Acemannan Hydrogel’s Effects on Neutrophils at Concentrations of 25%, 50%, 75% Counts in Wistar Rats with Periodontitis Induced by Alloxan

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Abstract: Neutrophils occur in periodontitis, an infection of the periodontal tissue that may increase the risk of diabetes. Because of its excellent physicochemical properties and biological capabilities, hydrogel is a promising biomaterial for treating periodontal disease. This study consisted of four groups of Acemannan hydrogel 25%, 50%, 75%, and the Federer formula was used to determine the sample size and six replicate experiments were performed for each group to form a positive control group. Considering the investigation’s conclusions, in the acemannan hydrogel administration group, the average ± SD levels of neutrophils in samples on day 3 were 25%, 50%, and 75%, and the positive control group was 3.08 ± 0.736. 2.46 ± 0.459; 1.54 ± 0.510; 1.17 ± 0.258. This value decreased to 1.83 ± 0.258 by day 5 were 1.29 ± 0.459; 0.50±0.447; 0.21 ± 0.292. As a result of the Kruskal-Wallis statistical test, there was a significant difference in the average number of neutrophils after administering 25%, 50%, 75%, and 75% Acemannan hydrogel and the positive control group to rats with alloxan-induced Wistar periodontitis on the third and fifth days. The study’s findings suggest that 75% of acemannan hydrogel is the most effective concentration for reducing neutrophils.

Keywords: Acemannan hydrogel; Diabetes; Neutrophils; Periodontitis.

Introduction

Diabetes is a chronic condition that is prevalent in society and is caused by metabolic problems that result in elevated blood sugar levels that are higher than usual (hyperglycemia) (Kemenkes, 2020). Increased blood sugar levels occur when pancreatic cells do not function properly in producing the insulin hormone or when the body rejects the insulin hormone (Husain et al, 2020).

The prevalence of diabetes continues to increase every year (Himammi and Hartomo, 2020). The International Diabetes Facility (IDF) estimates that at least 463 million people aged 20 to 79 years had diabetes in 2019, equivalent to 9.3% of the global population in the same age group (Kementerian Kesehatan, 2020; Dachi et al., 2022). In developing countries, this disease is the sixth cause of death (Husain et al, 2020). With 10.7 million diabetes cases in 2019, Indonesia ranked seventh in the world among the nations with the largest number of diabetes cases (Kemenkes, 2020).

Patients with diabetes have a higher incidence of periodontitis, which might worsen with time (Sari, et al., 2017). In uncontrolled diabetic patients, PMN functions like monocytes and macrophages in defense against pathogens causing periodontitis. (Kurniawan et al, 2018). One of the PMN cells is a neutrophil. Neutrophils respond to bacterial infection by producing toxic oxidative substances. (Andriani dan Chairunnisa, 2019). Within 24 to 36 hours of bacterial infection, many of the functioning neutrophils remove foreign substances,
bacteria, dead cells, and the extracellular matrix is destroyed through phagocytosis. (Santi, 2018).

To destroy the remaining extracellular matrix, neutrophils release pro-inflammatory proteases and cytokines such as TNF-α, IL-1β, and IL-6. Neutrophils serve to prevent infection, but when they are done performing their phagocytosis functions, they are difagositosed or killed by macrophages. However, neutrophils that remain in the wound can make it harder to heal (Ellis et al., 2018; Tyavambiza et al., 2022).

The main focus of biomedical research is the use of hydrogel with antibacterial properties because the substance has a high hydrophilicity and cell rigidity (Yang et al., 2018; Li et al., 2018a; Susanto, et al. 2021). Its excellent physicochemical properties and biological functions make it an ideal type of biomaterial for periodontal disease in recent years. (Lee et al, 2021).

