The Effectiveness of Moringa Leaf Extract (*Moringa Oleifera*)Against *Porphyromonas gingivalis* Bacteria in Periodontitis Cases Through IL-1 Cytokine Analysis

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Abstract

Periodontitis is caused by bacteria that adhere to and grow on the tooth surface. The "red complex" bacteria consisted of Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia. These bacteria will trigger an inflammatory response in the body. Interleukin 1 is an interleukin that acts both as a pro-inflammatory cytokine, Interleukin 1 is a stimulator for MMP production. Treating periodontal disease can be done through non-surgical therapy as well as surgical therapy, to maximize therapy it is accompanied by antimicrobial therapy, but because of the frequent use of antimicrobials causing antimicrobial resistance in patients so that the use of natural ingredients as additional therapy is very necessary in this study using leaves. Moringa as a substitute for antimicrobials.

This study aimed to determine the effectiveness of Moringa leaf (Moringa oleifera) in influencing the anti- inflammatory cytokine IL-1. The first benefit of this research is to provide scientific information in the field of dentistry regarding the effectiveness of Moringa leaves against red complex bacteria Porphyromonas gingivalis as a cause of chronic periodontitis through anti-inflammatory cytokine analysis.

The type of research that will be used is quasi-experimental with a post-test research design with a control group design. This study used a sample consisted of 30 Wistars (Rattus Novergicus) and was divided into 2 groups based on periodontal tissue sampling as follows treatment group which were treated with extracts Moringa and control group with aquadest irrigation after bacterial induction in the gingival sulcus. Blood samples were taken on days 0,1,3,5,7 and centrifuged obtain blood serum and serum cytokine levels (pg/mL) were quantified using a commercial ELISA IL-1 kit.

There is a significant difference in the levels of IL 1 on which day with the p value 0.000 ($p<0,05$). Moringa leaf extract can reduce pro-inflammatory cytokine IL 1 cells, seen after being given treatment using Moringa extract on the day of observation from D0, D1, D3, D5 and D7 in experimental animals wistar rats and induced using Porphyromonas gingivalis bacteria.

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Introduction

Periodontal diseases constitute a group of conditions that are considered nowadays ubiquitous among children, adolescents, and adults. The term "periodontal diseases" includes any inherited or acquired disorders of the tissues that are investing and supporting the teeth (gingiva, cementum, PDL, and alveolar bone)[1,2].

Another researcher defined periodontal diseases as chronic infectious disorders caused primarily by bacteria. Periodontitis is an inflammation of the supporting tissues of the teeth, caused by microorganisms and can cause progressive damage to the periodontal ligament, alveolar bone and accompanied by pocket formation. connective tissue and alveolar bone loss. The clinical picture of periodontitis is a change in color to bright red, accompanied by swelling of the margins.[1] Bleeding on probing and a probing depth of 4 mm is caused by apical migration of the fused epithelium.[1,2] There is loss of alveolar bone and tooth mobility[1].

Periodontitis is a multifactorial disease. Many studies have proven that the occurrence of periodontitis involves the presence of dental plaque, individuals who are genetically susceptible to periodontitis and the presence of one or more risk factors such as stress or depression that can alter immune responses and behaviors related to dental health including oral hygiene[2,3,4]. Risks is the chance of developing a specific disease in an individual over a period of time. Risk factors are environmental, behavioral and biological factors that have a certain causation with the disease process and can increase the chance of a disease occurring[3,4].

Periodontal tissue damage is mainly caused by the interaction of bacterial antigens and inflammatory cells resulting in the production of cytokines. IL-1 is secreted by macrophages in response to inflammation and is involved in leukocyte recruitment and apoptosis and T cell activation[5] IL-1 is a pleitropic cytokine that acts as an anti-inflammatory as well as pro-inflammatory[6]. IL-1 exhibits anti-inflammatory properties through increased production of tissue inhibitor matrix metalloproteinase (TIMP) and suppression of the proinflammatory cytokines IL-1β and TNF- IL-1 and its receptors induce bone resorption by increasing the nuclear factor K ligand (RANKL) receptor activator or by directly inducing osteoclast formation [7]. In the study of Kou et al, Mengel et al and Buhlin et al reported that periodontitis has been associated with increased circulating IL-1 levels. This increase appears to be related to the severity of the disease. However, the study of Teles et al showed lower salivary IL-1 levels but there was no significant difference in patients with chronic periodontitis compared with healthy subjects[8].

