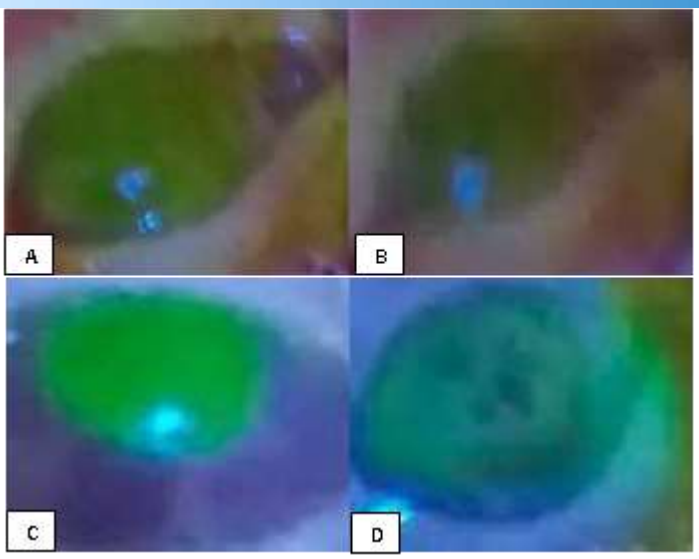

Figure 2. White Rat Cornea Day 3 Post Chemical Trauma Induction.
a. Negative Control, b. Dose 1, c. Dose 2, d. Dose 3

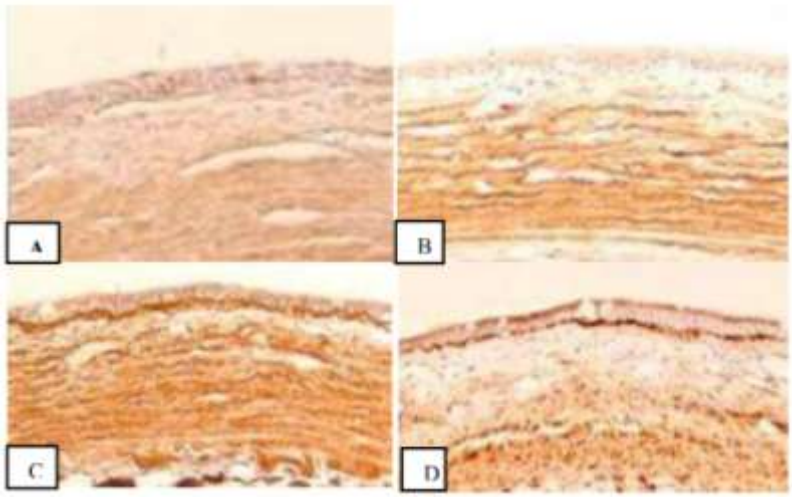

Figure 2. Immunohistochemistry TGF-B1 Corneal Rat. A. Negative Control; B. Aloe Vera Dosage 1; C. Aloe Vera Dose 2; D. Aloe Vera Dose 3. 400x magnification

Discussion

Effects of Aloe Vera on the Healing of Corneal Lesions in White Rats Wistar Strain Model of Chemical Trauma of Bases Given Aloe Vera Eye Drops. In this study after 72 hours treated with normal saline and Aloe Vera (AV) with concentrations of 10%, 20% and 40%, a very significant difference was seen in the size of the corneal lesions between these groups ($p = 0.000$) where the aloe vera extract 20% and 40% are more effective in healing corneal wounds and are

proven by decreasing the extent of corneal lesions in rats after 72 hours of treatment. But the 20% aloe vera extract has a higher level of effectiveness based on the reduction in the size of the corneal lesion with a size reduction of 0.62 mm or a reduction of 50.2% compared with the extract of aloe vera 40% also a significant reduction in the size of the corneal lesion of 0.13 mm (10.52%) after 72 weeks.

This study is not much different from Ayman et al. 2015 which concluded that aloe vera extract in the normal and controlled diabetic rats group both had a significant cure rate ($p < 0.05$) after 72 hours of treatment. In vitro research (culture media on corneas) conducted by Curto et al in 2013 found that aloe vera extract with a concentration of ≥175µg / mL had a significant relationship on wound healing in corneal epithelium or fibroblasts.[21]

Mechanical studies conducted by Atiba et al on the healing of corneal epithelial defects due to alkaline chemical trauma in diabetic rats have clearly demonstrated that AV can inhibit the inflammatory process, not only by reducing the level of proinflammatory cytokines, but also by reducing leukocytes, adhesions and site infiltration injuries, and decreased edema. 19 This study is not much different from Woźniak et al found that the use of aloevera ethanol extract ≥125µg / ml can reduce cytokine levels (IL-1β, IL-6, TNF-α and IL-10) in wounds in corneal cells, Woźniak et al concluded that aloe vera ethanol extract used as eye drops can reduce inflammation or help heal wounds in the cornea of the eye.[30]

According to Ayman et al. 2019 Corneal injuries induced by alkaline burns usually require more time for wound closure than mechanical re-epithelialization. This difference is due to alkaline burns causing inflammation that damages corneal stromal collagen and scar tissue formation by inducing immune cell infiltration and myofibroblast formation from keratocytes. This inflammatory response does not go away on its own and is associated with degradation of the epithelium and stroma.