Acemannan is a water-soluble polysaccharide β-(1,4)-acetate is a primary bioactive polysaccharide obtained by extracting crocodile leather and gel and is a form of glucomannan acetyl storage located in the protoplasts of parenchymal cells that contain some intracellular polysaccharides. Matrix walls. Acemannan has a more dominant stimulating activity on leukocytes and lymphoid cellularity on the absolute number of lymphocytes, neutrophils, and monocyte in mice experiencing myelosuppressed induced radiation. (Liu et al., 2019). This is evident from the results of a Susanto dkk study (2022) which showed that collagen density scores increased In addition to increased concentrations of acemannan hydrogel by 25 percent, 50 percent, and 75 percent, the group given acemannan hydroglasses by 25 per cent, 50 per cent and 75 per cent showed a very different collagen density score from the group not given Acemannan Hydrogel.

In the pancreatic, aloxane results in beta-cell necrosis. Furthermore, a variety of animals, including dogs, rats, and squirrels, are frequently employed as test subjects in the study of diabetes. In diabetes-related research, aloxane, one of the most widely utilised diabetogens, is frequently used to assess the antidiabetic potential of pure chemicals and plant extracts. (Dachi, et al., 2022).

Base on the statements above, researchers are Based on the statement above, researchers wanted to know the effect of hydrogel with acemannan concentrations of 25%, 50% and 75% on the number of neutrophils in Wistar rats with alloxan-induced periodontitis.

**Method**

**Research Design**

This type of analysis consists of a posttest control group-only laboratory experiment.

**Time and Place Research**

The research was carried out at: Herbarium Laboratory of the University of North Sumatra for Aloevera inspection site, Physiology and Crop Culture Laboratory FMIPa Plant Networks of North sumatra University for acemannan extract and acemannan hydrogel manufacture site, Laboratory Centre for Animal Development of the University of North Sumatera for place to make wistar mice in the condition of diabetes and periodontitis, and Pathology Lab of the Faculty of Medicine of the University Sumatra North for neutrophil examination site. The research was conducted from July to October 2023.

**Research Sample**

The sample in this study was a white Wistar rat. (Rattus norvegicus). The Federer formula is used to determine the size of the sample. The study involved four groups: the treatment group (25%, 50%, and 75% Hydrogel Acemannan) and the positive control group. (AllochlearTM). Because the observations were done on the third and fifth days, and the number of samples needed for this study was 48 samples.

**Tools and materials used**

These research tools include a digital scale, Buchner's corong, filing paper, delta, vacuum evaporator, petri cup, microbrush, animal cage, diagnostic set, kidney beetle, 3cc spuit, needle holder, microcentrifuge, filings paper, gloves, swabs, masks, digital cameras, micro-Brushes, histological manufacturing tool set, lens glass, storage container, optical microscope, and microscopes.

Substances used in the study include aloe vera, male wistar rat, 70% ethanol, Aloclair TM, sodium alginate, pouch foil, 10% formalin, 10% paraffin, glycerol, adhesive strip, ketamine HCl, CaCl2, xylol, HE, and edta.

**Preparation of acemannan extract by using a tested**

Stir tested Aloe Vera, wash with clean water and insert 65% Hypochlorite solution into a container that contains aloe vera. Clean the aloe Vera and cut the dots. Insert Aloe vera into the blender to smooth. After smooth mixing Aloe Vera and ethanol in a ratio of 1:4 mix until dissolved for 10 minutes at a temperature of 10°C. After that insert into the refrigerator leave for 10 hours for sedimentation. The formed reservoir is separated from the solution by using a filter coated with filter paper. The filtered reserve is then inserted into a vacuum dryer at a temperature of 50°C for 24 hours or a day.

**Production of acemannan extract into acemannan hydrogel**

First weighed Acemannan extract that was obtained at 25 gr, 50 gr, as well as 75 gr. Separate 25
grams of CaCl2 dissolved in 500 ml of Aquades to make 25% of the CaCl2, mixed using a stirrer until homogeneous. Then, alginate weighs 15 grams for each treatment. To make acemannan hydrogel 25% use 25 gr Acemannan and 100 gr aquades then mix with a stirrer until homogeneous, then take using spuid then insert into CaCl2 and mix with stirrer till homogenous then after it is finished hold up to 1 day, as well as the next to make Acemanan Hydrogel 50% using 50 gr Acesannan and to make Hydro Gel Acesanan 75% using 75 g Acesannen.

