Sys Rev Pharm 2020;11(5):571-573
A multifaceted review journal in the field of pharmacy

Effect of *Aloe vera* Extracts towards the Fibroblast Number and Collagen Thickness on Clean Skin Wound Healing on *Rattus Novergicus*

Rezky Sagita Girsang, Marjono Dwi Wibowo, Ariandi Setiawan*, Edwin Danardono

Department of Surgery, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya 60131, Indonesia

ABSTRACT

Currently, there are many agents that can improve the healing process, one of which is *Aloe vera* extract. This study aimed to prove the effect of *Aloe vera* and tulle extracts on the healing of clean wounds on rat skin. This was a laboratory experimental study in white rats using the treatment of *Aloe vera*, tulle, and normal saline extract on clean skin wounds. Data on body weight and age of the sample used in this study were all homogeneous (p >0.05). The mean number of fibroblasts in control group was 72.18±28.35, *Aloe vera* group was 129.3±33.27, and tulle group was 84±18.85. The *Aloe vera* administration group had more fibroblasts and was statistically significant compared to the other groups (p = 0.011), although there was no significant difference in the collagen thickness score among three groups (p = 0.801). *Aloe vera* extract can increase the number of fibroblasts in the clean wound healing process.

Keywords: Fibroblast Amount, Collagen Thickness Score, *Aloe vera* Extract, Tulle.

Correspondence:
Ariandi Setiawan, MD
Department of Surgery, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya 60131, Indonesia
Tel: +6231-550-1327; Fax: +6231-504-9256
E-mail: ariandi.setiawan@gmail.com

INTRODUCTION

The wound is the most common case of humans' injury. It can be caused by sharp or blunt trauma, temperature changes, chemicals, explosions, electric shock or animal bites (1). The wound healing process is a complex cellular process and focuses on returning the integrity of the damaged tissue. In general, the wound healing process occurs in three phases, inflammatory phase, proliferation phase, and remodeling phase (2,3).

The proliferation phase is the most important in wounds healing, where fibroblasts play a major role. Fibroblasts will produce the basic ingredients of collagen fibers that will link the edges of the wound. With the influence of growth factors and hydrolytic enzymes released by macrophages, it causes proliferation of fibroblasts and produces a lot of collagen to form granulation tissue that contains many new capillaries (3).

Long-term injury can lead to infection, hematoma, scarring, keloids and more complications. This can increase the length of stay and the cost of care. Many efforts have been developed throughout the world to find agents that can improve wound healing, expected to reduce these complications, for example, with the tulle application [1]. Tulle is a net coated made of polymer matrix, which are soft and elastic compounds, tar oil, and hydrocolloids. When the hydrocolloid particles come into contact with the wound exudate, a gel will form, making the wound environment moist and conducive to the wound healing process (4). On the other hand, more than 80% of the world's population still relies on traditional medicine, including wound healing, for example, by using *Aloe vera*. In some studies, *Aloe vera* has an effect in the process of wound healing, such as anti-inflammatory, antibacterial, anti-allergic, and analgesic effects (5).

Modern medicine combined with traditional medicine traditional can have an impact on reduced maintenance costs, length of care, and mortality rates (6). Based on this background, the authors wanted to find out the effect of giving *Aloe vera* and tulle extracts on the healing of clean wounds on rat skin [2].

MATERIALS AND METHODS

A laboratory experimental study with a randomized post-test only control group design was conducted to prove the administration of *Aloe vera* and tulle extracts can affect the number of fibroblasts and collagen thickness scores. The administration of *Aloe vera* extract further increases the number of fibroblasts and collagen thickness on clean wound healing full thickness white mouse skin. This study used experimental male Wistar strain *Rattus novergicus* about 12-16 weeks old with a body weight between 250-300 grams, carried out by the process of making full thickness clean wounds. In the study, there were 3 groups: the control group treated with wounds by giving normal saline fluid, the treatment group 1 given *Aloe vera* extract gel, and the treatment group 2 applied with tulle. The three groups carried out the research sampling process on the 6th day (proliferation phase), and the specimens were put into bottles with 10% formalin fixed. Hematoxylin-eosin staining was carried out, and the thickness measurements were made on the wound edge area with a micrometer at 400x to see the number of fibroblasts. The collagen thickness score was used. This study was approved by the Ethics Committee before this research was conducted. All experimental animals were treated according to the rules of the Animal Care and Use Committee of Universitas Airlangga.

