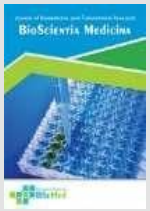
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Potential of Hydrogel Acemannan Aloe Vera (*Aloe vera*) on Wound Healing After Tooth Extraction In vivo Via Regulation of Inflammatory Response

Firdha Muharraran[1*], Gusbakti Rusip[2], Rosa Paradita Dalimunthe[3]

[1] Department of Dental Sciences, Faculty of Dentistry, Universitas Prima Indonesia, Medan, Indonesia
[2] Department of Physiology, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia
[3] Undergraduate Student of Dental Science, Faculty of Dentistry, Universitas Prima Indonesia, Medan, Indonesia.

ARTICLE INFO

Keywords:
Aloe vera
Hydrogel
Macrophage
Inflammation
Hematopoiesis

***Corresponding author:**
Firdha Muharraran

E-mail address:
firdhamuharraran@unprimdn.ac.id

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v6i6.532

A B S T R A C T

Background: Tooth extraction is a surgical procedure that involves hard tissue such as bone and soft tissue. Wounds on soft tissue are then followed by a healing process that occurs through three phases, namely the inflammatory phase, the proliferative phase, and the remodeling phase. Acemannan has the ability to stimulate hematopoiesis and antioxidant effects. Acemannan is able to reduce inflammation through prostaglandin synthesis and increase leukocyte infiltration and play a significant role in the oral wound healing process. **Methods**: This study is an in vivo experimental study. A total of 30 rats (5 each/group) were divided into groups that received 1%, 2%, 4%, 8%, povidone-iodine, and carbopol acemannan Aloe vera hydrogel. Furthermore, the number of macrophage cells in the tissue after tooth extraction was assessed. Data analysis was carried out with the help of SPSS with ANOVA and post hoc LSD tests. **Results:** There was a significant difference between the application of acemannan 1% hydrogel and 4% acemannan hydrogel, 8%, povidone-iodine, and carbopol, between the application of acemannan 2% hydrogel 4% acemannan hydrogel, 8%, povidone-iodine and carbopol, applications acemannan hydrogel 8% with carbopol, and between povidone-iodine and carbopol ($p \leq 0.05$). **Conclusion:** Acemannan Aloe vera hydrogel is able to accelerate wound healing after tooth extraction in vivo by suppressing macrophage cell activity.

1. Introduction

One of the treatment actions that are often carried out in the field of dentistry is extraction or tooth extraction.[1] Tooth extraction is a surgical procedure that involves hard tissues such as bone and soft tissue wounds on soft tissue are then followed by a healing process that occurs through three phases, namely the inflammatory phase, the proliferative phase, and the remodeling phase.[2] A few hours after the wound, there is an invasion of inflammatory cells, namely neutrophil cells or polymorphonuclear cells (PMN) in the wound tissue that occurs in the first 6-24 hours, then polymorphonuclear cells (PMN) migrate to the wound area, and after 24-48 hours PMN cells will be replaced by macrophages which are the most dominant cells in inflammation with the highest number on day 2 to day 3. Macrophages will remain in the wound until healing is complete, and macrophages will gradually decrease and will be replaced by lymphocytes.[2,3] Macrophages are cell elements that are important in the formation of granulation tissue derived from monocytes. Monocytes are derived from progenitor cells in the bone marrow. Macrophages function to phagocytose pathogens, dead cells, some

components in the extracellular matrix, and fibrin. The decrease in the number of macrophages on day 5 indicates that the inflammatory process has been greatly reduced.[1,4,5]

Aloe vera contains 20 minerals, 12 vitamins, 18 amino acids, and 200 active compounds, including enzymes, triterpenes, polysaccharides, flavonoids, and glycoside groups. Aloe vera stimulates epidermal growth factors, increases fibroblasts, and the formation of new blood vessels, thereby promoting wound healing. The active substances in aloe vera are very useful in accelerating wound healing because they contain glucomannan, lignin, vitamin A, vitamin C, enzymes, and amino acids which are very important for cell regeneration.[6] Aloe vera gel contains polysaccharide components, namely acemannan and glucomannan. Glucomannan affects fibroblast growth factor and stimulates cell activity and proliferation, and increases collagen production and secretion. Acemannan accelerates wound healing by influencing fibroblast proliferation and stimulating KGF-1 and VEGF expression. Acemannan has the ability to stimulate hematopoiesis and antioxidant effects. Acemannan is able to reduce inflammation through prostaglandin synthesis and increase leukocyte infiltration and play a significant role in the oral wound healing process via induction of fibroblast proliferation and stimulating KGF-1, VEGF and type I collagen expression.[6,7]

This study is one of the first studies to evaluate the effect of Acemannan in wound healing after tooth extraction. In this study, an evaluation of the potency of the acemannan hydrogel preparation on the inflammatory response in wound tissue after tooth extraction was carried out in experimental animals.

