

Combined Effects Of Aloe Vera And Manuka Honey On IL-6 Level In Periodontitis-Induced Rats

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ABSTRACT

Background: Periodontitis is a multifactorial inflammatory condition that progressively damages the tooth-supporting structures, including the alveolar bone and periodontal ligament. This process involves a rise in pro-inflammatory cytokines, such as IL-6, which are essential agents in the inflammation and tissue destruction. Aloe vera (Aloe vera) and manuka honey are recognized for their ability to reduce inflammation and suppress the release of pro-inflammatory signaling molecules, such as IL-6. This study aims to evaluate the effectiveness of the combination of Aloe vera and Manuka honey in reducing IL-6 level in periodontitis rats.

Method: Thirty rats were randomly assigned to four experimental groups: a control group, Aloe vera group, Manuka Honey group, and a group receiving a combination of Aloe vera and Manuka Honey. The Aloe vera and Manuka Honey treatments were administered via a gingival injection at a dose of 150 uL. After five days treatment, the rats were euthanized. An ELISA (Enzyme-Linked Immunosorbent Assay) was conducted on the gingival tissue to measure the concentration of Interleukin-6 (IL-6).

Result: The combination of Manuka honey and Aloe Vera significantly reduced IL-6 levels compared to the control group ($p=0.000$).

Conclusion: The combination of Aloe Vera and manuka honey are effectively and significantly reduced on IL-6 level.

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INTRODUCTION

Periodontitis is a common oral disease that can be found in developed and developing countries. In Indonesia, dental health remains a pressing issue for the government. According to the 2023 Indonesian Health Survey (SKI), more than 40% of Indonesians experience serious problems related to damaged, decayed, or diseased teeth. Approximately 6.8% of the population suffers from loose teeth, one of the causes of which is periodontal disease. Loose teeth indicate problems with the health of the gums and supporting tissues that require immediate medical attention. This demonstrates the high risk of periodontal disease in Indonesia¹.

Periodontal disease is a chronic inflammation of the periodontal tissues caused by certain microorganisms². This disease causes progressive damage to the periodontal ligament and alveolar bone which is indicated by increasing probing depth and recessive inflammation. Periodontal disease arises from a complex interaction between pathogenic subgingival biofilm and the host's immune response³.

Bacterial cell components and proinflammatory cytokines induce PMN responses and activation which can accelerate the production of *reactive oxygen species* (ROS). After activation of this pathway, various pro-inflammatory cytokines can be produced, including *Interleukin-6* (IL-6), a key mediator of the inflammatory response. Conversely, IL-6 can also stimulate ROS production, creating a positive feedback that can worsen the inflammatory response, which may impact the alveolar bone resorption pathway during periodontitis⁴.

Herbal ingredients have been a choice in traditional medicine for treating various human disease. One herbal ingredient widely used in traditional medicine is aloe vera leaves due to their

numerous benefits. Monosaccharide compounds such as mannose-6-phosphate, polysaccharide compounds, acemannan and glucomannan contained in aloe vera have strong anti-inflammatory properties. Therefore, aloe vera can reduce inflammation by stimulating prostaglandin synthesis and increasing leukocyte infiltration⁵.

Honey is an alternative ingredient frequently used by Indonesians due to its benefits. One type of honey frequently used for medicinal purposes is manuka honey, due to its anti-inflammatory properties. Manuka honey contains phenolic and non-phenolic antioxidant compounds which is making it an effective anti-inflammatory⁶. Manuka honey's unique characteristics are related to its high methylglyoxal (MGO) content, which is four times higher than local honey. Furthermore, the high viscosity of manuka honey allows this honey to adhere well to the oral mucosa, helping to create a protective layer that can reduce irritation and aid in wound healing⁷. According to previous research, the anti-inflammatory properties of honey are related to its antioxidant and cytoprotective content which can inhibit the production of peroxides, namely free radicals formed from oxygen and play a role in reducing inflammation⁸.

RESEARCH METHOD

Procedure for Making Aloe Vera Juice

The process of producing aloe vera juice starts by washing the aloe vera stems with clean water to remove any dirt. Afterwards the stems are peeled to extract the inner flesh. The peeled aloe vera flesh is then rinsed again under running water. After that, the flesh is blended at medium speed for about 3 minutes until it becomes a smooth juice. Finally, the aloe vera juice is placed in a cool container and avoid the sun⁹.