The sample data analysis was performed by the Shapiro Wilk normality test and Levene homogeneity test. The ANOVA comparison test was carried out for hypothesis testing for the number of fibroblasts if the data were normally distributed and homogeneous. Meanwhile, Kruskal-Wallis comparative test was performed for collagen thickness scores on clean wounds between the control group, treatment group 1, and treatment group 2. If there were significant differences, the Post hoc Test was then proceeded to find out more clearly the significant differences between the sample groups.

RESULTS

Based on age characteristics, the age of the samples in the three groups ranged from 12 to 16 weeks, as seen in Table 1. In the control group, the mean age was 14.00±1.342 weeks. In the treatment group 1, the mean age was 14.55±1.229 weeks. Meanwhile, in the treatment group 2, the mean age was 14.36±1.502 weeks. The age between the three groups was homogeneous and did not have a significant difference (p = 0.645).

Based on the characteristics of body weight, the sample weight in the group ranged from 250 to 300 grams. In the control group, the average body weight was 269.09±15.783 grams. In the treatment group 1, the average body weight was 270.9±17.003 grams. Meanwhile, in the treatment group 2, the average body weight was 271.82±17.215 grams. The weight between the three groups was homogeneous and did not have a significant difference (p = 0.927).

Table 1. Characteristics of Subjects

Characteristics	Control (n=11)	Treatment 1 (n=11)	Treatment 2 (n=11)	P-value
Age (week)				0.645
Mean±SD	14.00±1.342	14.55±1.293	14.36±1.502	
Min-max	12-16	12-16	12-16	
Weight (gram)				0.927
Mean±SD	269.09±15.783	270.91 ±17.003	271.82±17.215	
Min-max	250-300	12-16	12-16	

The number of fibroblasts and collagen thickness scores between the three groups is shown in Table 2. The mean number of fibroblasts in the control group was 72.18±28.35, treatment group 1 was 129.3±33.27 and treatment group 2 was 84±18.85. In the normality and homogeneity test in 3 groups, the number of fibroblasts was normally distributed and homogeneous, followed by ANOVA statistical test.

There were significant differences in the number of fibroblasts between the three groups (p = 0.011). While the collagen thickness score was found in score 3, the control group was 6 samples (54.5%), treatment group 1 was 7 samples (63.6%) and treatment group 2 was 6 samples (54.5%). There was no significant difference in the collagen scores of the three groups (p = 0.801).

Table 2. The difference of fibroblasts count and collagen thickness score

Characteristics	Control (n=11)	Treatment 1 (n=11)	Treatment 2 (n=11)	P-value
Number of fibroblasts				0.011
Mean±SD	72.18±28.35	129.3±33.27	84±18.85	
Min-max	26-112	98-199	63-112	
Collagen Thickness Score				0.801
Score 1	0	0	0	
Score 2	5 (45.5%)	4 (36.4%)	5 (45.5%)	
Score 3	6 (54.5%)	7 (63.6%)	6 (54.5%)	

DISCUSSION

In this study, we found that the number of fibroblasts and collagen thickness scores increased more in the *Aloe vera* treatment group than in the other groups. This increase in the number of fibroblasts is caused by increased stimulation of fibroblastic growth factors (FGF) secretory proteins resulting from platelet degranulation (7). The entire wound healing process involves a series of complex events that begin at the time of the injury and can continue for months to years (8). In normal conditions, the wound can heal by itself, but how fast it is can be affected by many factors (9). The wound healing process occurs in three phases, including the inflammatory phase, the proliferation phase, and the remodeling phase. The proliferation phase is the most important in wounds healing, where fibroblasts will produce the basic ingredients of collagen fibers to form granulation tissue containing many new capillaries (3).

