

ANTI-INFLAMMATORY EFFECTIVENESS OF *ALOE VERA* EXTRACT GEL BASED ON THE NUMBER OF MACROPHAGES IN POST TOOTH EXTRACTION SOCKET WOUND OF WISTAR WHITE RATS

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ABSTRACT

Objective: This study aims to determine the effectiveness of anti-inflammatory gel *aloe vera* extract (*Aloe vera*) with the concentration of 25%, 50%, 75% on the number of *macrophage* cells in the socket wound after tooth extraction in white wistar rats.

Methods: This research is a laboratory experimental research. The research design used was a simple post-test experimental design. Forty-six male wistar rats were divided into four groups, namely the group given 25%, 50% and 75% *Aloe vera* extract gel after extraction. On the rat tooth that had been extracted, 0.1 ml of *Aloe vera* gel was applied to cover the socket wound. Experimental animals were sacrificed after the first and third day of treatment. The sacrifice was made decapitation. After that, tissue sampling and staining were carried out and the number of *macrophage* cells counted in the smear was observed using a binocular microscope with 400x magnification to obtain a counting area. Data processing was carried out using the SPSS application.

Results: Normality test with Shapiro Wilk test followed by one way ANOVA and Post hoc LSD tests ($p < 0.05$). Data analysis showed some significant differences in the number of *macrophages* on the first and third day's socket wounds in the 25%, 50% and 75% *Aloe vera* extract gel groups.

Conclusion: The results of this research is the 75% *Aloe vera* extract gel group had more effective anti-inflammatory results against the number of *macrophages* than the control group and the other *Aloe vera* extract gel groups.

Keywords: Socket wound, *Aloe vera*, *Macrophages*

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INTRODUCTION

Tooth extraction is a surgical procedure with the aim of removing a tooth from its socket. This action is performed to prevent tissue injury caused by pathological conditions or trauma [1]. Tooth extraction procedures often experience difficulties which cause post-extraction complications as the most common and cause severe pain after tooth extraction is dry socket. The pathophysiology of dry socket results from increased local fibrinolytic activity in the alveoli caused by the release of mediators during inflammation by direct or indirect activation of plasminogen into the blood [2].

When a surgical incision or wound is formed, macrovascular or microvascular injury occurs. The body responds to prevent bleeding and stimulate hemostasis. After bleeding is controlled, inflammatory cells migrate into the wound (chemotaxis) and stimulate the start of the inflammatory phase which is characterized by sequential infiltration of neutrophils, macrophages and lymphocytes. Inflammation begins with cell damage which then releases various mediators. These inflammatory mediators cause vasodilation, increased vascular permeability, and migration of leukocytes to the site of inflammation. The most important inflammatory mediators are vasoactive amines, lipid products (prostaglandins and leukotrienes), cytokines including chemokines and the complement system. Prostaglandins and leukotrienes produced from arachidonic acid present in membrane phospholipids stimulate vascular and cellular reactions in acute inflammation [3].

Macrophages are phagocytic cells that have many lysosomes and are a change from mature monocytes. Histologically, macrophages are irregular in shape, with a cross-section of about 10–30 μm , the cell surface is uneven and has finger-like projections. There is basophilic cytoplasm, there are a number of small vesicles and also small and dense granules. The nucleus is oval or kidney-shaped, eccentrically located, smaller, and darker than the fibroblast [4]. Macrophages have an important role during the transition from inflammation to

the proliferative period during the healing process. In particular, macrophages are important cellular constituents of granulation tissue that clean up extracellular debris and fibrin at the site of injury, eliminate foreign bodies and macrophages secrete proteinases to degrade extracellular matrix (ECM), and act as a host mediator that induces fibroblast proliferation and stimulates growth factors to triggers neovascularization which is the process of forming new blood vessels that will fill the wound gaps and establish a balance between scar tissue formation and tissue regeneration and this is important in the wound healing process. Macrophages secrete pro-inflammatory cytokines such as Interleukin (IL)-1 β , IL-6, IL-8, and TNF- α . Macrophage cells increase on the 2nd day after the injury and their number will remain stable until the 5th day which among other things encourages inflammation and is followed by the process of angiogenesis in which macrophages induce the formation of new blood vessel cells, and play a role in fibroblast proliferation by secreting factors which will stimulate the formation of collagen and elastin by fibroblasts and begin to decline gradually to their original state on day 14 [5].

