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Effect of *Aloe vera* Hydrogel Application on Increasing the Number of Fibroblasts in Socket Wounds Post-Tooth Extraction: An In Vivo Study

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ABSTRACT

Background. *Aloe vera* has been used as an anti-inflammatory, antimicrobial, and immune-boosting agent since ancient time. Hydrogel is a polymeric material that can retain a significant amount of water component and can last as long as possible in the gingival sulcus because the hydrogel is hydrophilic. This study is the first study that seeks to explore the potential of Aloe vera in healing socket wounds after tooth extraction.
Methods. Twenty-five white rats of the Wistar strain were acclimatized for seven days before being included in the study. After acclimatization, the experimental animals were grouped into five groups. Evaluation of fibroblast response process with HE staining.
Results. There were differences in the mean number of fibroblasts in all groups. From the results of this study, it can be stated that there is an effect of the application of hydrogel *Aloe vera* 1%, 2.5%, 5%, 10%, and xylazine on the increase in the number of fibroblasts in socket wounds after tooth extraction in *Rattus novergicus.*
Conclusion. There is an effect of *Aloe vera* hydrogel application on increasing the number of fibroblasts in socket wounds after tooth extraction in *Rattus novergicus.*

1. Introduction

Tooth extraction will cause injury in alveolar bone of the oral cavity.[1] The wound healing process is divided into 3 phases, namely the inflammatory phase, the proliferative phase and the maturation phase.[2] The inflammatory phase begins a few minutes and lasts approximately three days after the injury. The proliferative phase is characterized by angiogenesis, granulation tissue formation, fibroplasia, collagen deposition, re-epithelization, and wound contraction. Histological cross-section of the wound healing process showed changes in the wound area such as a decrease in the number of inflammatory cells, the formation of new blood vessels, an increase in the number of epithelial cells and fibroblasts, and the formation of collagen fibers. This phase occurs within 3-24 days. The maturation phase is the final stage of the wound healing process. This process can take more than a year, depending on the depth and extent of the wound.[2,3]

Fibroblast cells are cells commonly found in connective tissue that synthesize several extracellular matrix components such as collagen, elastin, reticular,

and some anionic macromolecules such as glycosaminoglycans and proteoglycans.[4] After the formation of a wound, there will be a complex process. Fibroblast cells significantly affect the wound healing process. The proliferation of fibroblasts determines the outcome of wound healing. Fibroblasts will produce collagen, which will link the wound, and fibroblasts will also affect reepithelization, which will close the wound.[5]

World Health Organization recommends using traditional medicines, including herbs, to maintain public health, prevent and treat diseases, especially chronic diseases, degenerative diseases, and cancer. One of the commonly used herbal ingredients to alternative antimicrobial and anti-inflammatory ingredients is *Aloe vera.*[6] *Aloe vera* is native plants of South Africa, Madagascar, and Saudi Arabia. *Aloe vera* plant belongs to the Liliaceae family. This plant can grow in hot and dry weather due to its high capacity to retain water. Since ancient times, *Aloe vera* has been used as an anti-inflammatory, antimicrobial, and immune-boosting agent. *Aloe vera* leaves contain about 98.5% water, and 1.5% contains a composition of vitamins, minerals, enzymes, polysaccharides, polysacharide compounds, and organic acids soluble in water fat-soluble.[7] *Aloe vera* contains acemannan, phytosterols, and their active components, resulting in faster wound healing through stimulation of growth factor production, angiogenesis, the proliferation of fibroblast deposition, and collagen.[8] Hydrogel is a polymeric material that can retain a significant amount of water component and can last as long as possible in the gingival sulcus because the hydrogel is hydrophilic. This study is the first study that seeks to explore the potential of *Aloe vera* in healing socket wounds after tooth extraction. In this study, an exploration of the role of *Aloe vera* on fibroblast cells was carried out in rats (in vivo study).