Glucomannan, the active ingredient of *Aloe vera*, can stimulate the activity of FGF and proliferation, which will also increase the synthesis of collagen. Increased angiogenesis also optimizes wound healing (10). Many active components contained in *Aloe vera*, such as vitamins, enzymes, minerals, proteins, fats, sugars, lignin, saponins, salicylic acid, and amino acids that can relieve inflammation similar to Mangosteen (*Garcinia mangostana* Linn.), provide a conducive environment and encourage collagen synthesis compared to tulle application which can only provide a conducive environment in the proliferation phase of the wound healing process (4,5,11). In addition, the increase in the number of fibroblasts in the *Aloe vera* extract is in line with previous studies [3]. The extract cream of *Aloe vera* gel plays a role in wound depth burning by increasing the number of lumen vessels, the amount of macrophages, the number of fibroblasts and the thickness of collagen in the inflammatory and proliferation phases. In the inflammatory reaction, this topical cream has an anti-inflammatory effect (12). *Aloe vera* extract has a beneficial effect on the proliferation phase and can significantly accelerate wound healing (7).

Fibroblasts are the main cells in the proliferation phase, with a role in providing the extracellular matrix as a framework for migrating keratinocytes [4]. Larger amounts of fibroblasts help to form denser and more compact extracellular matrices to stimulate epithelialization by keratinocytes (13). The higher number of fibrolasts in the wound treatment group cause the treatment group to experience epithelialization faster than the control wound group. Meanwhile, tulle also plays a role in the proliferation phase by creating a moist and conducive environment for the formation of new tissue and collagen [5]. Tulle does not work to heal wounds but facilitates wound healing by providing a conducive environment for the process of wound healing (14-20).

The treatment of giving *Aloe vera* gel seems to trigger collagen production. However, the process has not been proven. This can be caused by the time the sampling is too early (on day 6) where collagen is not yet optimally formed. Collagen begins to form on days 5 to 7 where fibroblasts migrates into the wound form new collagen tissue subtypes I

and III at the beginning of normal wound healing. Type III collagen is very dominant, then replaced by type I collagen where the collagen remodeling process lasts from 3 weeks to 2 years (13–15).

This study generally shows that the application of topical *Aloe vera* extract to clean wounds can accelerate the epithelialization process. This is proved by the increase in the number of fibroblasts at the observation of day 6 in the proliferation phase. The same thing happened in collagen. There was an increase in collagen density serving as the main structure of the new extracellular matrix of wound tissue in the treatment group with *Aloe vera*, although it was not statistically significant. To prove this, further research needs to be done with a longer observation time.

CONCLUSION

The administration of *Aloe vera* extract can increase the number of fibroblasts in the clean wound healing process. The administration of *Aloe vera* extract can be used as an additional product in the healing process to reduce maintenance costs, length of stay, and mortality rates in patients.

