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The Effect of Acemannan Hydrogel on Collagen Expression in Gingival Tissue of Diabetes Mellitus Animal Model

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ABSTRACT

Background: Diabetes mellitus is a comorbid disease that greatly interferes with the process of wound healing, including injuries to the gingival tissue. The *Aloe vera* plant, or *Aloe vera*, is one of Indonesia's natural wealth, which is often found and has been widely used by the community in overcoming various health problems, including to treat various wounds. This study aims to explore the potential of hydrogen acemannan to repair gingival tissue in rats with diabetes mellitus. **Methods:** An in vivo experimental study using 24 Wistar rats grouped into 4 groups (negative control, 25%, 50%, and 75% acemannan hydrogel). Collagen density was assessed histologically semiquantitatively. Data analysis was performed using SPSS using univariate, bivariate, and multivariate methods. **Results:** The administration of acemannan hydrogel showed an increase in the collagen density score along with an increase in the concentration of acemannan hydrogel. Administration of 25%, 50%, and 75% acemannan hydrogel showed significant differences in the collagen density score compared to the group that did not receive acemannan hydrogel treatment, $p < 0.05$. **Conclusion:** Acemannan hydrogel application can increase collagen density in gingival tissue of diabetes mellitus animal model.

1. Introduction

An invasive procedure on gingival tissue is an action that is quite often performed in dental practice. Various studies have shown that invasive procedures on gingival tissue have increased almost 3-fold in the last decade. Any invasive action on the gingival tissue triggers exposure to various microorganisms, both normal and pathogenic flora, to invade open wounds. Invasion of microorganisms in open wounds triggers various inflammatory reactions, which lead to impaired wound healing and physiological disorders of the gingival tissue. Diabetes mellitus is a comorbid disease that greatly interferes with the process of wound healing, including injuries to the gingival tissue. In diabetes mellitus, there is a significant

increase in blood glucose levels. However, the increase in glucose in the blood is not accompanied by the ability of the cells to enter blood sugar into the cells. Cells experience a shortage of glucose for various cell activities, including cells in the gingival tissue. The lack of glucose in the cells in the gingival tissue causes the cells to be unable to optimally secrete various proteins and various signaling molecules, including disturbances in the secretion of collagen protein which plays a role in the repair of gingival tissue after invasive procedures.¹⁻⁶

Efforts to optimize gingival tissue repair after invasive procedures have been widely pursued. There are substances that generally used to help repair

tissue after invasive procedures, such as zinc oxide and eugenol. However, the drawback of these two substances is the presence of allergic effects, which are generally caused by the use of these substances. Of course, this allergic effect is very disturbing and, in some cases, can cause serious clinical conditions in patients. Efforts to explore new therapeutic modalities to optimize gingival tissue healing after invasive procedures still need to be carried out.⁷⁻¹¹

Indonesia is a country with abundant biological wealth. This biodiversity is a great potential for Indonesia to explore various new bio-based therapeutic modalities. The *Aloe vera* plant, or *Aloe vera*, is one of Indonesia's natural wealth, which is often found and has been widely used by the community in overcoming various health problems. *Aloe vera* is widely used by the public for various skin and hair care modalities. *Aloe vera* is also used traditionally to treat various wounds. *Aloe vera* is rich in primary and secondary metabolite compounds with the potential for health. One of the ingredients of *Aloe vera* is acemannan, which is a polysaccharide that is rich in growth factors so that it can trigger a series of wound tissue repair processes.¹² This study aims to explore the potential of hydrogen acemannan to repair gingival tissue in rats with diabetes mellitus.

2. Methods

This study is an in vivo experimental study with a post-test with a control group approach. A total of 24 male Wistar rats (*Rattus norvegicus*), aged 8-10 weeks, with body weight 200-250 grams. Rats were acclimatized for 7 days, then grouped randomly into 4 groups (6 rats/group), namely group 1: negative control (induced diabetes mellitus), groups 2, 3, and 4: each received hydrogel acemannan 25%, 50% and 75% given for 7 days. This study was approved by the research ethics committee of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (No.0423/KEPH-FMIPA/2022).