2. Methods

Study design

The research design is an experimental study, post-test only with a control group design. This research was conducted from August to October 2021 at the Physiology and Plant Tissue Culture Laboratory, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara, Animal Development Center Laboratory, and PA Prospecta Laboratory. This research has been approved by the Health Research Ethics Committee (KEPK) Universitas Prima Indonesia, No. 042/KEPK/UNPRI/XI/2021.

Animal preparation

The subjects in this study were male rats (*Rattus norvegicus*) Wistar strain, which met the criteria for bodyweight between 150-200 grams, and age between 8-10 weeks. Twenty-five white rats of the Wistar strain were acclimatized for seven days before being included in the study. Rats were caged in a treatment facility with a light-dark cycle of 12 hours, room temperature 25^{0}C, access to food and drink ad libitum. After acclimatization, the experimental animals were grouped into five groups (each group consists of five rats), namely HAV1: the group that received 1% hydrogel *Aloe vera*, HAV2.5: the group that received 2.5% hydrogel *Aloe vera*, HAV5: the group that received hydrogel *Aloe vera* 5%, HAV10: group receiving 10% hydrogen *Aloe vera*, XLZ: group receiving xylazine.

Hydrogel *Aloe vera* preparation

Fresh and clean *Aloe vera* was prepared with a size of 30 cm x 2.5 cm. Then, 70% ethanol was added, stirred for 30 minutes with a magnetic stirrer, and stabilized for 48 hours. The results were filtered using a Buchner funnel, coated with filter paper, and then put into an Erlenmeyer. The filter results were evaporated with a vacuum evaporator and diluted with distilled water—preparation of hydrogel layer by solvent casting method. Sodium alginate (1.5% w/v) and *Aloe vera* (1.0% w/v) were added with 15% (w/w) glycerol according to the mass of the alginate. The ingredients were mixed to produce a concentration of 1%, 2.5%, 5%, 10% (v/v), 25 mL of each mixture was placed in a petri dish and allowed to dry at a temperature of 25^{o}C controlled humidity (50%). After drying, the mixture was added to 5.0% (w/v) $CaCl_2$ for 5 minutes, and a layer of hydrogel material was obtained.

Treatment

Each experimental animal was subjected to the extraction process of canine teeth by first being

anesthetized with ketamine 10 mg/kg BW. Then give a tampon to stop the bleeding in the wound for 3 minutes—1 aloe vera hydrogel application in each treatment group. After the treatment, the test animals were fed fine porridge with due observance of the health of the test animals. After day five post-extraction, rats were sacrificed by neck dislocation. Before the mice were anesthetized, ketamine was combined with xylazine. Tissue fixation was conducted with 10% formalin for 24 hours, then decalcified with 10% EDTA solution at room temperature. In the process of tissue dehydration with alcohol, the specimen is placed in toluene alcohol (1:1), then put into a saturated paraffine solution, infiltrated in the oven. Do the embedding process, then give a label/code. When finished, slice the tissue series with a thickness of ±6 microns with a microtome.

Fibroblast response evaluation

Evaluation of fibroblast response process with HE staining, deparaffinized with xylol and alcohol solution, rehydrated with alcohol. Washed and rinsed with distilled water, then wiped. Put the slide in Mayer's hematoxylin and wash. Rinse with distilled water. Staining is assessed under a light microscope. Suppose the staining is considered good, dehydrated with alcohol in stages, and wiped. Put in the xylol solution, and the glass object is covered with a glass deck, then make observations. Count the fibroblasts in 5 fields of view with a binocular microscope.

Data analysis

Data analysis was performed using SPSS version 25 software. The number of fibroblast cells was presented with mean ± SD. Univariate analysis was performed to determine the mean ± SD, followed by bivariate analysis with one-way ANOVA and multivariate analysis with post hoc LSD.

3. Results

The analysis results on the effect of 1%, 2.5%, 5%, 10% *Aloe vera* hydrogel application on the increase in fibroblasts in socket wounds after tooth extraction in *Rattus novergicus* using the one-way ANOVA test are shown in Table 1.