Porphyromonas gingivalis is a gram-negative anaerobic bacteria which in normal numbers is a normal microflora that can be found in the oral cavity. *Porphyromonas gingivalis* is often associated with chronic periodontitis in adults[9]. This bacterium produces a number of unique virulence factors that can be genetically manipulated[10,11]. The presence of genomic sequences can help understand the biological nature of *Porphyromonas gingivalis* and how it interacts with the environment, other bacteria, and the host[12]. The ability of *Porphyromonas gingivalis* to cause periodontitis is determined by its arsenal of virulence factors. Biofilm formation and bacterial dipeptidyl peptidase IV (DPPIV) contribute to the pathogenic potential of *Porphyromonas gingivalis*[13,14,15]. Furthermore, biofilm formation can increase the virulence of *Porphyromonas gingivalis* by increasing DPPIV activity[15,16]. *Porphyromonas gingivalis* is closely associated with chronic periodontitis. Its presence in periodontal tissues depends on its ability to evade host immunity without inhibiting the overall inflammatory response[17,18].

These bacteria can be reduced by using an appropriate mouthwash, one of the mouthwashes that can be used is chlorhexidine digluconate 0.2%[19,20]. However, the use of chlorhexidine as an antiseptic turns out to have side effects if used continuously. Side effects that occur are the presence of staining on the teeth, sensation and an unpleasant taste.[21] Another alternative is needed as a mouthwash raw material with minimal side effects. Alternatives that qualify as antiplaque and antibacterial are herbal ingredients. One of the plants in herbal medicine is Moringa leaves[22].

Moringa leaves (*Moringa oleifera*) is a native plant of Indonesia that can be used as medicine, and as an antioxidant[23]. Moringa plants grow in the lowlands and highlands [24]. Moringa is known to contain more than 90 types of nutrients in the form of essential vitamins, minerals, amino acids, anti-aging and anti-inflammatory[25]. Data regarding the content of active compounds in Moringa leaves are still very rare, some literature states that Moringa leaves contain flavonoids, saponins, alkaloids, tannins, and phenols. Moringa leaves are very rich in nutrients, including calcium, iron, protein, vitamin A, vitamin B and vitamin C[26]. Moringa leaves contain higher iron than other vegetables, which is 17.2 mg/100 g. In addition, Moringa leaves also contain a variety of amino acids, including amino acids in

the form of aspartic acid, glutamic acid, alanine, valine, leucine, isoleucine, histidine, lysine, arginine, venylalanine, tryptophan, cysteine and methionine[14].

The use of Moringa plants in dentistry is also widely found and researched for its development[27,28]. Moringa plants can be used as toothpaste, mouthwash, root canal irrigation, wound healing after tooth extraction, medicine for gingivitis, canker sores, and can be used to prevent dental caries with its antibacterial properties. Recent research on the effect of Moringa leaves (*Moringa oleifera*) in accelerating the reduction of signs of inflammation of erythema in sterile wounds of guinea pigs (*Cavia porcellus*) stated that the results of the analysis of the ability of Moringa leaves were proven to accelerate the wound healing process in this case what was observed was a decrease in signs of inflammation erythema[29,30].

Periodontal disease has an inflammatory process that involves both natural and adaptive immune responses[30]. The natural immune system is the initial protective immune system to fight infection or inflammation[31]. The natural immune system also functions to activate adaptive immune cells. Natural immune cells are phagocytic cells such as polymorphonuclear neutrophils, monocytes, and macrophages that trigger the release of chemical mediators such as the cytokine tumor necrosis factor (TNF-α), Interleukin (1β)[16,32]. While adaptive immune cells such as T and B lymphocyte cells. Interleukin 1β is produced in response to microorganisms, bacterial toxins, complement components or tissue injury. IL-1β has an important role, one of which is inducing other inflammatory cytokines, stimulating fibroblasts to produce collagenase enzymes, inducing bone demineralization processes in stimulating bone resorption, especially in changing the connective tissue matrix. IL-1β levels were increased in the crevicular fluid of periodontitis patients compared to healthy subjects or had mild gingivtis[12,33].