Aloe vera contains amino acids such as phenylalanine and tryptophan which have anti-inflammatory activity. Salicylic acid in *aloe vera* prevents the biosynthesis of prostaglandins from arachidonic acid. This explains how *Aloe vera* reduces vasodilation and reduces the vascular effects of histamine, serotonin and other inflammatory mediators. *Aloe vera* can modulate both immune reactions and inflammatory reactions can act as a stimulator of wound healing, antibody production, blocking prostaglandin synthesis. Vitamin C in *aloe vera* inhibits inflammation, picks up oxygen radicals to block the inflammatory process. The anti-inflammatory agents that *aloe vera* has include salicylic acid, indomethacin, which can reduce edema [6]. This study aims to determine the effectiveness of anti-inflammatory gel *aloe vera* extract (*Aloe vera*) with the concentration of 25%, 50%, 75% on the number of *macrophage* cells in the socket wound after tooth extraction in white wistar rats.

MATERIALS AND METHODS

This research is a laboratory experimental research with ethical approval and protocol from animal research ethics committees/AREC "0426/KEPH-FMIPA/2022". the research design "post-test only control group design". The population in this study were white wistar rats (*Rattus norvegicus*) which met the inclusion criteria, including male Wistar rats with an average body weight of 150-300 g and in healthy condition, which was characterized by active movements, hair that did not fall off easily, and no sores on the body and oral cavity.

The research process began with making aloe vera extract by washing, peeling and then cutting aloe vera into cubes after that, it was mashed using a blender with the addition of ethanol until it was homogeneous, then poured into a closed container and stirred after 6 h and left to stand again for 18 h while stirring occasionally and filtered through filter paper. The process is continued by making the basic gel by sprinkling CMC-Na (Carboxyl Methyl Cellulose-Natrium) onto 30 ml of distilled water in a mortar and then crushed by adding distilled water little by little until it is homogeneous. Add glycerin and homogenize to form a basic gel. After the basic gel is finished, proceed with making aloe vera extract gel with 25%, 50%, and 75% by putting 30 ml of distilled water into the mortar, then evenly sprinkling CMC-Na (Carboxyl Methyl Cellulose-Sodium) onto the surface of the distilled water. Cover and let stand for 15 min. Grind, then add the remaining distilled water little by little while grinding until homogeneous. Add glycerin and homogenize. Then weigh the extract as much as 25, 50 and 75 grams, put it in the mortar. Add little by little the base gel mass up to 25.50 and 75 grams while grinding until homogeneous.

Prior to extraction, the rats underwent an acclimatization process for 7 d. For extraction, the mice were administered intraperitoneally, namely anesthesia in the left or right lower quadrant of the abdomen, with an injection angle of 30-45 degrees. The anesthetic dose of xylazine ketamine was given to rats at a dose of 70 mg/kg. Then, the left incisor of the lower jaw was extracted using an artery clamp with careful luxation movements so that the teeth would not fracture. After tooth extraction, irrigation of the socket with distilled water was carried out to remove debris or remnants of tooth extraction.

The procedure was continued with the application of Aloe vera gel extract with concentrations of 25%, 50%, and 75%. In the treatment group, 0.1 ml of Aloe vera gel was applied to the rat teeth that had been extracted to cover the socket wound. The application of Aloe vera gel to the post-extraction socket wound area is given twice a day, namely in the morning and evening, until the day before

decapitation. On the first day (after 24 h of application) and on the third day, the mice were excised and tissue samples were made using Hematoxylin Eosin (HE) staining, then looked at under a microscope to collect data. Then basic gel was applied to the control group as much as 0.1 ml to cover the socket wound twice a day, namely in the morning and evening, until the day before decapitation was carried out. On the first day (after 24 h of application) and on the third day, the rats were excised and tissue samples were made stained with Hematoxylin Eosin (HE). The sacrifice was made decapitation (decapitation). After that, tissue samples were taken.