Periodontitis Induction

Laboratory rats (*Rattus Novergicus*) underwent for 7 day acclimatization period. Prior to periodontitis induction on day seven, the rats received intramuscular anesthesia via ketamine HCl injection into the hamstring at 0.2 ml per 200 grams body weight. *P. gingivalis* bacteria were inducted into the distobuccal and distolingual gingival sulcus of the mandibular left incisor at a volume of 0.05 ml¹⁰. This bacterial inoculation was repeated every three days over fourteen days using a tuberculin syringe fitted with a 30-gauge needle. The periodontitis model development required 14 days. Successful periodontitis induction was confirmed by clinical indicators including periodontal pocket formation, alveolar bone loss, and inflammatory signs such as gingival swelling and erythema¹¹.

Rat Treatment Procedure

The rats were grouped into four experimental groups. The first group served as the negative control and received distilled water. The second group was administered aloe vera at a dosage of 0.2 cc per gram of body weight. The third group received manuka honey at the same dose of 0.2 cc per gram of body weight. The fourth group was treated with a combination of both aloe vera and manuka honey at 0.2 cc per gram of body weight. The therapeutic agents were administered through gingival injection over a four-day period. After the treatment has been finished, the rats were euthanized by placing them in a sealed chamber with chloroform for 10-30 minutes until the rats died. The rat mandibles were dissected using a scalpel and scissors, and gingival tissue from the region surrounding the anterior teeth was collected. The next procedure involved processing the gingival samples to create tissue homogenate for measuring Interleukin-6 (IL-6) level. The gingival tissue was divided into small fragments and

transferred to microtubes containing suitable buffer solutions, such as phosphate buffer or PBS, to preserve protein integrity. The tissue was then mechanically disrupted using either a homogenizer or mortar and pestle to create a homogenate, facilitating the release of tissue proteins, including IL-6. Following mechanical disruption, the samples underwent centrifugation to remove insoluble materials, allowing the supernatant containing soluble proteins, including IL-6, to be collected for further analysis.

Detection of IL-6

Serum interleukin-6 levels was measured through enzyme-linked immunosorbent assay (ELISA) methodology, utilizing immunoserology testing equipment (Human Interleukin 6 ELISA kit Cat.No E0090Hu and Elisa reader PHO MO Autobio). The testing protocol followed the Bioassay Technology Laboratory's standard operating procedure for the Human Interleukin 6 ELISA kit Cat.No E0090Hu, which features a detection sensitivity of 1.03 ng/L in a 96-well format. The study was carried out at the Laboratory of the Center for Food and Nutrition Studies at Gajah Mada University during December 2024.

RESULT

The results of the ELISA reading provide data on IL-6 levels as follows :

Table 1. Mean and Standard Deviation of IL-6 amount

Number	Group	Mean±Standar Deviasi
1	Aquades	72.18±0.72
2	Aloevera	53.37±0.78
3	Manuka Honey	59±1.21
4	Aloevera + Manuka Honey	50.03±0.76

Based on the table, it can be seen that the average number of IL-6 in the group given distilled water was the highest compared to the other three groups, namely 72.18. The average number of IL-6 in the group given manuka honey was 59, the group given Aloe Vera was 53.37, and the group given the combination of Aloe Vera + Manuka Honey showed the lowest value, namely 50.03.

Picture 1



Periodontitis Rat Treatment Aloe vera+manukaHoney

The next step is conducting normality tests to adjust the test that will be used:

Table 2. Normality Test

Number	Group	Sig
1	Aquades	0.958
2	Aloevera	0.688
3	Manuka Honey	0.175
4	Aloe vera+Manuka Honey	0.945

Because the research data was less than 50, the Shapiro-Wilk test was used to test for normality. The table above shows that all data were normally distributed ($p > 0.05$). The data were then tested for homogeneity using Levene's test.

Table. 3 Homogeneity Test

	Sig
Amount of IL-6	0.157

The significance value in the table is 0.157 ($p > 0.05$), indicating that the data is homogeneous. Because the data is normally distributed and homogeneous, meaning it meets the requirements of the parametric test, then a One-Way ANOVA parametric test was conducted to determine whether there is a significant difference in the amount of IL-6 in each group.

Table.4 One-way Annova Test

<i>One-Way Anova</i>

df	Sig
3	0,000

Based on the table, the significance figure in the One-Way ANOVA test shows a p value = 0.000 ($p < 0.05$), so it can be concluded that there is a significant difference in the amount of IL-6 in the 3 test groups. To determine the differences in each group, a post hoc LSD test was conducted.