2. Methods

The research design is an experimental study, posttest only with a control group design. This research was conducted at the Laboratory of Physiology and Plant Tissue Culture of USU's Faculty of Mathematics and Natural Sciences, Laboratory of the Animal Development Center, and the PA Prospecta Lab from September 2021 – to January 2022. The research subjects in this study were White Rats (Rattus norvegicus) Wistar strain, which met the criteria for the male sex, bodyweight between 150-200 grams, and age between 8-10 weeks. A total of 30 rats of the Wistar line were acclimatized for 7 days before being included in the study. Rats were kept in a treatment facility with a 12-hour dark-light cycle, room temperature 250C, and access to food and drink ad libitum. After acclimatization, the experimental animals were grouped into 6 groups (5 rats each), namely HA1: the group that received Aloe vera, HA2: the group that received Aloe vera, HA4: the group that received the Aloe vera hydrogel acemannan Aloe vera 4%, HA8: a group receiving 8% acemannan Aloe Vera hydrogel, PI: a group receiving povidone-iodine and KP: a group receiving Carbopol. This research has been approved by the Health Research Ethics Committee (KEPK) Universitas Prima Indonesia, No. 042/KEPK/UNPRI/XI/2021.

After the Aloe vera is cut from the plant, then it is washed with a solution of calcium hypochlorite, peeled, and cut into small pieces for inclusion in the juicer. Aloe vera was then added with 96% ethanol in a ratio of 1:4. In this case, 50 ccs of Aloe vera juice was added with 200 ccs of 96% ethanol. The mixture of Aloe vera juice and ethanol was stirred for 10 minutes at 30°C, then allowed to settle for 10 hours at a temperature of 10°C. The precipitate formed is separated from the solution by a suction filter. Next, the precipitate is placed in a vacuum dryer at a temperature of 50°C. Preparation of hydrogel layer by solvent casting method. Sodium alginate, Acemannan, added glycerol 15% (w/w) according to the mass of the alginate. The ingredients were mixed to produce concentrations of 1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml (v/v). 25 ml of each mixture was placed in a petri dish and allowed to dry at a temperature of 25oCand controlled humidity (50%). After drying, the mixture was put into 5% CaCl2 (w/v) for 5 minutes and a layer of Acemannan.

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. Application of 1 acemannan aloe vera hydrogel in each treatment group. After the treatment, the test animals were fed fine porridge with due observance of their health of the test animals. After day 5 post-extraction, rats were sacrificed by neck dislocation. Before the mice were anesthetized, ketamine was combined with xylazine. Tissue fixation with 10% formalin for 24 hours at room temperature, then decalcification with 10% EDTA solution at room temperature. In the process of tissue dehydration with alcohol, the specimen is placed in toluol alcohol (1:1), then put into a saturated paraffin toluol solution. Infiltration 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. Evaluation of fibroblast response process by HE staining, deparaffination of xylol and alcohol solution, dehydration 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 number of macrophages in 5 fields of view with a binocular microscope.

Data analysis was performed using SPSS version 25 software. The number of macrophage 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

Tables 1 and 2 show that there are significant differences between the application of hydrogel acemannan 1% 4% acemannan hydrogel, 8%, povidone-iodine, and carbopol, between the application of hydrogel acemannan 2% hydrogel, 8%, povidone-iodine, and carbopol. , between the application of hydrogel acemannan 8% carbopol, and between povidone-iodine and carbopol ($p \leq 0.05$), while between acemannan 1% hydrogel and 2% acemannan hydrogel and between acemannan hydrogel and povidone-iodine there was no significant difference in effect. ($p > 0.05$).

Table 1. Effects of acemannan hydrogel on the number of macrophage cells

Group	**Mean number of macrophage cells**	**p-value**
Acemannan hydrogel 1%	23.68±0.576	0.000*
Acemannan hydrogel 2%	22.16±0.684	
Acemannan hydrogel 4%	15.84±2.239	
Acemannan hydrogel 8%	10,20±2,293	
Povidone-iodine	11.96±1,314	
Carbopol	25,72±1,119	

*ANOVA test, $p<0.05$

Table 2. Effect of acemannan hydrogel between test groups on the number of macrophage cells