The procedure is followed by Hematoxylin Eosin (HE). The body tissue is identified macroscopically. The next step is that the tissue will be wet cut and processed automatically using a tissue processor. The procedure was followed by counting the number of macrophage cells in the smear and observed using a binocular microscope with 400x magnification to obtain the counting area. Then a drop of immersion oil is placed on the preparation to be examined. After that, it was continued with data processing and analysis, data processing was carried out using the SPSS application. The data normality test was carried out with the Shapiro-Wilk test followed by a one-way ANOVA to see the effectiveness of the Aloe vera material on the number of macrophages in the socket wound after tooth extraction in white rats wistar strain. If the results of the statistical test using one-way ANOVA show significant results, then a further statistical test is carried out using the Post Hoc Test to find out which treatment group shows the most significant results. If the data obtained does not show a normal distribution, then the analysis will be changed using the Kruskal Wallis

RESULTS

This experimental study was to determine the anti-inflammatory effectiveness of aloe vera extract gel (Aloe vera L) with a concentration of 25%, 50%, and 75% on the number of macrophages in the socket wound after tooth extraction in white wistar rats. Based on table 1, it shows that on day-1 in the negative control group, namely placebo or basic gel, had the largest mean of 23.00 (± 2.52). In the administration of 25% aloe vera extract gel, which was 14.85 (± 0.76), 50% aloe vera extract gel had an average of 10.95 (± 0.48), and in the aloe vera extract (Aloe vera) 75% has an average of 7.30 (± 0.83). And the mean results of calculations on day-3 in the treatment group giving negative control, namely placebo or basic gel, had the largest mean, namely 28.20 (± 4.54). In the administration of 25% aloe vera extract gel, which was 17.15 (± 1.31), 50% aloe vera extract gel had an average of 13.75 (± 0.88), and in the 75% aloe vera extract has the smallest average of 10.2 (± 1.52).

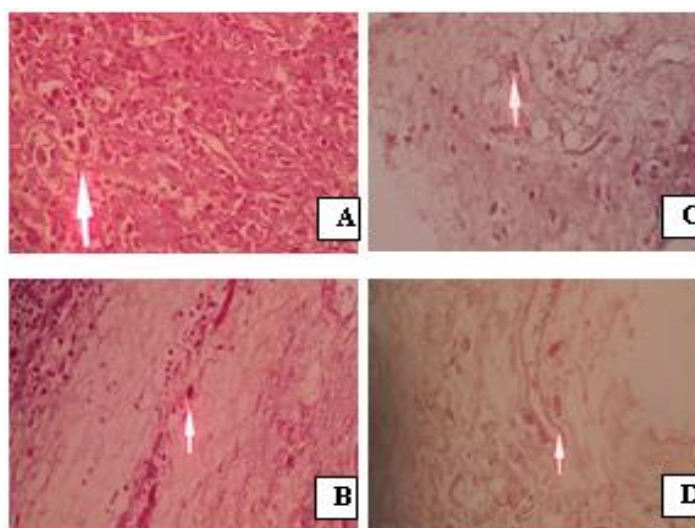


Fig. 1: Histology of macrophages on the first day (a) Control group; (b) The group given Aloe vera extract gel 25%; (c) The group given Aloe vera extract gel 50%; (d) The group given Aloe vera extract gel 75%

Table 1: Basic test data

Duration	Group	Number of samples	Average	Minimum	Maximum	Std. deviation
Number of Macrophages day-1	Negative control	5	23.00	20.00	27.00	2.52
	Aloe vera 25%	5	14.85	14.25	16.00	0.76
	Aloe vera 50%	5	10.95	10.25	11.50	0.48
	Aloe vera 75%	5	7.30	6.25	8.25	0.83
Number of Macrophages day-3	Negative control	5	28.20	24.50	35.75	4.54
	Aloe vera 25%	5	17.15	15.50	18.50	1.31
	Aloe vera 50%	5	13.75	12.75	15.00	0.88
	Aloe vera 75%	5	10.20	8.75	12.75	1.52

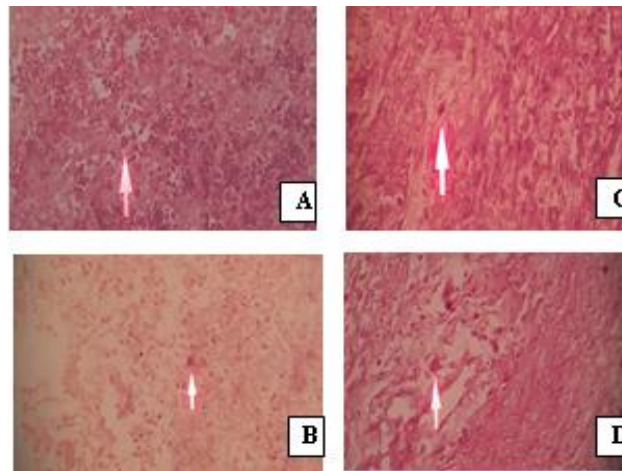


Fig. 2: Histology of macrophages on the third day (a) Control group; (b) The group given aloe vera extract gel 25%; (c) The group given aloe vera extract gel 50%; (d) The group given aloe vera extract gel 75%