Table.5 Post Hoc LSD

Kelompok	Aquades	Aloevera	Madu manuka	Aloevera + Madu manuka
Aquades	-	0.000	0.000	0.000
Aloevera	0.000	-	0.000	0.000
Madu manuka	0.000	0.000	-	0.000

<i>Aloevera</i>	0.000	0.000	0.000	-
+ Madu manuka				

Based on the LSD post hoc test results, there was a significant difference in IL-6 levels across all groups. Comparison of IL-6 levels between groups given distilled water, aloe vera, manuka honey, and the combination of aloe vera and manuka honey showed a significant difference in each group ($p=0.000$).

DISCUSSION

The results of this study indicate that the combination of aloe vera and manuka honey can reduce IL-6 level in rat induced by periodontitis. The results of the calculations obtained showed that the amount of IL-6 in the group given the combination of aloe vera and manuka honey showed a lower amount of IL-6 compared to the group given each ingredient separately and the group given distilled water.

The Previous research report that *aloe vera* can reduce IL-6 expression due to its anti-inflammatory properties. Aloe vera itself contains various active compounds, such as polysaccharides, particularly acemannan, the main compound in aloe vera known to stimulate the immune system and accelerate wound healing¹². In terms of antibacterial, several studies have shown that aloe vera can inhibit the growth of various types of pathogenic bacteria, including *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, which are the main bacteria involved in the development of periodontitis.¹³ In addition, there is another research showed that aloe vera has the ability to inhibit the cyclooxygenase enzyme so that it can reduce the production of Prostaglandin E2 (PGE2) in the inflammatory tissue. Aloe vera tissue has anti-inflammatory properties that can reduce

inflammation by inhibiting the cyclooxygenase (COX) pathway which plays a role in the formation of prostaglandins, the main mediators of inflammation.¹⁴

Bioactive compounds in Aloe vera, such as acemannan, aloin, and flavonoids, can suppress NF- κ B activation, thereby reducing IL-6 expression. In addition, Aloe vera also plays a role in modulating the MAPK (Mitogen-Activated Protein Kinase) pathway, which consists of ERK1/2, JNK, and p38 MAPK, where Aloe vera can inhibit protein phosphorylation in this pathway, thereby suppressing IL-6 production.¹⁵

Manuka honey is known for its high methylglyoxal (MGO) content, which provides powerful antibacterial effects. The MGO in manuka honey can destroy bacterial cell walls and inhibit the growth of various pathogens, including periodontitis-causing bacteria like *Porphyromonas gingivalis*.¹⁶ Manuka honey also can reduce the production of pro-inflammatory cytokines such as IL-6 which plays a role in increasing inflammation and damaging tissue.¹⁶ Manuka honey works by inhibiting the signal transduction pathway that triggers the production of these cytokines and reducing the immune system's reactivity to pathogens in periodontal tissues. Manuka honey has the ability to reduce IL-6 level through several mechanisms, one of which is the role of the Methylglyoxal (MGO) compound contained in it.¹⁷

MGO is known as a potent antimicrobial agent, but it also has anti-inflammatory effects by inhibiting the activation of NF- κ B, a key transcription factor that controls the production of IL-6 and other pro-inflammatory cytokines. MGO's antioxidant effects also help reduce oxidative stress, a key trigger for IL-6 production in inflammatory cells. By reducing levels of IL-6 and other pro-inflammatory cytokines, manuka honey plays a role in reducing inflammation and accelerating the wound healing

process, especially in chronic inflammatory conditions.¹⁸

There was a previous study that compared the effectiveness of Manuka honey with Tualang honey against *P. gingivalis*, where the results were that Manuka honey was more effective detain *P. gingivalis* and had the ability to accelerate wound healing and regeneration of damaged tissue, so it can help repair damage to periodontal tissue that occurs due to infection.¹⁸

CONCLUSION

Combination of Aloe Vera and Manuka Honey showed a significant decrease in IL-6 level compared to the control group, aloe vera group and manuka honey group. These results conclude that the combination of Aloe Vera and Manuka Honey is devastating as an anti-inflammatory agent in reducing IL-6 levels in periodontitis.

The combined treatment of Aloe Vera and Manuka Honey resulted in a significant decrease in IL-6 level when compared to the control group, aloe vera group and manuka honey group. These findings demonstrate that using Aloe Vera and Manuka Honey is highly effective as an anti-inflammatory treatment for decreasing IL-6 levels in periodontitis cases.

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