REFERENCES

1. Irham WH, Tamrin, Marpaung L, Marpongahtun. Bioactive Compounds in Pegagan Leaf (Centella asiatica L. Urban) for Wound Healing. In: Journal of Physics: Conference Series. Institute of Physics Publishing; 2019.
2. Arijani E, Khoswanto C. The use of 90% Aloe vera freeze drying as the modulator of collagen density in extraction socket of incicivus Cavia cobaya. Dent J (Majalah Kedokt Gigi). 2008;41(2):74–6.
3. Junqueira LCU, Mescher AL. Junqueira's basic histology text & atlas. New York: McGraw-Hill Medical,; 2016.
4. Chamanga ET. Clinical management of non-healing wounds. Nurs Stand. 2018;32(29):48–62.
5. Surjushe A, Vasani R, Saple DG. Aloe vera: a short review. Indian J Dermatol. 2008;53(4):163.
6. World Health Organization. WHO traditional medicine strategy: 2014-2023. Geneva: World Health Organization; 2013.
7. Moriyama M, Moriyama H, Uda J, Kubo H, Nakajima Y, Goto A, et al. Beneficial effects of the genus Aloe on wound healing, cell proliferation, and differentiation of epidermal keratinocytes. PLoS One. 2016;11(10).
8. Hanafiah OA, Abidin T, Ilyas S, Nainggolan M, Syamsudin E. Wound healing activity of binahong (Anredera cordifolia (Ten.) Steenis) leaves extract towards NIH-3T3 fibroblast cells. J Int Dent Med Res. 2019;12(3):854–8.
9. Laila L, Febriyenti F, Salhimi SM, Baie S. Wound healing effect of Haruan (Channa striatus) spray. Int Wound J. 2011;8(5):484–91.
10. Machmud E, Jubhari EH, Ardiansyah M. Histometry analysis of osteoclasts, osteoblasts and fibroblasts in immediate placement implants with addition of aloe vera extract. Pesqui Bras Odontopediatria Clin Integr. 2019;19(1).
11. Sombolayuk HB, Djawad K, Wahab S, Miskad UA, Alam G, Pattelongi I. The effectivity of topical mangosteen pericarp extract cream on wound healing in Swiss albino mice. J Biol Res. 2019;92(2).
12. Hidayat T, Noer MS, Rizaliyana S. Role of Topical Extract Aloe Vera gel in Deep Burn Wound Healing in Rat. Media J Rekonstruksi Estet. 2013;2(2).
13. Gurtner GC. Wound healing: normal and abnormal. Grabb Smith's Plast Surgery-Philadelphia, LWW. 2014;13–9.
14. Schultz GS, Chin GA, Moldawer L, Diegelmann RF. Principles of Wound Healing. In: Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists [Internet]. University of Adelaide Press; 2011.
15. Goel SA, Guo L-W, Shi X-D, Kundi R, Sovinski G, Seedial S, et al. Preferential secretion of collagen type 3 versus type 1 from adventitial fibroblasts stimulated by TGF-β/Smad3-treated medial smooth muscle cells. Cell Signal. 2013;25(4):955–60.
16. de la Cerda Paolinelli, C., C. Martinez, and A. Tomicic Suner, *MENTALIZATION AS PROCEDURAL LEARNING IN PSYCHOTHERAPEUTIC INTERACTION: A RELATIONAL REFLECTIVE FUNCTIONING.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(3): p. 285-294.
17. Duffy, D., *ANALYSIS OF FORMAL AND INFORMAL HEALTH NETWORKS. INFORMATION FLOW AND DERIVATION OF USERS WITH EXCESSIVE CONSUMPTION OF ALCOHOL.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(3): p. 295-303.
18. Entenberg, G., et al., *ATTITuDES OF ARGENTINE ThERAPISTS TOwARDS ThE INCORPORATION OF NEw TEChNOLOGIES IN PSyChOThERAPy: TEN yEARS OF EVOLuTION.* Revista Argentina de Clínica Psicológica, 2019. **28**(3): p. 304-313.
19. Serino, S., *Usability and feasibility of virtual reality cognitive trainings in Alzheimer's disease: A narrative review.* Revista Argentina de Clínica Psicológica, 2019.
20. Peng, S., et al., *EXPERIMENTAL STUDY ON GROUP SANDPLAY INTERVENTION FOR SOCIAL ANXIETY AND LONELINESS IN OBESE CHILDREN/ESTUDIO EXPERIMENTAL DE UNA INTERVENCIÓN DE TERAPIA DE ARENA GRUPAL PARA ANSIEDAD SOCIAL Y SOLEDAD CON NIÑOS OBESOS.* Revista Argentina de Clínica Psicológica, 2019. **28**(I): p. 105.