The basis of this research is the ability of Moringa leaves (*Moringa oleifera*) as a plant herbs that have a variety of active substances such as flavonoids, saponins, alkaloids, tannins, and phenols, so that they have the potential to be agents for treating periodontitis[20,32]. However, until now there has been no research on the effect of the active substance *Moringa oleifera* on periodontitis in children through anti-inflammatory cytokine analysis. Therefore, researchers are interested in conducting research on this[17,33].The effectiveness of Moringa leaf (*Moringa oleifera*) on *Porphyromonas gingivalis* bacteria as a cause of periodontitis through analysis anti-inflammatory cytokines.

Materials and methods

Place and time of research at Toxicology Pharmacology Laboratory Faculty Of Pharmaceutical, Hasanuddin University, Makassar from March – May 2021, Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University from March – July 2021 Molecular Biology And Immunology Laboratory, Ministry Of Microbiology Hasanuddin University from July-August 2021, with research sample is Wistar rats induced by *Porphyromonas gingivalis.*

Procedures and stages of work : a) Wistar rat experimental animal maintenance b) Making Moringa (*Moringa oleifera*) leaf extract c) Induction of periodontitis in wistar rats with Preparing bacterial colonies, anesthesia and induction of periodontitis and observation d) blood sampling e) Blood serum sampling is then prepared for testing with Elisa's method f) The was then observed to see the expression of inflammatory cytokines cytokine expression interleukin 1β inflammation.

Data analysis using SPSS ver 22.0 with ANOVA test and presented in tabular form. Research Material is Moringa leaves (*Moringa oleifera*) come from the moringa cultivation garden in the Blora area of Central Java, Indonesia. Moringa leaves are then washed and dried in an oven to produce dry leaves. The dried moringa leaves are then ground and processed by maceration technique to produce a thick moringa extract in the form of a gel.

Animals and Research Design: This protocol has been approved by the Health Research Ethics Committee of the Dental and Oral Hospital, Faculty of Dentistry, Hasanuddin University, Ministry of Research, Technology and Higher Education, Indonesia (No. 0093/PL.09/KEPK FKG-RSGM UNHAS/2021). The male Wistar was previously acclimatized (adapted) for about 1 week in the animal cage to get used to the rats to their new environment and observed general conditions such as weighing and animal health. Wistar rats were placed in

cages in groups, light/dark cycle for 12 hours, temperature 26-29 C, humidity 60- 70%, and were given standard feed and drinking water ad libitum. The cage is in the form of a plastic box with a wire cover measuring 40 cm x 60 cm x 25 cm, covered with rice and cleaned regularly every three times a week to keep the cage dry and healthy. Research sampling was carried out using simple random sampling after meeting the inclusion and exclusion criteria.

The sample consisted of 30 wistars and divided into 2 groups based on periodontal tissue sampling as follows: • The control group consisted of 15 wistars with aquadest irrigation after induction of *Phorpyromonas gingivalis* bacteria in the gingival sulcus • The treatment group consisted of 15 wistars which were administered application of Moringa Extract and aquadest irrigation after induction of *Phorpyromonas gingivalis* bacteria in the gingival sulcus. Blood samples were taken on days 0,1,3,5,7 and centrifuged at 5000 *g* for 10 minutes at 4℃ to obtain blood serum. Serum cytokine levels (pg/mL) were quantified using a commercial ELISA IL-1 kit following the manufacturer's instructions (Elabscience).

Results

Before conducting statistical tests, conducted tests of normality and homogeneity first, the homogeneity using Levene test, test results obtained showed p> 0.05, which means the data were normally distributed and homogeneous, so that then do parametric statistical tests. IL-1 levels in each group were measured for 7 days of observation and the values in each group are presented in Figures 1 and 2. The average value of IL-1 levels in both groups was found to be the highest on the third day of observation and the concentration of IL-1 began to decrease on the fifth (D5) to the seventh (D7) day of observation after induction of periodontal tissue with *Porphyromonas gingivalis* bacteria. The highest mean ± SD value of IL-1 levels was observed in the control group on the third day of observation and the lowest IL-1 level of mean ± SD was observed in the treatment group on the seventh day of observation.

The observations on Day 0 the average IL 1 in the Treatment Group was 10.74, Day 1 = 11.44, Day 3 = 10.16 and day 5 = 9.15 and Day 7 = 7.89 . It can be seen that all treatment groups have a significance value of p<0.05, which means that there are significant differences in each treatment group. On Day 0 the value was 11.00 and increased on day 1 with a value of 12.05 and continued to increase on day 3 until it reached 14.05 after that a decrease in IL 1 began in the control group on day 5 with a value of 12.02 and will there was a decrease in IL 1 again on day 7 with a value of 10.05. Observations on Day 0 until Day 7 in the group using 2% Moringa leaf extract, showed a significance value of p<0.05, which means that there was a significant difference in the decrease in IL 1 starting from Days 1,3 5 and 7. In the control group without treatment, Moringa extract decreased only after the 3rd day of observation.