Table 1. Effect of *Aloe vera* hydrogel application on increasing the number of fibroblasts in socket wounds after tooth extraction in *Rattus novergicus*

Groups	Mean±SD	*p value*
HAV1	37,6±16,07	0,000*
HAV2,5	76,5±19,28	
HAV5	113,0±17,43	
HAV10	128,0±28,81	
XLZ	64,7±31,70	

*p<0.05, one-way ANOVA

Based on the study results, there were differences in the mean number of fibroblasts in all groups. From the results of this study, it can be stated that there is an effect of the application of hydrogel *Aloe vera* 1%, 2.5%, 5%, 10%, and xylazine on the increase in the number of fibroblasts in socket wounds after tooth extraction in *Rattus novergicus*.

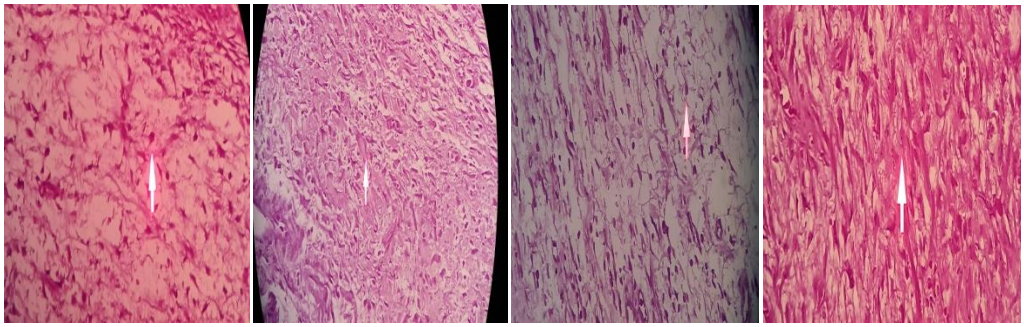
Data analysis was continued with the post-hoc LSD test aimed at analyzing the differences in the effect of 1%, 2.5%, 5%, 10%, and xylazine *Aloe vera* hydrogel application on the number of fibroblasts in socket wounds after tooth extraction on *Rattus novergicus* between the two treatment groups as shown in Table 2.

Table 2. Differences in the effect of *Aloe vera* and xylazine hydrogel application on the number of fibroblasts in socket wounds after tooth extraction in *Rattus novergicus* between the two treatment groups

Groups		Mean difference	p value
HAV 1	HAV2,5	-42,4	0,020*
	HAV5	-75,4	0,000*
	HAV10	-90,4	0,000*
	XLZ	-27,12	0,120
HAV2,5	HAV5	-33,0	0,049*
	HAV10	-48,0	0,007*
	XLZ	15,3	0,343
HAV5	HAV10	-15,0	0,352
	XLZ	48,3	0,006*
HAV10	XLZ	63,3	0,001*

Note: * there is a significant difference

Based on the results of the study, it was found that there was a significant difference in the effect of the application of *Aloe vera* hydrogel 1%, 2.5%, 5%, 10%, and xylazine on the number of fibroblasts in the socket wound after tooth extraction in *Rattus novergicus* which was significant between the 1% hid *Aloe vera* group and the hid *Aloe vera* two groups. ,5% (p=0.020; mean diff=-42.4), *Aloe vera* hydrogel group 1% and *Aloe vera* hydrogel 5% (p=0.000; mean diff= -75.4), *Aloe vera* hydrogel group 1% and *Aloe vera* hydrogel 10 % (p=0.000; mean diff= -90.4).



(a) (b) (c) (d)

Figure 1. Longitudinal section of the tooth socket on *Rattus novergicus* with HE staining and observation using a 400x binocular microscope (a) 1% *Aloe vera* hydrogel, (b) 2.5% *Aloe vera* hydrogel, (c) 5% *Aloe vera* hydrogel, (d) *Aloe vera* hydrogel 10%.