Aloe vera contains many pharmacologically active substances including polysaccharides, anthraquinones, lectins, superoxide dismutase, glycoproteins, vitamins A, C, and E, and minerals which are reported to have anti-inflammatory, immunomodulatory and wound healing effects. Aloe vera is famous for its wound healing properties. Aloe vera has been reported to stimulate wound healing in different wound models. In addition, aloe vera can also accelerate the healing of skin wounds in diabetes, radiation, and burn models by increasing fibroblast proliferation,

collagen production, and re-epithelialization. Recently, an in vitro study showed that aloe vera solutions are beneficial in healing superficial wounds by reducing fibrosis and accelerating epithelialization. [20-21]

Oryan et al's research in 2016 using aloe vera extracts 10.20 and 30% compared to normal saline as a placebo in rats's skin lesions. During 10 days of treatment, aloe vera was 20% and 30% more effective for healing rat wounds but the CPI results showed that aloe vera was 30% faster to heal than 20% .40 Ibrahim et al in 2019 stated that the use of aloe vera still has side effects in sensitive groups even at the right dose there are still side effects such as irritation or diarrhea. Aloe vera used for more effective wound healing is used at lower doses with frequent frequency, because at high doses it will create an adverse effect to irritation.[41]

Edward et al in 2015 found that the right dose of aloe vera was very effective in healing level 1 and level 2 burned skin tissue, frost-bitten, and aloe vera were also suitable for the treatment of wounds in diabetics. Edward et al. Conducted pre-clinical studies in in vivo and clinical studies. In vivo studies found that the use of aloe vera at the right dose according to the degree of severity of the wound would be very effective, but excessive doses of aloe vera can have adverse effects such as nephritis.[42]

Not much different from Edward et al, Kanat et al in 2006 found excessive use of aloe vera extract in rats that had been given a wound in the stomach, causing gastrointestinal irritation until there were several subjects with rats suffering from gastrointestinal cancer.43 National Toxixity program Year 2010 also states that the use of excessive doses of aloe vera can have adverse effects such as allergies, and irritation, but because there is not much research on the effects of aloe vera on wounds so there is no specific dosage on the use of aloe vera extracts, either gel or topical appropriately.[44]

Samarh et al, in 2012 revealed that the aloe vera plant has been used as a treatment for wounds since ancient Egypt, all content of aloe vera from the skin to each leaf consists of three layers: 1) Inner clear gel containing 99% water and the rest is made of glucomannan , amino acids, lipids, sterols, and vitamins. 2) The middle layer of latex which is a bitter yellow sap and contains anthraquinone and glycosides. 3) The thick outer layer of cells 15-20 is called skin which has a protective function and synthesizes carbohydrates and proteins. Inside the skin there are bundles of vessels that are responsible for the transportation of substances such as water (xylem)

and starch (phloem). All of them contain 75 potentially active constituents: vitamins, enzymes, minerals, sugar, lignin, saponins, salicylic acid, and amino acids which are very effective as an anti-inflammatory, antiseptic, anti-viral, antioxidant, and wound treatment.[45]

Effect of Aloe Vera on the Transforming Growth Factor (TGF-β) Expression in White Rat Cornea Wistar Strain Model for Chemical Base Trauma. In this study the highest levels of TGFβ after 72 hours were seen in the normal control group without lesions, which was 54.94 (53.21-56-12). TGFβ levels in the BSS group were 10.44, the 10% aloe vera group was 25.43, 47.99 for the 20% aloe vera group and 37.95 for the 40% aloe vera group. Researchers did not find similar studies on the effect of aloe vera extract on the expression of transforming growth factor (TGF-β) in pure rat corneas of ordinary chemical trauma models, but Cohen et al's 2013 study conducted in vivo research by combining aloe vera extract with stem cells such as umbilical cord blood stem cells, or embryonic germ cell derivatives, and / or embryonic stem cells and find effective results to increase TGF-β levels which play an important role in healingvwounds, one of which is in the wound in the corneal area of the eye.[31]

The 2012 Banu et al study found that besides being able to increase TGF-β levels, the addition of aloe vera extract could be used to inhibit bacterial growth in wounds.32 This is in line with Vandana et al's 2014 study which found that aloe vera extract which was used as a transemulgel could increases TGF-β levels and is anti- inflammatory in wounds.33 The basic mechanism of corneal epithelialization is similar to that seen in other mucous membranes and consists of epithelial migration and proliferation. Several biochemical factors, including epidermal growth factor, insulin-like 1 (IGF-1) growth factor, growth factors derived from platelets, transforming growth factor (TGF-β), and basic fibroblast growth factor (BFGF), are known to be involved in re -epitelization. These factors have been identified as the main ingredients in epithelialization, especially in cell migration and

mitosis.28 Nakamura et al have revealed that the topical application of P substances and IGF-1 accelerates the healing process of corneal epithelial wounds in diabetic animals. In previous in vivo studies, we reported that aloe vera accelerates healing of skin wounds and increases epithelialization through increased TGF and bFGF expression.10 Therefore, current results indicate that rapid corneal epithelialization caused by topical aloe vera is also mediated through increased production growth factor.[5]

Conclusion

Aloe vera extract used as eye drops with concentrations of 20% and 40% is more influential in healing basic chemical corneal trauma.

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