Figure 1. Results of hydrogel acemannan 25%, 50%, 75% and control Positive

Animal Test Preparation

a. Mice are allocated into four groups using the randomization process. These groups included the treatment group, which was subsequently divided into three subgroups receiving Acemannan hydrogel at different concentrations (25%, 50%, and 75%), and the positive control group, receiving xylazine.

b. Before receiving therapy, given an adaptation period of one week for all mice.

c. The induction of aloxane is done on the seventh day after the animal trial is disclimatised. The recommended dose of aloxane for Wistar rats is 4.2 mg per gram of body weight, administered by intraperitoneal injection. After the injection, a waiting period of 72 hours was performed, after which the mice were given high-fat food. Before assessing blood glucose levels, mice have a fasting period of between 8 and 12 hours. According to Pertiwi dkk. (2021), the presence of diabetes in wistar rats can be determined when blood glucose levels are above 175 mg/dl.

d. Blood glucose levels in Wistar rats were assessed by the researchers using established protocols. First, the tail end of the rat was infected with alcohol, and then a small wound was made at the tails end. Finally, the blood droplets from the injection were applied to a glucometer test strip called AutocheckTM. (Pertiwi, dkk., 2021).

e. Next, subgingiva ligation technique is performed using silk ligature until periodontitis occurs, a condition characterized by inflammation of the gingiva. (Hariyani, as al., 2021; Prasetya, et al., 2021; Kurniawati, et al., 2023).

f. Substance administration is done topically using a microbrush on the labial surface of the mandibular tooth with a duration of 1 minute. According to Kartikaningtyas dkk. (2015), the application was done once in two days, i.e. in the morning and in the afternoon, with an interval of 7 hours.

g. On the third and fifth days of the experiment, rats were humanly executed by dislocating their necks. Next, the part of the periodontal tissue that was previously tied with a silk bond, was cut off from each animal using a surgical knife. The section is immersed in a 10% neutral formalin buffer solution (BNF) for fixation. Subsequently, the material was dyed with the procedure of dyeing Hematoxylin and Eosin. (HE).

h. The study aims to analyze the quantification of neutrophils using a light microscope equipped with a 400x magnification capability, including four different fields of view. (Wulandari, dkk., 2017)

i. All data is collected, then statistical testing is carried out in order to obtain the results and conclusions of the research.

Data Collection and Analysis Methods

The statistical techniques used in this study include one-way variance analysis (ANOVA) and post-hoc LSD analysis for data analysis.

Results and Discussion

Average number of neutrophils on day 3 and 5

Based on the results shown (Table 1.1) below, the average and raw concentration of the 3rd day neutrophil count in wistar rats with periodontitis due to excusor treated with acemannan hydrogel was 25%, 50%, and 75%, and positive. The control group was 3,08 ± 3,08, i.e. ±1,0736; 2,46 ±0,459; 1,54 ± 0,510; 1,17 ± 0,258. This decreased to 1.83 ±0.258 on day 5.

Table 1. Average number of neutrophils on days 3 and 5

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acemannan</td>
<td>3.08 ± 0.736</td>
<td>1.83 ± 0.258</td>
</tr>
<tr>
<td>Hydrogel25%</td>
<td>2.46 ± 0.459</td>
<td>1.29 ± 0.459</td>
</tr>
<tr>
<td>Acemannan</td>
<td>1.54 ± 0.510</td>
<td>0.50±0.447</td>
</tr>
<tr>
<td>Hydrogel50%</td>
<td>1.17 ± 0.258</td>
<td>0.21 ± 0.292</td>
</tr>
</tbody>
</table>
Normality and Homogenity Test Results

Based on the results of the normality and homogeneity tests are shown (Table 1.2) below, the (Table 1.2) Normality and homogeneity tests data can be said to be homogeneous and not normally distributed, so the statistical tests used in this study are Kruskal-Wallis and Mann-Whitney.