This study used a normality test using Shapiro-Wilk and based on table 2 it can be seen that the averages of all groups on day 1 and day 3 were normally distributed. Whereas in the homogeneity test, the value in the 1st d group was 0.210 ($p>0.05$) and 0.135 ($p>0.05$)

on the 3rd d, so it was concluded that the data on the first and third-day groups were homogeneous, and continued with data processing using a one-way ANOVA test to see a comparison between the results of the first day and third-day groups.

Table 2: Normality test data

Duration	Group	Number of samples	P-value*
Number of Macrophages day-1	Negative control	5	0.375
	Aloe vera 25%	5	0.190
	Aloe vera 50%	5	0.928
	Aloe vera 75%	5	0.737
Number of Macrophages day-3	Negative control	5	0.155
	Aloe vera 25%	5	0.321
	Aloe vera 50%	5	0.844
	Aloe vera 75%	5	0.255

*Shapiro-Wilk test: $p>0.05$ data is normally distributed

The one-way ANOVA test on the first and third days obtained a p value of 0.000 where $p<0.05$, which concluded that there was a significant difference between the control group and the treatment group, then, it was obtained that H_0 was rejected and H_a was accepted. Based on these data it was concluded that Aloe vera had an anti-inflammatory effect on the number of macrophages in the socket after tooth extraction and then an LSD (Least Significance Different) test was carried out to determine the significance of the difference in the number of macrophages on each group

The results of the LSD post-hoc test on the first day of the 25%, 50% and 75% aloe vera extract gel group had anti-inflammatory results that were more effective against reducing the number of macrophages in post-tooth extraction socket wounds than the negative control group. In the gel 50% and 75% aloe vera extract gel group, the results were more effective in reducing the number of macrophages than the 25% aloe vera extract group. In the 75% aloe

vera extract gel group, the anti-inflammatory results were more effective against the number of macrophages in post-tooth extraction socket wounds than the other Aloe vera extract gel group and on the third day the difference in the number of macrophages between each the control and treatment groups had the same results as the first day, wherein the 75% aloe vera extract gel group had more effective anti-inflammatory results against the number of macrophages than the control group and the other aloe vera extract gel groups.

DISCUSSION

Inflammation is a response to tissue injury or infection. Inflammation is a natural process to maintain the body's homeostasis due to the presence of foreign agents or compounds. The main signs of inflammation are redness (erythema), swelling (edema), heat (calor), pain (dolor), and loss of local function [7].

Inflammation causes many substances to be released endogenously, known as inflammatory mediators. Arachidonic acid is one of the important inflammatory mediators, arachidonic acid plays a role in the biosynthesis of prostaglandins through the cyclooxygenase pathway. Arachidonic acid derivatives have the potential as inflammatory mediators, namely cyclooxygenase. Cyclooxygenase-2 (COX-2) is an enzyme whose existence is influenced by stimulation of the tissue. These stimuli can be cytokines, lipopolysaccharide bacteria, inflammation or other pathological conditions. Inflammation also causes the accumulation of white blood cells, especially macrophages. Macrophages will perform margination, emigration, chemotaxis and phagocytosis [8].

Macrophages have a role in mediating the immune response against microorganisms. When microorganisms such as bacteria penetrate the body's epithelial surface, they are the first to be encountered by cells or molecules that play a role in the innate immune response. Macrophages are large cells with folded or indented nuclei containing fine chromatin resembling threadlike cytoplasm and usually containing fine azurophilic granules. Macrophages are responsible for defense against bacteria or can be interpreted as surface receptors that are able to recognize and bind to the surface of bacteria. The activated macrophages will secrete proteins released by cells due to activation or what are called cytokines. Macrophages mainly play a role in phagocytosis of chronic infection stages and have a function as an antigen-presenting cell (APC) to lymphocytes. This process is necessary for the initiation of an adaptive immune response from the host. Cytokines and chemokines released by macrophages in response to bacterial components initiate the inflammatory process. Macrophages have a dual function, namely as innate immunity and specific immunity. Macrophages also actively secrete biological products, including IL-1 which regulates the tasks of T-cells and B-cells and mobilizes other body defense systems [9-11].