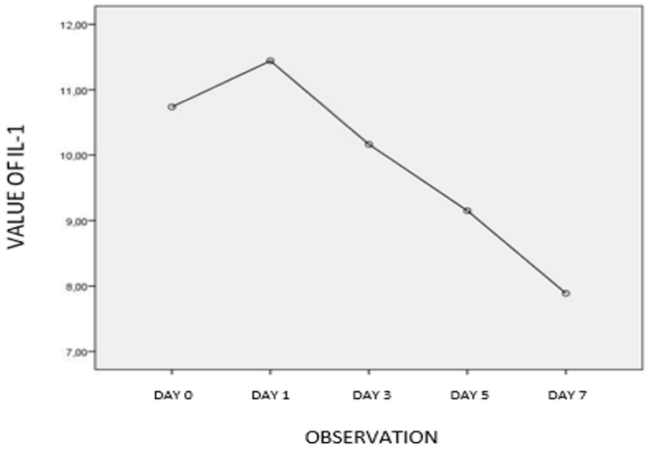

Figure 1. The average value of IL-1 levels in the Treatment Group on observations D0, D1, D3, D5 and D7.

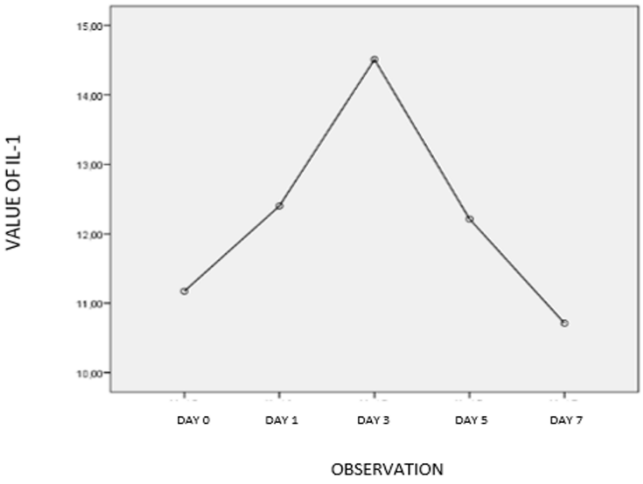

Figure 2. The average value of IL-1 levels in the control group on observations D0, D1, D3, D5 and D7.

On the day of Observation Day 0 the value of IL 1 in the treatment group was at a value of 10.05 and increased on Day 1 after that it decreased on day 3 and continued to decrease on day 5 until the observation on day 7.

The difference between each group between the control group and the treatment group IL 1 where in the control group D0 was at 10.05 while the Treatment group was at 10.07. On Day 1 in the control group were in the value of 11.07, while the treatment group at a value of 12.03 and decreased in the control group on the value of 10.01 on Day 3, while the treatment group actually increased at their highest value of 14.03 and on Day 5 in the control and treatment groups decreased until the 7th day of observation.

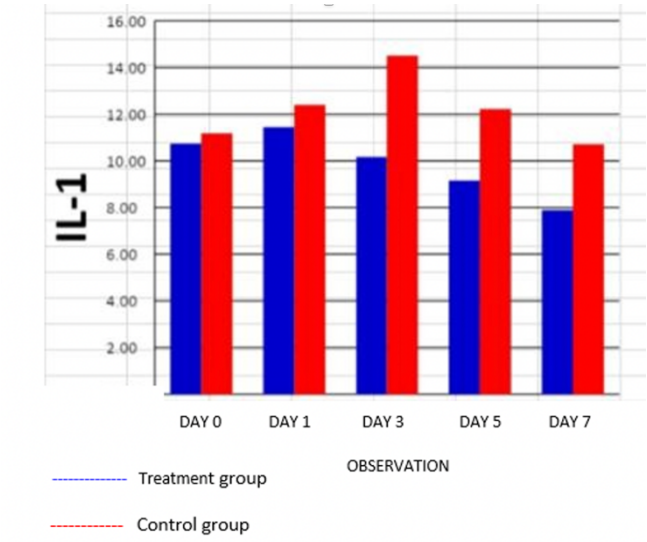

Figure 3. The different between control group and treatment group IL 1 on observation D0, D1, D3, D5, D7.

