ORIGINAL ARTICLE

Increasing Macrophages in Tooth Extraction Wound Healing after induction of Freeze-drying Gel Aloe vera 90% on Cavia cobaya

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ABSTRACT

Introduction: Tooth extraction is a regular method in dentistry in which it will result a lesion on the socket. The healing process of the lesion might lead to complication for instance bleeding, swelling, socket drying, and spreading of the infection. However, the healing process could be helped by the use of herbal medicine for it can reducing the complication risk. 90% freeze-drying Aloe vera gel, can grow the number of macrophages cells in which they play a significant role in the healing process. The purpose of this study was to determine an increasing number of macrophage cells in the wound healing process after tooth extraction after applying 90% freeze-drying Aloe vera gel. **Method:** freeze-drying Aloe vera consisted in CMC Na. Cavia cobaya divided into control group, day 1 and day 3, consists of the two groups without Aloe vera treatment and Treatment group, day 1 and day 3, consists of the two group treated with Aloe vera. **Results:** There is significant dissimilarity in both control and treatment group. In the treatment group, the number of macrophages between days 1 and 3 has been grown. It is because of the anti-inflammatory properties of Aloe vera. The activities and number of macrophages in tissue would be disrupted, if it is inhibited. **Conclusion:** 90% freeze-drying Aloe vera gel extract could increase number of macrophages in the healing process after tooth extraction on Cavia cobaya in observation days 1 and 3.

Keywords: Freeze-drying gel Aloe vera, Wound healing, Macrophage

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INTRODUCTION

Tooth extraction is a procedure that combines operating principles and basic physical mechanics. Tooth extraction is a commonly used procedure in dentistry(1). The results of the 2013 RISKESDAS (Basic Health Research), imply that the average tooth decay is 5 to 6 teeth per person and the biggest cause of tooth decay is tooth extraction at 2.62% (2).

Tooth extraction procedure will cause a wound in the socket. A wound is an injury to human body tissue caused by abnormal disruption of the tissue continuity structure. Wound healing occurs if there are complications or effects such as bleeding, swelling, or bacteria spreading. During the inflammation stage, acute inflammation and chronic inflammation stages occur. In chronic inflammation stage, one of the cells that actively contribute is macrophages (1,3,4,5).

The functions of macrophages include phagocytosis, collagen synthesis, granulation tissue formation in conjunction with fibroblasts, growth factors production, and new capillaries formation or angiogenesis (5,6). These functions affect the stages of wound healing. In wound healing process, small number macrophages begin to appear on the initial day. The activity and number of macrophages increased on day 3 and decreased on day 5 (6,7).

The direction of pharmaceutical-health technology development nowadays has focused its attention on natural ingredients. It is relatively safer to use compared to the use of drugs that contain chemicals that are considered harmful to human body. One of the medicinal plants in Indonesia is Aloe vera (8,9). Aloe vera is one of the plants included in the Liliaceae family, growing in dry areas. Aloe vera contains ingredients that act as antibacterial, antidiabetic, antifungal, anti-inflammation and contributes in wound healing. Some active Aloe vera ingredients, acemannan and lectin, are able to increase the accumulation of macrophages that helps the wound healing process (10,11).

Previous research on cytotoxicity test of Aloe vera in various concentrations showed that 90% Aloe vera concentration is appropriate to use. Another study

conducted the research used freeze-drying 90% Aloe vera as a modulator of collagen density after tooth extraction of incisors on guinea pigs (Cavia cobaya), showed that Aloe vera is able to stimulate the formation of new collagen in wound healing process (12,13). Increasing number of macrophages in wound healing process of post tooth extraction after giving 90% freeze-drying Aloe vera gel on observation day 1 and day 3 was examined in this research to prove the role of Aloe Vera in wound healing process.

MATERIALS AND METHODS

Animal model of tooth extraction

The number of sample used are 6 samples, Male Cavia cobaya, 2-3 months old, and weighs 300-500 grams. The samples were divided into 4 groups. The control group on day 1, the treatment group on day 1, the control group on day 3, and the treatment on group day 3. They were maintained and placed in a cage in accordance with their body conditions.

Aloe vera gel preparation

One thousand grams of Aloe vera leaves are washed thoroughly with aquadest and then peeled, then the Aloe vera gel is washed thoroughly with aquadest until the mucus fades, then weighed and obtained its 617 grams gel, then processed with a blender. Thus, 500 ml of processed Aloe vera was obtained. In order to obtain 2.9 grams of dry Aloe vera gel, the processed Aloe vera was put into a bottle, froze in a feezer until -35 ° C, then put in a vacuum and evaporated until it became dry (14). To make 90% Aloe vera gel, the 100% Aloe vera gel was weighed and then freeze-dried. Another step conducted was mixing 9 grams of Aloe vera extract with 1 gram of Sodium Carboxyl Methyl Cellulose (15).

