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Ethanolic Extract of Moringa oleifera Leaves Influences NF-κB Signaling Pathway for periodontal tissue regeneration in rats

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ABSTRACT

Periodontitis is a chronic inflammatory disease that causes damage to the supporting structures of the teeth, and if left untreated this disease will lead to impaired function, appearance, pain, and loss of teeth. Periodontitis is caused by bacteria adhering to and growing on the tooth surface and will trigger an inflammatory response in the body. NF-κB has a very important role in the immune system. NF-κB can control transcription, cytokines, antimicrobial effectors, and genes that regulate cell differentiation, cell survival, and proliferation so that they can regulate various aspects of innate and adaptive immune responses. Therefore, down-regulating the NF-κB signaling pathway is one way to reduce chronic inflammation. Moringa leaf extract as a natural ingredient for anti-inflammatory adjunctive therapy can be an alternative in the treatment of periodontitis.

Objective. This study aims to determine the effectiveness of Moringa oleifera leaves in influencing NF-κB expression in the pathway of inflammatory pathways.

Methods. Experimental laboratory research and clinical trials with posttest-only control group design. Twenty- four Wistar rats were divided into two groups. Then the periodontitis was made with an injection of Porphyromonas gingivalis bacteria on the mandible, the control group was given aquades and the treatment group was given a Moringa oleifera gel extract. Wistar rats were sacrificed on days 0,7, 14, and 21, and the mandible bone was then taken for immunohistochemical analysis to determine the levels of NF-κB.

Result. On days 7, 14, and 21, the expression levels of NF-κB were significantly different between the two groups.The group added with Moringa oleifera leaf extract showed a faster decrease in NF-κB expression than the control group.

Conclusion. Moringa oilefera extract can inhibit NF-κB expression in inflammatory pathways.

Keywords: *ALP, Hypercolestrolemia ,Antioxadinat*

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INTRODUCTION

Dental and oral health is one of the health problems in society that requires comprehensive treatment because of its broad impact [1,2]. Based on the 2018 Basic Health Research, Indonesia's dental and oral health problems show a fairly high prevalence of 57.6% [2]. Oral disease is the most common is periodontal disease[3]. According to WHO, periodontal disease is the second largest prevalence after dental caries, reaching a prevalence of 96.58% in all age groups including children and adolescents. Based on 2018 RISKESDAS data, the prevalence of periodontitis in Indonesia is around 74.1% [2].

Periodontitis is inflammation that affects the supporting tissues of the teeth, is caused by microorganisms, and can cause progressive damage to the periodontal ligament, and alveolar bone and is accompanied by pocket formation [4,5]. Periodontitis causes permanent tissue destruction, characterized by chronic inflammation, the epithelium's coalescing apical migration, loss of connective tissue, and loss of alveolar bone[1,3]. The clinical picture of periodontitis is a change in color to bright red, accompanied by swelling of the gingival margin. Bleeding on probing and a probing depth of ≥ 4 mm is due to apical migration of the unifying epithelium. There is a loss of alveolar bone and loose teeth[4-6].

The main cause of periodontal disease is the presence of microorganisms that colon the dental plaque[3,4]. Dental plaque is a structured, soft, yellow substance that adheres to the tooth surface[4]. The contents of dental plaque are various types of microorganisms, especially bacteria, the rest are fungi, protozoa, and viruses[7,8]. Plaque staining these pathogenic microorganisms plays an important role in causing and exacerbating periodontal infection[3,5]. An increase in the number of gram-negative organisms in the subgingival plaque such as Porphiromonas gingivalis, Actinobacillus actinomycetemcomitans, Tannerela forsythia, and Treponema denticola initiates periodontal infection[5-7].

Approximately 40-90% of periodontitis is caused by the opportunistic bacterium Porphyromonas gingivalis is an anaerobic gram-negative bacterium that is the main cause of periodontitis[5,8]. In a person with poor oral hygiene, Porphyromonas gingivalis bacteria in the gingival sulcus has the potential to damage epithelial tissue so periodontitis causes loss of attachment to the periodontal tissue[4,8]. Porphyromonas gingivalis produces LPS and activates macrophages and neutrophils, activating Nuclear Factor Kappa Beta (NF-κB) is a transcription factor that plays an important role in inducing the regulation of various genes in the inflammatory response and cell proliferation[3,6].