Group		Mean difference	p-value
Acemannan hydrogel 1%	Acemannan Hydrogel 2%	1.52	0.129
	Acemannan hydrogel 4%	8.20	0.000*
	Acemannan hydrogel 8%	13.48	0.000*
	Povidone-iodine	11.72	0.000*
	Carbopol	-2.04	0.046*
Acemannan hydrogel 2%	Acemannan hydrogel 4%	6.68	0.000*
	Acemannan hydrogel 8%	11.96	0.000*
	Povidone-iodine	10.20	0.000*
	Carbopol	-3.56	0.001*
Acemannan hydrogel 4%	Acemannan hydrogel 8%	5.28	0.000*
	Povidone-iodine	3.52	0.001*
	Carbopol	-10.24	0.000*
Acemannan Hydrogel 8%	Povidone-iodine	-1.76	0.082
	Carbopol	-15.52	0.00*
Povidone iodine	Carbopol	-13.76	0.000*

*LSD post hoc, p<0.05

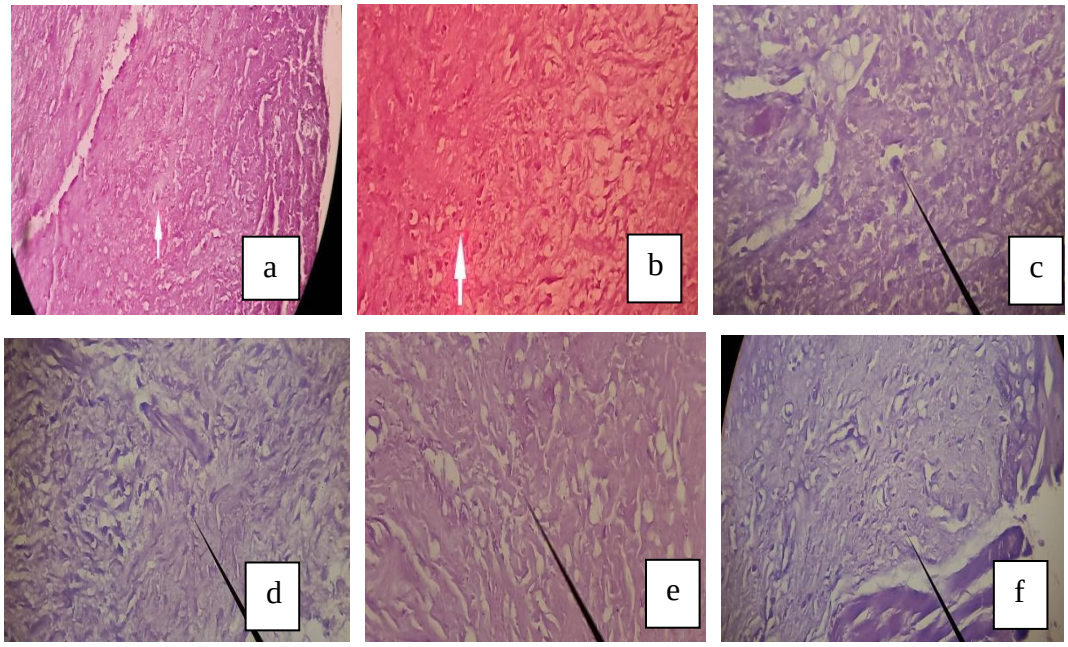


Figure 1. Histopathological cross-sectional view (a) positive control, (b) negative control, (c) 1% acemannan hydrogel, (d) 2% acemannan hydrogel, (e) 4% acemannan hydrogel, (f) 8% acemannan hydrogel.

Figure 1 shows the expression of macrophage cells in tissue preparations. The arrows indicate the presence of macrophages in each preparation. The presence of macrophage cells indicates an inflammatory process that occurs in the tissue due to the trauma process after tooth extraction.