Cavia cobaya were anesthetized by using 10% ether. The lower right incisors were cleaned from food scraps by using water and then dried. Then, the extraction of the lower right incisor was carefully performed using a needle holder and elevator in order to avoid root fracture so that the teeth were completely extracted. The socket was irrigated with sterile aquadest solution. The samples were given topical 90% Aloe vera gel in each treatment Group. 90% Aloe vera gel was put into a syringe and dropped into the socket until the socket was filled with ± 0.1 ml volume. The socket was then sutured. On the other hand, the control group was directly sutured after being irrigated without applying the 90% Aloe vera gel (13).

After suturing, animal was sacrified using 10% ether in lethal doses on days 1 and 3. The mandibular was removed by detaching it from its mandibular angle. Mandibular pieces were plunged into formalin buffer and then decalcified by using nitric acid for approximately 1-2 days. The soft mandible around the right lower incisor was cut into small rectangular pieces. Then, the mandible was plunged into a buffer solution.Then, it was dehydrated by using alcohol, cleared by using xylol, and then embedded. The paraffin making was done and painted with Hematoxylin Eosin (HE) stain ing. Finally, histological observation of macrophages was conducted by using a light microscope with 400x magnification and number of macrophages was calculated.

RESULTS

The number of macrophages in wound healing post tooth extraction on Cavia cobaya can be seen in Tables I. In observation results, it can be seen that the control group on day 1 its average number of cells is 0.83 whereas in the control group on day 3, the average number of cells is 2.67. Based on these data, it can be concluded that there is an increasing number of macrophage cells on day 3 and there are differences in number of macrophage cells between observations days 1 and 3 in the control group.

Table I: The mean of the number of macrophage cells and standard deviation of control and treatment groups on day 1 and day 3

Day	Sample	Average	Standard Deviation
Control group day 1	6	0,83	0,83±0,7527
Control group day 3	6	2,67	2,67±3,0110
Treatment group day 1	6	2,5	2,5±1,224
Treatment group day 3	6	3,8	3,8±3,710

The observations of the treatment group on day 1 showed that the average number of cells is 2.5 while in the treatment group on day 3 the average number of cells is 3.83. Based on these data, it can be concluded that the number of macrophage cells is mostly found on day 3 and there are differences in the number of macrophage cells on days 1 and 3 in the treatment group.

Histopathology results of macrophage cells in the mandibular lower right incisor socket on days 1 and 3 was determining uses a certain criteria in which the macrophage has an eccentric nucleus, golgi complex, a number of lysosomes and a clear rough endoplasmic reticulum, large cell size, flat, and has black granules (16). The obtained results can be seen in the fig 1

In the control group on day 1, the macrophages begin to appear in a relatively small number. As pointed by the arrows, a number of granular macrophage appears on the sample. While in the control group on day 3, there is an increasing number of macrophages.

On the other hand, many macrophages appear in the treatment group on day 1 and day 3, shown by the arrows in Fig 2. In accordance with the theories used in this study, the number of macrophages increases on day 3.

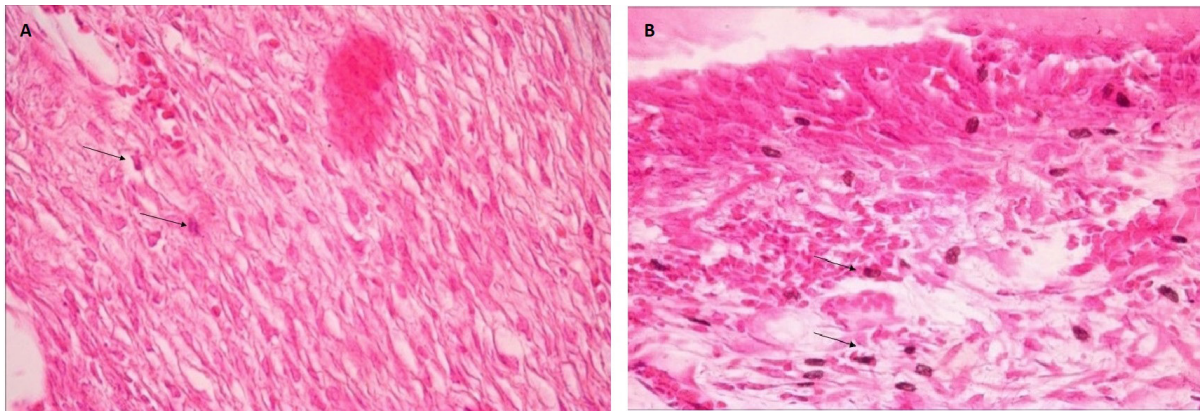

Figure 1: A. Macrophage cells in the control group day 1, B. Macrophage cells in control group day 3 (HE, 400x magnification)