Aloe vera is cleaned and washed thoroughly. Next, mix *Aloe vera* with 65% hypochlorite. Then, the

mixture was mashed and mixed with 96% ethanol in a ratio of 1:4, stirred until dissolved for 10 minutes at 10°C. After that, leave it at 8°C overnight. The precipitate that has formed is separated from the solution using a filter covered with filter paper. Then the precipitate that has been filtered is put into a vacuum dryer at 50°C. The extract formed was then mixed with calcium chloride to obtain acemannan hydrogel. Wistar rats were then induced with alloxan at a dose of 110 mg/kgBW to develop diabetes mellitus. After the induction of diabetes mellitus was successful, further surgery was performed on the gingival tissue of the rats by first administering anesthesia using biopenthyll at a dose of 0.1 ml/kgBW. After 7 days of treatment, the gingival tissue was evacuated for histological examination to evaluate the density of collagen types III and IV semiquantitatively with a score of 1-3, where the higher the score, the denser the collagen types III and IV.

Data analysis was performed with the help of SPSS version 25 software. Univariate analysis was performed to present the average collagen density score between treatment groups. Bivariate and multivariate analyzes using ANOVA and Post hoc Bonferroni were used to determine differences in mean collagen density scores between treatment groups, $p < 0.05$.

3. Results

Table 1 shows a comparison of collagen density scores between groups. Administration of acemannan hydrogel showed an increase in the collagen density score along with an increase in the concentration of acemannan hydrogel. Administration of 25%, 50%, and 75% acemannan hydrogel showed significant differences in the collagen density score compared to the group that did not receive acemannan hydrogel treatment, $p < 0.05$. Figure 1 shows the histological picture regarding the density of collagen in the gingival tissue. Figure 1 shows that the density of collagen is getting denser with increasing concentration of acemannan hydrogel.

Table 1. Comparison of collagen density scores between groups.

Group	Mean collagen density score \pm SD	p-value
1	1 \pm 0.0	-
2	1.7 \pm 0.2	0.01*
3	2.3 \pm 0.2	0.01*
4	2.7 \pm 0.1	0.01*

*Post hoc Bonferroni, $p < 0, 05$ VS Group 1.