Table 2. Normality and homogeneity tests

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Normality</th>
<th>Homogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acemannan</td>
<td>0.804</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydrogel25%</td>
<td>0.158</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Acemannan</td>
<td>0.393</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>Hydrogel50%</td>
<td>0.566</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acemannan</td>
<td>0.167</td>
<td>0.377</td>
</tr>
<tr>
<td></td>
<td>Hydrogel75%</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Periodontitis Impact of 25%, 50%, 75% Concentrations of Acemannan Hydrogel on the Neutrophil Count in Wistar Rats with Alloxan-Induced Periodontitis

The study's findings regarding the effects of 25%, 50%, and 75% acemannan hydrogel concentrations on the quantity of neutrophils in wistar mice with aloxan-induced periodontitis are displayed in (Table 1.3).

The results of the Kruskal-Wallis statistical test in (Table 1.3) above show a p=0.001 value (p≤0.05) which means that there is a significant difference in the average number of neutrophils in wistar rats with periodontitis. With aloxan periodontitis, wistar rats were treated with 25%, 50% and 75% acemannan hydrogel with positive controls on the third and fifth days. According to the findings, the concentrations of 25%, 50%, and 75% of acemanan hydrogels affected the number of neutrophils in wistar mice (p≤0.05).

Table 3. Kruskal-Wallis test results

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>3rd</th>
<th>5th</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acemannan</td>
<td></td>
<td>0.08±0.736</td>
<td>1.83±0.258</td>
<td></td>
</tr>
<tr>
<td>Hydrogel25%</td>
<td></td>
<td>0.46±0.459</td>
<td>1.29±0.459</td>
<td></td>
</tr>
<tr>
<td>Acemannan</td>
<td></td>
<td>0.54±0.510</td>
<td>0.50±0.447</td>
<td>0.001*</td>
</tr>
<tr>
<td>Hydrogel50%</td>
<td></td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acemannan</td>
<td></td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogel75%</td>
<td></td>
<td>0.54±0.510</td>
<td>0.50±0.447</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td>0.17±0.258</td>
<td>0.21±0.292</td>
<td></td>
</tr>
</tbody>
</table>

Description: *Significant (p≤0.05)

Variations in the Impact of 25%, 50%, 75% Acemannan Hydrogel Counts in Wistar Rats with Periodontitis Induced by Alloxan Between Two Groups

Results of both groups of studies on the influence of 25%, 50% and 75% acemannan hydrogel concentrations on the number of neutrophils found in wistar rats suffering from aloxan-related periodontitis as shown below in (Table 1.4).

The Mann-Whitney test results showed that there were substantial differences in influence between acemannan hydrogel concentrations of 25% and 50% with acemanan hydrogel concentration of 75% as well as positive control group (p≤0.05).

No significant influence was observed between the positive control groups of acemannan hydrogels of concentrations 25%, 50%, and 75% (p>0.05).

Table 1.4 Mann-Whitney test results

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>K is positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25%</td>
<td>0.121</td>
<td>0.008*</td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0.121</td>
<td>0.019*</td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>0.008*</td>
<td>0.019*</td>
<td>0.163</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K is positive</td>
<td>0.003*</td>
<td>0.003*</td>
<td>0.163</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25%</td>
<td>0.042*</td>
<td>0.003*</td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0.042*</td>
<td>0.022*</td>
<td>0.004*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>0.003*</td>
<td>0.022*</td>
<td>0.242</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K is positive</td>
<td>0.003*</td>
<td>0.004*</td>
<td>0.242</td>
<td></td>
</tr>
</tbody>
</table>

Description: *Significant (p≤0.05)

Discussion

Based on the results of the study, the average number of neutrophil cells decreased after the treatment of Wistar mouse periodontitis induced by aloxan with acemannan hydrogel concentrations of 25%, 50%, and 75% for 3 and 5 days. The greatest decrease in the number of aloxan-induced neutrophil cells in periodontal mice was found in the group of acemanan hydrogels at a concentration of 75%. The higher the concentration, the greater the reduction in neutrophy cells. The results of previous research by Susanto dkk (2021) on the influence of aloe vera hydroglass on the count of neutrophiles in aggressive parodontitis caused by Aggregatibacter actinomycetemcomitans, which was studied in vivo in Wistar mice. There's a number of neutrophilic cells found. The highest concentration of Aloe Vera Hydrogel is 20%.