4. Discussion

Aloe vera is an herbal ingredient that can change its consistency into a hydrogel without adding other ingredients. The study results stated that there was an effect of the application of hydrogel aloe vera 1%, 2.5%, 5%, 10%, and xylazine on the increase in the number of fibroblasts in socket wounds after tooth extraction in *Rattus novergicus*. These results were supported by the observation of post-extraction tooth socket preparations on *Rattus novergicus* after HE staining, which showed an increase in the number of fibroblasts.

The 10% *Aloe vera* hydrogel group experienced the most significant fibroblasts compared to the other groups. The results of this study are in line with studies that state that *Aloe vera* gel extract 90% freeze-drying can increase the number of macrophages in the healing process after tooth extraction in *Cavia cobaya* on observation days 1 and 3.[9] Topical application has been shown to have promising effects on the wound healing process. In another study revealed that *Aloe vera* can

heal acute and chronic burns.[10] Other studies have also proven that *Aloe vera* can heal postoperative wounds.[9,11] In this study, the application of *Aloe vera* hydrogel 10%, 5%, and 2.5% effectively increased fibroblasts faster than the control group (xylazine).

Fibroblast response after applying *Aloe vera* hydrogel was evaluated by hematoxylin-eosin (HE) staining. HE staining is the most common type of routine staining and the standard for histological examination of tissues.[12] This stain is suitable for examining the structure of cells and tissues. The normal wound healing process involves a process that is interconnected with one another, namely hemostasis, inflammation, proliferation, and remodeling.[2] The number of fibroblasts evaluates wound healing parameters. Fibroblasts produce an extracellular matrix that fills the wound cavity and provides a platform for keratinocyte migration. From the study results, it was found that *Aloe vera* hydrogel was proven to increase the number of fibroblasts in socket wounds after tooth extraction in *Rattus novergicus*.

This study also follows the theory that *Aloe vera* can stimulate fibroblast proliferation in vitro.[6] The increase in the number of fibroblasts in the aloe vera hydrogel group was caused by the activity of the mannose-6-phosphate component binding to the IGF-2/mannose-6-phosphate receptor on the cell surface. This attachment causes the stimulation of fibroblasts to proliferate, differentiate into myofibroblasts, or produce large amounts of collagen and other matrix proteins. The proliferation of fibroblasts will determine the outcome of wound healing. The increasing number of fibroblast cells will speed up the wound healing process.[11]

Aloe vera is a lance-shaped plant where the leaves contain a clear and slimy gel. The benefits of *Aloe vera* are often associated with the polysaccharides contained in the leaf gel.[13] Other secondary metabolites that affect the acceleration of fibroblast proliferation are flavonoids. Flavonoids are anti-inflammatory which works by reducing the inflammatory process by inhibiting the formation of prostaglandins formed by arachidonic acid and other inflammatory mediators.[14] The inflammatory phase causes the migration of neutrophils, then continues to the wound area, replaced by monocytes. An increase in the number of monocytes will increase the number of macrophages. These macrophages are in charge of secreting growth factors such as FGF, PDGF, TGF-β, and EGF, which can attract more fibroblasts to the wound area, synthesize collagen, and increase the proliferation of capillary blood vessels so that the distance of fibroblasts increases. Saponins can accelerate the wound healing process by increasing vascular endothelial growth factor and interleukin (IL)-1β, an inflammatory mediator, which can induce macrophages to the injured area and accelerate the wound healing process. In addition, Vitamin C also plays a role in cell differentiation, collagen synthesis, and increasing fibroblast proliferation. Vitamin C plays a role in increasing immunity which will accelerate the proliferation of fibroblasts. Tannins can be used as a preventive against wound infections because they have antiseptic and burn healing properties.[13-15]

5. Conclusion

There is an effect of *Aloe vera* hydrogel application on increasing the number of fibroblasts in socket wounds after tooth extraction in *Rattus novergicus.*

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