The Effect of Hydrogel Aloe vera (Aloe vera (L.) Burm) on the Number of Neutrophil Cells in Aggressive Periodontitis Induced by Aggregatibacter actinomycetemcomitans (In Vivo Study on Wistar Rats)

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1. Introduction
One of the health problems that people complain about is oral and dental problems. According to the results of the Basic Health Research Data (Riskesdas) in 2018, the Indonesian population experiences dental and oral health problems as much as 57.6 percent.¹ The most common dental and oral health problems are dental caries and periodontal disease.² Periodontal disease is commonly found in patients with poor oral hygiene.³ Tooth unsteadiness is a symptom of periodontal disease.⁴ The American Academy of Periodontology classifies periodontal disease and its condition into 2 forms, namely chronic periodontitis and aggressive periodontitis. Aggressive periodontitis is periodontitis that starts early (early-onset periodontitis) and progresses rapidly (rapidly progressive periodontitis).⁵ Aggressive periodontitis is a multifactorial disease, the causes of which can be caused by many things such as hypersensitive response to the body’s immune response, genetic, area aspects.⁶ Aggressive periodontitis is caused by constraints from the bacteria Porphyromonas gingivalis, Actinomycetem Comitans, and Tannerella forsythia.⁶

Aggregatibacter actinomycetemcomitans are gram-negative, facultative anaerobic bacteria, short (0.4–1 µm), rod-shaped with rounded tips. These bacteria use epithelial cells as reservoirs when initial attachments and move
to the tooth surface. These bacteria are pathogenic bacteria for periodontal tissue and cause the formation of aggressive periodontitis, not only that these bacteria can also cause inflammation in the supporting tissues of the teeth resulting in rapid destruction of the ligamentous tissue to the alveolar bone.

Neutrophils are inflammatory cells that appear first, mostly because of their great mobility and also because they are present in large numbers in the blood circulation. Neutrophils are cells that first migrate from blood vessels when there is inflammation which functions as a defense from the body to phagocyte incoming microorganisms. By a process called phagocytosis, neutrophils have the ability to move actively like amoeba and are able to ingest various substances.

Periodontitis treatment includes mechanical therapy such as scaling and root planning which is supported by the provision of antibiotics and anti-inflammatory drugs. One of the anti-inflammatory drugs that can be used is the non-steroidal anti-inflammatory drug, namely ibuprofen. The plants that can be used as anti-inflammatory in periodontitis are aloe vera (Aloe vera (L.) Burm.f).

Aloe vera (Aloe vera (L.) Burm.f.) contains vitamins, essential and non-essential amino acids, proteins, hormones, anthraxes, organic compounds, saccharides, carbohydrates, fats and active compounds including enzymes, triterpenes, polysaccharides, and groups. glycosides. It is known that aloe vera contains alkaloid compounds, flavonoids, steroids, anthraquinones, phenols, tannins. Aloe vera has anti-inflammatory, antioxidant, wound healing, antimicrobial and laxative effects.

Hydrogel is a pharmaceutical dosage form that is used topically or applied topically. Natural hydrogel is an attractive material for the treatment of various types of wounds, because of its properties such as smoothness, high water content, elasticity, flexibility.

Some of the gelling agents are sodium alginate. Alginate is a natural polymer that is widely used for the treatment of several types of wounds, due to its biocompatibility, biodegradability and ability to form hydrogels, for example through interactions with calcium ion. Aloe vera gel with alginate, to explore its therapeutic properties, which includes antibacterial, antiseptic, anti-inflammatory.

2. Method

This research has received approval from the Ethics Commission with Letter Number: 035 / KEPK / UNPRI / III / 2021. This research is a laboratory experimental study with a post-test only design with a control group design. The population in this study were the experimental animals of Wistar rats, in the form of 24 male Wistar rats.

The research sample was taken by using purposive sampling technique, namely the sampling was based on inclusion and exclusion criteria. The inclusion criteria were 24 Wistar rats, male sex, healthy rats, body weight 100-200 grams, ages 2-3 months. Exclusion criteria were male rats who were sick, male rats with infections and physical disabilities. To take the sample size, Federer's formula was used, namely: \((n-1)(r-1) \geq 15\). Then the sample size for each treatment was 4 wistar rats. which were divided into 6 groups, namely, the negative control group (Basic hydrogel), the ibuprofen group and the Aloe vera hydrogel group 2.5%, 5%, 10%, 20% against Wistar rats with aggressive periodontitis.