Aloe Vera contains amino acids that have anti-inflammatory activity such as phenylalanine and tryptophane. Salicylic acid in aloe vera works as an inhibitor of the cyclooxygenase enzyme to prevent the biosynthesis of prostaglandins from arachidonic acid so that prostaglandins, which are inflammatory mediators fail to form and the release of leukocytes from the spinal cord to the circulation decreases. Aloe vera reduces vasodilation and reduces the vascular effects of histamine, serotonin and other inflammatory mediators. Prostaglandins play an integral role in regulating both inflammation and immune reactions. Aloe Vera can affect the work of these two systems by blocking the synthesis of prostaglandins. Besides having an effect on inflammatory reactions and immune reactions, aloe vera also reduces oxygen-free radicals produced by PMN. The presence of sterol compounds, anthraquinones, and other natural substances, including polysaccharides in aloe vera, work synergistically to cause anti-inflammatory effects; aloe vera has antioxidant enzymes that play a role in inhibiting inflammatory mediators [12].

Based on the results of observations and data analysis in this study, it was stated that there were differences in the number of macrophages produced by the negative control group and the treatment group. The treatment group had fewer macrophage cells than the negative control group on the same day. On the third day there was an increase in macrophages; this was due to the proliferative phase, which began on the third day after the wound occurred; granulation tissue would form consisting of new blood vessels (neovascular), fibroblasts and macrophages [13]. But along with the increase in concentration in the treatment there was a decrease in the number of macrophages.

In the control group, the basic gel did not decrease the number of macrophages because they did not have an active substance as an anti-inflammatory and also, on the 3rd day there was an increase in the number of macrophages due to factors that were not controlled by the researchers such as the sensitivity of the wistar rats to a substance, psychological conditions or other factors like mice that have low immunity so the possibility of infection is higher. The control group on the third day also experienced an increase in the number of macrophages due to the absence of wound-healing materials. The basic gel also does not contain active ingredients that

are susceptible to enzymatic degradation by pathogenic microorganisms, so there are still microbes that must be phagocytosed and tissue damage by cells in the wound area, but basic gel can be used as preparation because it has high viscosity and adhesiveness so that does not flow easily, has thixotropic properties so that it is easily spread evenly when smeared, does not leave marks, gives a cooling sensation and is able to penetrate further than cream [14-16].

Based on the research results, it can be seen that the anti-inflammatory effect of the active substance contained in aloe vera extract gel (Aloe vera) is indicated by a decrease in the number of macrophage cells in post-extraction socket wounds. In this study, an increase in the number of macrophages was found on the third day. However, the number of macrophages decreased as the concentration of the extract gel increased in the treatment, whereas in the negative control with the administration of basic gel there was no decrease in the number of macrophages as in the group that was given the extract gel treatment. The concentration that showed significant results in reducing the number of macrophages in this study was aloe vera extract gel with a concentration of 75%. The reduction in vitamins, enzymes, proteins, carbohydrates, minerals (calcium, sodium, magnesium, zinc, iron) and amino acids contained in aloe vera plays a major role in treating socket wounds. In addition, various anti-inflammatory agents, including salicylic acid, indomethacin, mannose-6-phosphate, B sitosterol, as well as components of lignin, saponins and anthaquinones consisting of aloin, barbaloin, anthranol, anthracene, aloetic acid, aloe emodin are basic ingredients. Drugs that act as antibiotics and painkillers. Aloe vera extract gel (Aloe vera) has relatively few side effects from traditional medicine and requires accuracy in use, which consists of the accuracy of the ingredients, the accuracy of the dosage, the accuracy of the time of use, the accuracy of the method of use, the accuracy of information review and without misuse of the traditional medicine itself.

CONCLUSION

This study aims to determine the effectiveness of anti-inflammatory gel aloe vera extract (Aloe vera) with the concentration of 25%, 50%, 75% on the number of macrophage cells in the socket wound after tooth extraction in white wistar rats and the results of this research is the 75% aloe vera extract gel group had more effective anti-inflammatory results against the number of macrophages than the control group and the other aloe vera extract gel groups.

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AUTHORS CONTRIBUTIONS

RS conceived of the presented idea, developed the theory design of the study and verified the analytical methods with support from DC and KB. RS, DC and KB carried out the experiment. DC wrote the manuscript with support from KB, SMG, AG, AS. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTERESTS

Declared none

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