4. Discussion

Aloe vera is rich in glucomannans and acemannan, which are quite high where these compounds are growth factors that are able to suppress the inflammatory process and stimulate the growth of fibroblasts. Acemannan (acetylated mannan) is the largest polysaccharide in the flesh of aloe vera. Acemannan acts as an immunomodulator that can stimulate macrophages, lymphocytes, interleukins, cytokines, and TNF. It also functions as anti-inflammatory, antibacterial, antiviral, and antifungal. Cells stimulated by acemannan, such as macrophages and lymphocytes, will stimulate TGF-ß, PDGF, bFGF, and VEGF, which will induce fibroblast migration and proliferation.[8-11] These results are consistent with the theory that aloe vera can stimulate fibroblast proliferation in vitro. The increase in the number of fibroblasts in the group treated with aloe vera was due to the activity of the mannose-6-phosphate component, which can bind to the IGF-2/mannose-6-phosphate receptor on the surface of fibroblast cells. 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 final outcome of wound healing. The increasing number of fibroblast cells will speed up the wound healing process.[12-14] Aloe vera is a biogenic stimulator that can stimulate activated cells in the alveolar and also accelerate the healing of scars from extractions. Tooth extraction trauma can cause inflammation, which causes osteoclastogenesis, namely the growth of osteoclasts caused by inflammation due to extraction trauma. Aloe vera plays a role in preventing inflammation, reducing osteoclastogenesis, and preventing bone cell resorption. Increased expression of collagen with aloe vera indicates that there is an increase in bone growth activity carried out by osteoblasts.[15] Other secondary metabolites that affect the acceleration of fibroblast proliferation are flavonoids. Flavonoids are anti-inflammatory and work by reducing the inflammatory process by inhibiting the formation of prostaglandins formed by arachidonic and other inflammatory mediators. The inflammatory phase causes the migration of neutrophils, then continues to the wound area, which is replaced by monocytes. An increase in the number of monocytes will increase the number of macrophages. Macrophages are a very important component of wound healing. These macrophages are responsible for secreting growth factors such as FGF, PDGF, TGF-beta, and EGF, which are able to attract more fibroblasts to the wound area, synthesize collagen and increase the proliferation of capillary blood vessels so that the distance of fibroblasts increases.[13,14] endothelial growth factor vascular and interleukin (IL)-1β, which is an inflammatory mediator which is able to induce macrophages to the injured area and accelerate the wound healing process.[16] 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.[17]

5. Conclusion

Acemannan Aloe vera hydrogel is able to accelerate wound healing after tooth extraction in vivo by suppressing macrophage cell activity.

6. References

1. Montandon A, Zuza E, Toledo B. Prevalence and reasons for tooth loss in a sample from a dental clinic in brazil. International J of Dentistry. 2012; 1-5.
2. M. Jafarian, A. Etebarian. Reasons for extraction of permanent teeth in general dental practices in Tehran, Iran. Medical Principles Practice. 2013; 22: 239–244.
3. Gurtner GC. Wound healing: Normal and abnormal. In: Thome CH, editor. Grabb and Smith's Plastic Surgery, 6th Ed. Philadelphia: Lippincott Williams & Wilkins. 2007; 15-22.
4. Prasetyono TOH. General concept of oral wound healing revisited. Med J Indonesia

2009; 18(3): 208-16.
5. Koh TJ, DiPietro LA. Inflammation and wound healing: The role of macrophage. Expert Rev Mol Med. 2013; 13(23): 23.
6. Darby IA, Laverdet B, Bonté F, Desmoulière A. Fibroblasts and myofibroblasts in wound healing. Clin Cosmet Investig Dermatol. 2014; 7: 301–11.
7. WHO. Global report on traditional and complementary medicine 2019 Geneva: World Health Organization; 2019.
8. Sawai MA. Aloe vera - a miracle herb. International Journal of Research in Dentistry 2014; 4(2): 1-7.
9. Joseph E. Pizzorno, Michael T. Murray. Textbook of natural medicine. 4th ed. Missouri: Elsevier, 2013: 577-80.
10. OA kayode. Effects of aloe vera gel application on epidermal wound healing in the domestic rabbit. Int J Res Med Sci. 2017; 5(1): 101-105.
11. Jain S, Rathod N, Nagi R, Sur J, Laheji S, Gupta N, et al. Antibacterial effect of aloe vera gel against oral pathogens: An in-vitro study. Journal of Clinical and Diagnostic Research. 2016: 10(11); ZC41-ZC44.
12. Arijani E, Khoswanto C. The Use of 90% Aloe vera freeze-drying as the modulator of collagen density on extraction socket of incisive Cavia cobaya. Dental Journal. 2009; 41(2): 74-6. 127
13. Yuza F, Wahyudi IA, Larnani S. Effect of aloe vera extract (aloe barbadensis miller) on the tooth socket on the density of collagen fibers after tooth extraction of guinea pigs (Cavia Porcellus). Maj Ked Gi. 2014; 21(2): 127 – 135.
14. Hemalatha. R, Hemagaran G. Effectiveness of honey and aloe vera on post-extraction healing. IOSR-JDMS. 2015; 14(5): 123-128.
15. Sihombing M, Rafizar. Nutritional status and liver function of mice (CBS-Swiss strain) and white rats (*Wistar strain*) in laboratory animals of the Research and Development Center for biomedical and pharmaceutical. Health Research and Development Media. 2010; 20(1): 33-40
16. Hupp JR, Ellis E, Tucker MR. Contemporary oral and maxillofacial surgery. 6th Ed. Missouri: Elsevier, 2014: 43-48.
17. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. J Int Med Res. 2009; 37:1528-42