Preparation of aloe vera extract (Aloe vera L. Burm.f.) by maceration method. The outer skin of the aloe vera is peeled, 500 grams of meat is taken, then cut into small pieces. The meat of aloe vera (Aloe vera (L.) Burm.f.) is mashed in a blender and put in a closed container. Add 1 liter 70% ethanol, stir for the first 6 hours. Let stand for 18 hours, stirring occasionally. Filter the aloe vera with cotton and filter paper, hold the filtrate (macerate I). Repeat the extraction process on the pulp with 70% ethanol 0.5 liters (macerate II). Combine the two maserates, then steam with a Rotavapor at 40 °C and obtain a thick extract.

The hydrogel base is made by a combination of 0.75 g carbopol, 0.55 g gelatin and 0.45 g Na-algininate which is developed first in aquadest for 24 hours. The entire base which has been expanded is homogenized by hand mixer. Enter 2 g of propylene glycol, 12.5 g of glycerol and 1 g of TEA in sequence into the base mass and...
homogenized so that they are completely mixed. Add distilled water so that the total weight of the preparation reaches 30 gr.\textsuperscript{15}

Wistar rats were adapted for 7 days in a laboratory setting. Wistar rats were anesthetized by intramuscular injection of ketamine HCl in the hamstring muscles at a dose of 0.2 ml \( / \) 200 g body weight.\textsuperscript{1} Induction of periodontitis by injecting the bacteria \textit{A. actinomycetemcomitans} into the interdental gingiva of the mandibular incisors of 200 microliter rats.\textsuperscript{16} On the 7\textsuperscript{th} day, clinical signs of aggressive periodontitis with inflammation appeared in the form of gingival color changing to redness, swelling, heat, bleeding and gingival recession, as a sign of periodontitis.\textsuperscript{1,17}

Apply the Aloe vera hydrogel topically with a microbrush and let it sit for 1 minute. The material was applied 2 times a day, morning and evening with a difference of 7 hours.\textsuperscript{1} On days 1, 3, 5 and 7 of Wistar rats, each group was sacrificed with ketamine at a dose of 0.5 ml intramuscularly until the Wistar rats died.\textsuperscript{1,17} Then performed periodontal tissue (gingival) collection around the mandibular incisors by cutting using a scalpel for histopathological preparations.

The tissue was fixed with 10\% formalin for 24 hours. carry out the decalcification process with a 10\% EDTA solution. Dehydrate with alcohol and liquid paraffin. do the embedding process, after which it is labeled. The tissue was sliced in series with a thickness of 5 microns with a microtome. The network is put on a waterbath. The tissue pieces were transferred to a glass object that had been previously applied with albuminglycerin as an adhesive. Let stand for 24 hours. The preparation was put into 2 tubes of xylol solution, then 3 tubes of alcohol each 1 minute, and washed in running water for up to 1 minute. After that, the preparations were stained with Hematoxylin for 8 minutes. The preparations were soaked in eosin for 2-3 minutes. and put into a xylol solution and the object glass was covered with a deck glass and observed under a microscope. Take a prep reading.\textsuperscript{7}

Data analysis using SPSS 21 program. First, the data was tested for normality with Shapiro Wilik. If the data is normal, data analysis using the Oneway ANOVA test aims to determine the effect of giving hydrogel Aloe vera concentrations of 2.5\%, 5\%, 10\%, 20\% with ibuprofen on Wistar rats induced by periodontitis. The data analysis was continued with the post hoc LSD test to determine the difference in the number of neutrophil cells between the two groups.

3. Results and discussion

The statistical results show that the data for all groups are normally distributed. Table of the average number of neutrophil cells in the negative control group, ibuprofen and hydrogel aloe vera 2.5\%, 5\%, 10\%, 20\% against Wistar rats induced by aggressive periodontitis by \textit{A. actinomycetemcomitans} bacteria can be seen in table 1. Table 1 shows that the average number of neutrophil cells of Wistar rats induced by aggressive periodontitis in the hydrogel aloe vera group is 2.5\%, 5\%, 10\%, 20\%, ibuprofen and negative controls is 120.50 ± 12.92, 78.25 ± 7.37, 47.25 ± 4.03, 1.00 ± 0.82, 0.50 ± 0.58, and 217.25 ± 62.26.