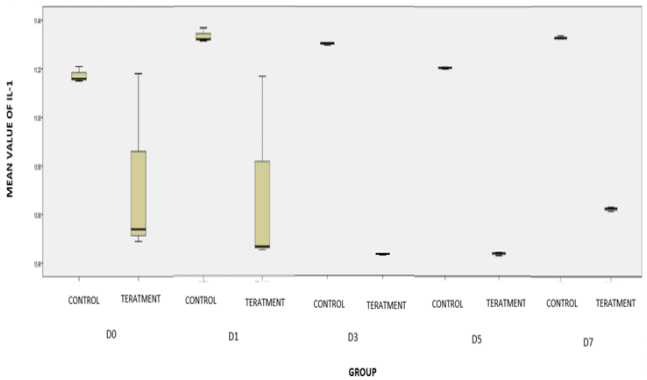

Figure 4. The Mean value and standard deviation of IL-1 levels in the treatment and control groups.

Discussion

In this study, the comparison factor of IL 1 expression from Day 0 until Day 7 there was a significant difference on the day of observation as well as the control. The P value of IL-1 Day 0 of the treatment group and the control group was significant, namely 0.023 and the homogeneity value was used to determine the homogeneity of the data using the level test and determine each group using the T test, the IL value of 1 on Day 5 and day 7. The treatment group for IL1 expression levels from H0 - H7 whether there is a significant difference[12]. The ANOVA test compared H0 between the treatment and control groups. The value of IL 1 between the control and treatment groups was 1.95 not significant on day 0 between the 2 treatment and control groups. On Day 1 there was also no significant difference. On Day 3 observations already showed a significant difference[13].

From the results, it was found that IL 1 was pro inflammatory. Look for the theory of IL 1 briefly. In 2 groups there was a decrease in IL 1 levels but the decrease was faster in the treatment of Moringa extract. Look for anti-inflammatory effects on Moringa look for other studies though not IL 1. In addition, the induction of anti-inflammatory mediators by phytochemicals is important to avoid triggering inflammation by cells. Activation of the MAP-kinase group will also activate NFkB signaling which will trigger inflammation. Therefore, plant components such as Moringa leaves can be used to reduce inflammation because they have the ability to inhibit mediators and signals. Previous studies have stated that *Moringa Oleifera* leaf extract can provide anti-inflammatory effects in male albino rats against carrageenan caused by pawoedema[15,17,18].

Moringa leaves contain several chemical compounds in the form of several bioactive compounds, one of which is flavonoids. Flavonoids are polyphenolic compounds produced from secondary metabolism in plants. The main flavonoids from Moringa leaves are myresitin, quercetin and kaempferol. Bioactive compounds involved in the anti-inflammatory properties of Moringa leaves, such as quercetin, can inhibit NFkB activation, and release the chain of inflammatory processes[19].

A number of inflammatory mediators including proinflammatory cytokines,

chemokines, free radicals, and certain enzymes are involved in the inflammatory process mediated by activated immune cells such as monocytes and macrophages. Macrophages play an important role in initiation and modulation host defense mechanism through the production of proinflammatory mediators (such as nitric oxide, cytokines, and prostaglandin E2 (PGE2)[25,28].

Induction of anti-inflammatory mediators by *phytochemicals is* important to avoid triggering inflammation by cells because inflammation can lead to carcinogenesis through the activation of inflammatory mediators such as prostaglandins, cytokines, chemokines, and nitric oxide[30]. Moringa contains flavonoid compounds, especially kaempferol and quercetin which can inhibit prostaglandin synthesis, especially PGE-2 which reduces macrophage infiltration. The decrease in macrophage cells will then be followed by a decrease in inflammatory mediators, such as histamine, serotonin, and the three proinflammatory cytokines (TNF a, IL-1, IL-6)[5,33].

Conclusions

The conclusions of this study are: Moringa leaf extract (*Moringa oleifera*) can reduce proinflammatory cytokine IL 1 cells, seen after being given treatment using Moringa extract on the day of observation from D0, D1, D3, D5 and D7 in experimental animals wistar rats and induced using *Phorpyromonas gingivalis* bacteria.

Declaration of Interest

The authors report no conflict of interest.

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