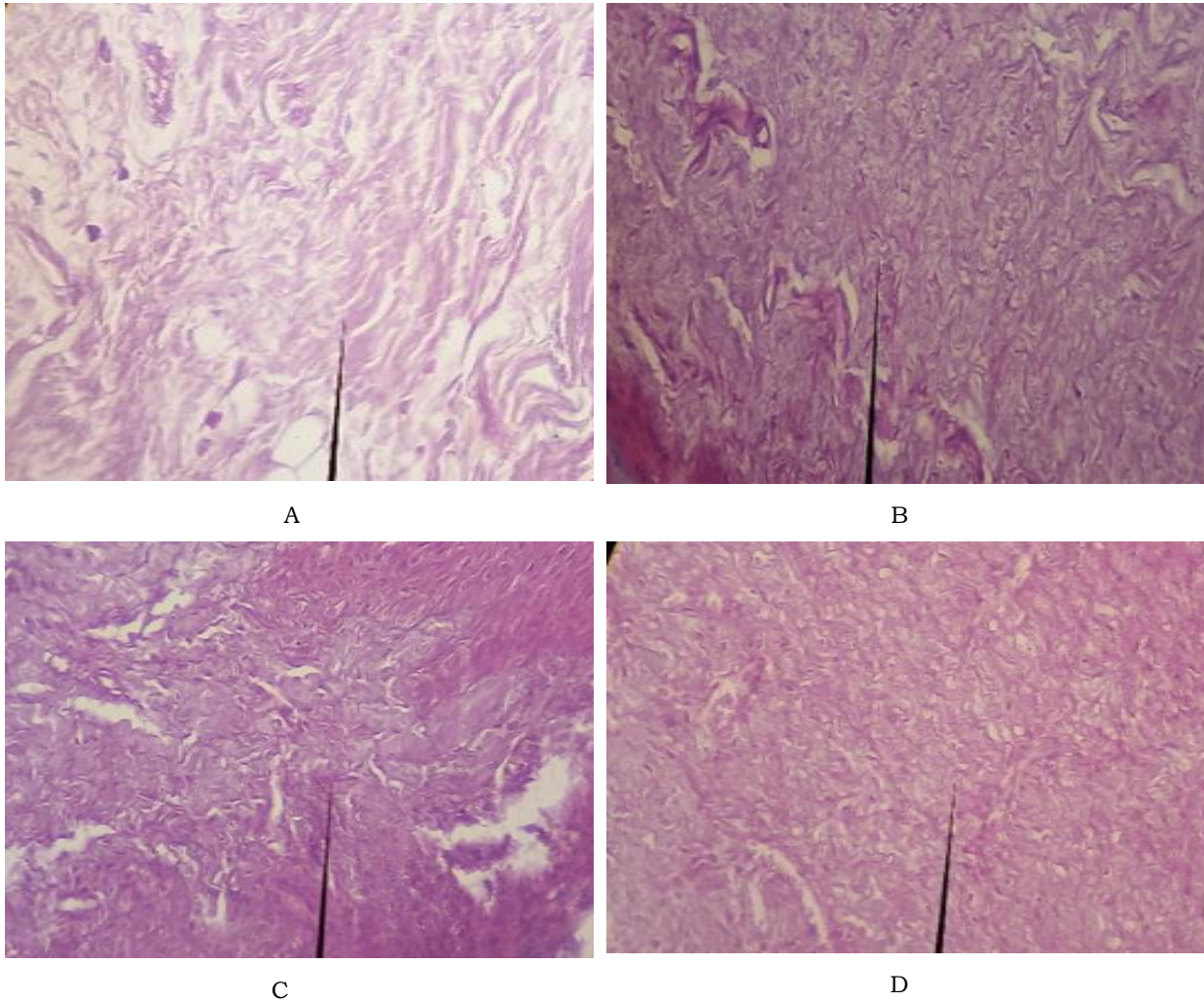


Figure 1. Histological appearance of collagen density. A. Group 1; B. Group 2; C. Group 3; D. Group 4. 400x magnification.

4. Discussion

Acemannan is a high molecular weight polysaccharide isolated from Aloe vera. These polysaccharides can induce favorable immune responses, making them attractive for a variety of biomedical applications. Various studies have shown that acemannan has the ability to initiate TGF-beta 1 cytokine activation. TGF-beta 1 is an anti-

inflammatory cytokine as well as a growth factor that has an important role in the process of tissue repair. Activation of TGF-beta 1 will then trigger the activation of various activator proteins, such as the SMAD protein. The SMAD protein will trigger the activation of the alpha-SMA protein, which plays a role in triggering fibroblast differentiation. Fibroblast differentiation then triggers the formation of mature fibrocytes and

the production of collagen. This study shows that acemannan is able to increase collagen density with increasing doses.¹³⁻¹⁶

The condition diabetes mellitus is a comorbid disease that clinically makes it more difficult for the wound healing process. Various studies show that diabetes mellitus reduces cell performance due to the inability to take up glucose for cellular activity. Glucose is the main source of energy for cells to trigger various cellular activities. The condition of diabetes mellitus will reduce the ability of fibroblast cells to differentiate from fibrocytes. This condition will reduce the ability of fibrocytes to produce collagen.¹⁷⁻²⁰ This study shows that the condition of diabetes mellitus reduces the ability to produce collagen. This study also showed that acemannan was able to significantly increase collagen density with increasing doses.

5. Conclusion

Acemannan hydrogel application can increase collagen density in gingival tissue of diabetes mellitus animal model.

6. References

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