The effect of giving hydrogel aloe vera concentrations of 2.5\%, 5\%, 10\%, 20\% with ibuprofen on Wistar rats induced by aggressive periodontitis by \textit{A. actinomycetemcomitans} can be seen in table 2. \( = \) 0.001, which means that there is a difference in the number of neutrophils of Wistar rats in all treatment groups. From the results of this study it can be stated that there is an effect of giving hydrogel aloe vera concentrations of 2.5\%, 5\%, 10\%, 20\% with ibuprofen on Wistar rats induced by aggressive periodontitis by \textit{A. actinomycetemcomitans} bacteria.

The difference in the effect of giving hydrogel aloe vera concentrations of 2.5\%, 5\%, 10\%, 20\% with ibuprofen against Wistar rats induced by aggressive periodontitis by \textit{A. actinomycetemcomitans} can be seen in table 3. Based on table 3, the results of the LSD posthoc statistical test showed that There was an effect of giving hydrogel aloe vera concentrations of 2.5\%, 5\%, 10\%, 20\% with ibuprofen on Wistar rats induced by aggressive periodontitis by \textit{A. actinomycetemcomitans} bacteria.

Periodontitis is a chronic inflammation caused by periodontopathogenic bacteria.\textsuperscript{18} In this study, the sample was induced by periodontitis by injecting A.
actinomycetemcomitans bacteria into the interdental gingiva of the mandibular incisors. Periodontitis can be confirmed by the appearance of an inflammatory infiltrate in the gingiva that initiates bone resorption. Host response will be reduced due to decreased tolerance to endotoxins produced by A. actinomycetemcomitans.19

In periodontitis there is an increase in neutrophil infiltration which functions to phagocytose bacteria that infiltrate the gingival tissue.18 Neutrophils are inflammatory cells that appear first when there is inflammation.1,20 This is because active neutrophils produce various chemokines and cytokines that lead to inflammation.20 It is also in accordance with the research of Agustin et al (2016) which states that neutrophil cells work by phagocytic foreign bodies when acute inflammation occurs, so that the number increases on the first day. The inflammatory phase generally occurs at 0-3 days and the proliferative phase starts on the 4th day.1

One of the anti-inflammatory drugs commonly used in the therapy of periodontal disease is ibuprofen.18 In this study, ibuprofen was used as a control group. Based on the results of the study, it was found that the number of neutrophils in Wistar rats that had been induced by periodontitis after being given ibuprofen was 0.50 ± 0.58. The composition of ibuprofen gel used in this study consisted of 5% ibuprofen, purified water, ethanol, propylene glycol, carboxomer and diisopropanolamine which are safe for use in the oral cavity.1

The administration of ibuprofen which acts as an anti-inflammatory will relieve inflammation by suppressing the migration of neutrophils and macrophages and reducing the production of prostaglandins. This disruption in the migration process of neutrophils and macrophages also causes interference with cytokine secretion, resulting in decreased distribution of chronic inflammatory cells.21

In this study, researchers used natural ingredients, namely hydrogel aloe vera to reduce the number of neutrophils of Wistar rats that have been induced by aggressive periodontitis by A. actinomycetemcomitans bacteria. The results showed that the average number of neutrophil cells of Wistar rats induced by aggressive periodontitis by A. actinomycetemcomitans in the hydrogel aloe vera group of 2.5%, 5%, 10%, 20% was 120.50 ± 12.92, 78.25 ± 7.37, 47.25 ± 4.03, 1.00 ± 0.82. The higher the concentration of the test material, the smaller the number of neutrophil cells in Wistar rats that had been induced by aggressive periodontitis. The results of this study are in accordance with the research of Andayani et al. (2016) which states that the neutrophil count of white Wistar rats in the 50% red ginger group is 3.00 and the 100% red ginger group is 2.60.22

Aloe vera is one of the most widely used healing plants. Research on the ability of aloe vera gel shows that aloe vera gel can heal wounds, ulcers and burns.23 From the results of this study it can be stated that there is an effect of giving hydrogel aloe vera concentrations of 2.5%, 5%, 10%, 20% with ibuprofen on Wistar rats induced by aggressive periodontitis by A. actinomycetemcomitans. The results of this study are in line with research conducted by Prasetya (2014) which states that the administration of mangosteen peel ethanolic extract 60 and 30 mg / kg BW is able to reduce neutrophil cell infiltration in mice induced by periodontitis.18

The decrease in the number of neutrophil cells in the group given hydrogel aloe vera is thought to be caused by the activity of the aloe vera content. The two main polysaccharides contained therein are glucomannan and acemanan. Glucomannan plays a role in replacing skin tissue and reducing pain caused by wounds. Acemanan is able to accelerate wound healing.23