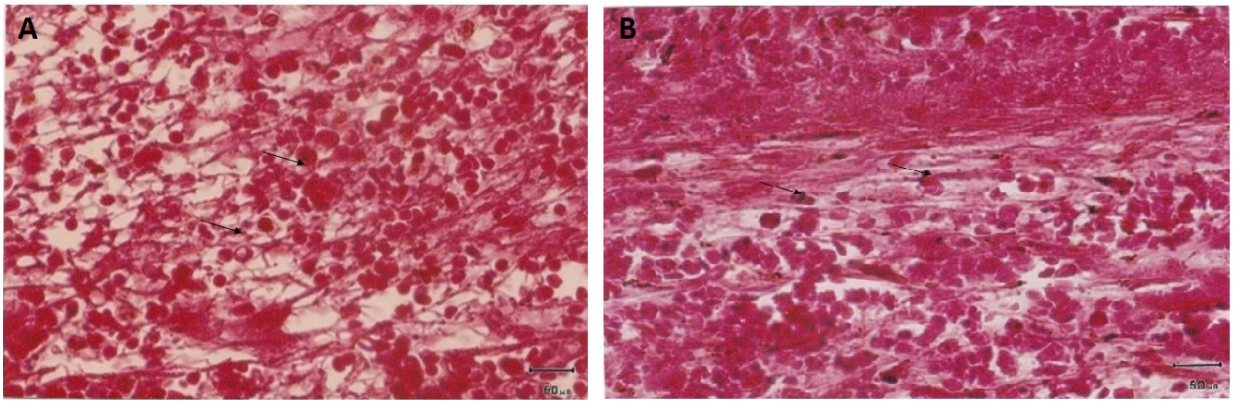

Figure 2: A. Macrophage cells in the treatment group day 1, B. Macrophage cells in control group day 3 (HE painting with 400x magnification)

One-way Annova, value is obtained as 0.150. It is greater than the normal limit. a Post Hoc HSD test is conducted in order to find out which groups have no meaning difference. The results can be seen in table 2. The p value for each group is various. Therefore, it can be concluded that there is no significant difference if the p value is $p > \alpha = 0.05$.

The turkey HSD test states the value of the pair of treatment group on days 1 and 3 is $p > \alpha = 0.05$. Hence, there is no significant difference regarding the meaning and the value in each group.

DISCUSSION

The findings implies that there are an increasing number of macrophages. In accordance with the theories used, some Aloe vera compounds, such as acemannan and lectin, can increase the number of macrophages on day 3 (4,11). Prior to inflammation stage, there are several stages occurred, namely hemostasis. During the hemostasis stage, mast cells release histamine and bradykinin. In this action, C3a and C5a complement are activated. Additionally, arachidonic acid cascade (cyclooxygenase and lipoxygenase pathways) are also activated. Histamine and bradykinin actively contribute in vasodilatation and increased tissue permeability(17,18). The activation of the arachidonic acid cascade occurs through two pathways, namely cyclooxygenase and lipoxygenase. The lipoxygenase pathway produces leukotrienes containing LTB4 and affects vasoconstriction and platelet aggregation. On the other hand, cyclooxygenase pathway produces thromboxane A2 (TX A2) which affects the increasing platelet aggregation, PGE2, and PGI2 (Prostacyclin) which affects vasodilation and increases vascular permeability. C3a and C5a take a role in PMN migration to the tissue (19).

The inflammation stage is characterized by the involvement of the mediators to the emergence of PMN and macrophage cells. PMN migration to the tissues is affected by the presence of chemotactic from C3a and C5a. In contrast, macrophage migration is affected by LTB4 activation which affects lymphocyte cell activation in order to produce IFN-γ and IL-6 during the macrophages migration to tissue (20).

This study examines the increasing number of macrophages after being given the active ingredient from Aloe vera extract. Macrophage cells begin to enter the wounded area about 48-72 hours after the injury. At the initial time of injury, before entering the tissue, macrophages are in the form of monocytes which located in blood vessels. When defect or damage occurred on blood vessels as the result of tooth

extraction, it stimulates the defect to convey certain signals to monocytes. This signal is also conveyed to several cytokines, such as IFN-γ and IL-6. The signal causes the monocytes activation and migration to the tissue in which later become macrophages. Some active ingredients, such as *Acemannan* and *Lecin*, have immunomodulatory properties that activate IFN-γ and IL-6 which affect macrophages (11,21).

There is no significant difference resulted from Aloe vera active ingredients of Chromone and β-sitosterol. Both of these are anti-inflammation. Both of these inhibit the work of mediators in the cyclooxygenation pathway. Chromone inhibits the release of Thromboxane A2. In contrast, β-sitosterol inhibits the release of PGI2 and PGE2. Therefore, the process of the inflammation stage is inhibited (22).

Another ingredient in Aloe vera is emodin that inhibits the performance of LTB4. LTB4 functions to activate T cells that contributes for phagocytosis (defense). T cells produce IFN-γ and IL-6 products that stimulate monocytes to become macrophages. In addition, thromboxane A2 inhibition affects platelet aggregation. When platelet aggregation is disrupted and decreased, LTB4 would not be formed because of increased platelet aggregation causes a change in LTA4 to LTB4. Thus, when T cell performance is inhibited, products that produce macrophages decrease and macrophages that reach the tissue are also insignificant. Thus, it disrupts the wound healing mechanism (18,22).

CONCLUSION

The administration of freeze drying Aloe vera gel has been shown to increase the number of macrophage cells with a concentration of 90% in wound healing after extraction of guinea pigs on day 1 and 3 observations.

ACKNOWLEDGEMENTS

Our high appreciation to our research team and our research analyst who participated in this study.

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