Neutrophilic cells were the first cells to increase within 24 to 72 hours of inflammation (Tamara et al., 2019; Soliman and Barreda, 2023). Tamara et al. (2019) found that an increase in the number of neutrophils on the first day was a form of body defense against pro-
inflammatory pathogens and was a sign that inflammations were occurring. It’s just started.

Neutrophilic cells act by encrypting foreign substances during acute inflammation, and the number increases in the first few days. Neutrophils can increase or decrease inflammation during the inflammatory phase of periodontitis. From the statistical results of the Kruskal-Wallis trial, there was a noticeable difference in the mean neutrophil count in Wistar mouse periodontitis induced by aloxan on day 3 and 5 in all groups. Therefore, the results of the study show that the concentrations of acemannan hydrogel 25%, 50%, and 75% alter the number of neutrophils in Wistar rats with aloxan-induced periodontitis (p = 0.001; p = 0.05).

The results of this study are consistent with previous studies. Susanto dkk (2021) obtained a one-way ANOVA statistical p value of 0.001 which means there is a difference in the number of neutrophils. The mice wistar after being treated with the hydrogel of the crocodile tongue.

Although neutrophils play an important role in the body’s defence system against inflammation, the infiltration of neutrophils in the early stages of inflammations is essential for the cycling of pathogenic microorganisms and the production of proteases. However, remaining neutrophils at the site of inflammation can inhibit healing. (Ellis et al., 2018; Wang, 2018). In this study, acemannan hydrogel made with the active ingredient coconut tongue polysaccharide at a concentration of 25%, 50%, and 75% was shown to affect the number of neutrophils in periodontitis, which was shown by a decrease in neutrophilic cell count after aloxan induction in Wistar rats between three and five days.

An additional statistical test called Mann-Whitney showed that there was no significant difference in the influence on the number of neutrophil cells between the positive control of AloclearTM and the concentration of 75% acemannan. Therefore, based on the results of this test, we can report as follows. Acemannan 75% hydrogel has a balancing effect of AloclearTM in reducing the number of neutrophils in Wistar rats.

Acemannan, is a major bioactive natural polysaccharide obtained from the extracting of the skin and gel of the crocodile tongue. It consists of monomers called manose, glucose, and galactose. Biodegradability and good biocompatibility (Redjeki dkk., 2021; Bai dkk., 2021). This acid can enhance the regulation of cytokines such as TNFα and IL-1 and enhance hematopoiesis in C57 injected mice. In addition, acemannan has a more dominant stimulating activity against leukocytes and lymphatic cellularity in the absolute number of lymphocytes, neutrophils, and monocytes on the scrotum (Liu et al., 2019; Wijaya et al., 2021).

Because of its good biocompatibility nature, Acemannan can also stimulate the formation of tooth tissue by stimulating cell proliferation, differentiation, formation of extra-cellular matrices, and mineralization. (Bai et al., 2023). This is demonstrated by the results of Susanto et al., (2022) research that dig- its potential. In a diabetic mouse model, an in vivo study was carried out on 24 diabetes mice divided into four groups with hydrogel and negative control to try to use acemannan to repair gingiva tissue. Acemannan 25%, 50%, 75%. The results of the study showed that the gingiva collagen density fraction of mice increased with increased concentration.

**Conclusion**

The results of this research indicate that in wistar rats with periodontitis, acemannan hydrogel at concentrations of 25%, 50%, and 75% affects neutrophil distribution.

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**Author Contribution**

Concepts and methods in research by Chandra Susanto; validation by Cindy Denhara Wijaya; remainder of research by Gabriela Lam Ulina Putri Turnip. The published version of the work has been read and approved by all of the writers.

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**Conflicts of interest**

The author states that there is no conflict in this research.

**References**


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