Flavonoids are the most abundant compounds found in medicinal plants. The flavonoids contained in aloe vera can improve the function of neutrophil cells. Flavonoids play a role in limiting the release of inflammatory mediators such as IL-1, IL-6, IL-8, Interleukin γ, and TNF-α. Flavonoids are the largest group of phenolic compounds that have anti-inflammatory activity through inhibition of cyclooxygenase and lipoxygenase so as to limit the number of inflammatory cells such as neutrophil cells to inflamed tissues. This causes the inflammatory
process to take place shorter so that the proliferation process occurs immediately.\textsuperscript{24} This is in line with the research conducted by Tamara et al (2019) that the flavonoid compounds found in kelulut propolis extract effectively reduce the number of neutrophils in Wistar rats induced by periodontitis.\textsuperscript{1}

Table 1. The average number of neutrophil cells in the negative control group, ibuprofen and hydrogel aloe vera 2.5%, 5%, 10%, 20% against Wistar rats induced by aggressive periodontitis by A. actinomycetemcomitans bacteria

<table>
<thead>
<tr>
<th>Group</th>
<th>Total of colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Hydrogel aloe vera 2.5%</td>
<td>139</td>
</tr>
<tr>
<td>Hydrogel aloe vera 5%</td>
<td>75</td>
</tr>
<tr>
<td>Hydrogel aloe vera 10%</td>
<td>45</td>
</tr>
<tr>
<td>Hydrogel aloe vera 20%</td>
<td>1</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1</td>
</tr>
<tr>
<td>Negative control</td>
<td>275</td>
</tr>
</tbody>
</table>

Table 2. Effect of giving hydrogel aloe vera concentrations of 2.5%, 5%, 10%, 20% with ibuprofen on Wistar rats induced by aggressive periodontitis by A. actinomycetemcomitans bacteria

<table>
<thead>
<tr>
<th>Group</th>
<th>( \bar{X} \pm SD )</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogel aloe vera 2.5%</td>
<td>120.50 ± 12.92</td>
<td></td>
</tr>
<tr>
<td>Hydrogel aloe vera 5%</td>
<td>78.25 ± 7.37</td>
<td></td>
</tr>
<tr>
<td>Hydrogel aloe vera 10%</td>
<td>47.25 ± 4.03</td>
<td>0.001*</td>
</tr>
<tr>
<td>Hydrogel aloe vera 20%</td>
<td>1.00 ± 0.82</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.50 ± 0.58</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>217.25 ± 62.26</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: * there is a significant difference

Table 3. Differences in the effect of giving hydrogel aloe vera concentrations of 2.5%, 5%, 10%, 20% with ibuprofen against Wistar rats induced by aggressive periodontitis by A. actinomycetemcomitans bacteria

<table>
<thead>
<tr>
<th>Group</th>
<th>Hydrogel aloe vera 2.5%</th>
<th>Hydrogel aloe vera 5%</th>
<th>Hydrogel aloe vera 10%</th>
<th>Hydrogel aloe vera 20%</th>
<th>Ibuprofen</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogel aloe vera 2.5%</td>
<td>-</td>
<td>0.035\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
</tr>
<tr>
<td>Hydrogel aloe vera 5%</td>
<td>0.035\textsuperscript{*}</td>
<td>-</td>
<td>0.111</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
</tr>
<tr>
<td>Hydrogel aloe vera 10%</td>
<td>0.001\textsuperscript{*}</td>
<td>0.111</td>
<td>-</td>
<td>0.022\textsuperscript{*}</td>
<td>0.021\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
</tr>
<tr>
<td>Hydrogel aloe vera 20%</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>0.022\textsuperscript{*}</td>
<td>-</td>
<td>0.979</td>
<td>0.001\textsuperscript{*}</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>0.021\textsuperscript{*}</td>
<td>0.979</td>
<td>-</td>
<td>0.001\textsuperscript{*}</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>0.001\textsuperscript{*}</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: * there is a significant difference
4. Conclusion

Based on the results of the research that has been done, it can be concluded that hydrogel aloe vera has an effect on the number of neutrophil cells in male Wistar rats induced by aggressive periodontitis by A. actinomycetemcomitans bacteria.

5. References

1. Tamara, A., Oktiani, B.W., Taufiqqurahman, I. 2019. Effect of flavonoid propolis kelulut (g.thoracica) extract on the number of neutrophil cells in peri-odontitis (in vivo study on male Wistar rats (rattus norvegicus)). Dentin (Jur Ked Gi), 3 (1), 10-6.
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