NF-κB contributes to the activation of a wide variety of genes, such as proinflammatory cytokines, TNF-α, IL-1, and chemokines[6,9]. NF-κB consists of a heterodimer between rel polypeptides and protein p50, which acts to control the expression of many adaptive genes, such as MHC proteins and genes important for the regulation of the apoptotic process [9,10]. NF-κB resides in the cytoplasm in an inactive form along with a regulatory protein I-κB. The interaction between LPS/TLR activates the transcription NF-κB which plays a role in activating the transcription of inflammatory mediators [3,9,11]. NF-κB is activated by LPS in THP-1 cells, the transcription factor NF-kB is associated with metabolic and inflammatory responses, including nuclear receptors, activators of protein (AP-1), and early growth response (EGR). Furthermore, it is suggested that IKK/NF-kB is a target for the treatment of periodontitis disorders [10-12].

Moringa oleifera or in Indonesian it is called Moringa leaf is a member of the Moringaceae tribe [12]. This plant has a high nutritional value, such as having more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yogurt, 15 times more potassium than bananas, and 25 times more iron than spinach [9,14,15]. All parts of this plant have biological benefits such as reducing hyperglycemia, anti-inflammatory, anti-diabetic, anti-cancer, anti-microbial, and antioxidant. Antioxidants contained in Moringa leaves include tannins, steroids, triterpenoids, flavonoids, saponins, interquinones, and alkaloids [14,16]. Flavonoid compounds, polyphenols, saponins, and tannins are active compounds and Moringa leaves that have antibacterial properties. Of the several properties that have been found, Moringa leaves have an important role in the inflammatory process [15-17]].

Given that inflammation is a major factor in the pathogenesis of periodontitis, prevention and treatment can begin by inhibiting the activation of an important protein that causes the inflammatory process, namely NF-κB as the target [14,17].

Moringa leaves can inhibit the production of cytokines by macrophages (Tumor Necrosis Factor-alpha (TNF-α), Interleukin-6 (IL-6), and IL-8)), caused by Lipopolysaccharide (LPS) [18,19]. Other studies have reported that concentrations of moringa leaves can reduce gene expression and production of inflammatory markers in macrophages [10,18,19]. Moringa leaf extract can stimulate cellular and humoral immune responses by increasing white blood cells, neutrophils, and serum immunoglobulins [18,19]. Quercetin, which is the flavanoid part of Moringa leaves, can be involved in reducing the inflammatory process by inhibiting the action of NF-κB [17,19]. Quercetin is also reported to stimulate osteoblasts and increase bone formation. The ethyl acetate fraction of Moringa oleifera has anti-inflammatory potential in regulating the NF-κB signaling pathway in macrophages stimulated by lipopolysaccharide [17,20]. The previous study found that bioactive compounds in MO leaf extract reduced the production of pro-inflammatory cytokines, including TNF, IL-6, IL-8, and COX-2 of LPS-induced human monocyte-derived macrophages (MDMs) via inactivation of NF-κB, blocking both IκB-α degradation and nuclear translocation of NF-κB (p65) [21,22].

Based on several studies, Moringa oleifera can reduce the degree of inflammation in the gingival junction epithelium due to the invasion of Porphyromonas gingivalis bacteria by increasing NF-κB levels so that inflammatory mediators produced by NF-κB which cause periodontitis can be reduced [2,23]. The purpose of this research is to measure expression NF-κB which physiologically plays a role in the immune system and processes inflammation on the gingival junction epithelium exposed to porphyromonas gingivalis bacteria

MATERIAL AND METHODS

This protocol was approved by the Health Research Ethics Committee for Dental and Oral Hospital, Faculty of Dentistry, Hasanuddin University, Ministry of Research, Technology and Higher Education, Indonesia (No.0104/PL.09/KEPK FKG-RSGM UNHAS /2022). This type of research is actual experimental laboratory research. The research subjects were divided into three major groups: the group that was given a Moringa oleifera extract and the control group that was given aquades. Each group consisted of 12 Wistar rats. Maintenance of experimental animals of Wistar rats, preparation of Moringa leaf extract (Moringa oleifera) and bacterial colony, anesthetized with ketamine, and induction of periodontitis. Mice were sacrificed and performed on days 7, 14, and 21 of each of the three rats. The samples were collected by cutting the jawbone and placed in a non-EDTA vacutainer tube. The tissue samples taken were then prepared for testing with the histochemical method. The samples were then observed to see NF-κB expression.

RESULT

After getting the results of the Moringa oleifera leaf extract in the form of moringa cream, followed by the FTIR test. Examination of the content of this research material so that it is known for its natural content that is still awake. Figure 1 shows the FTIR spectrum of moringa extract from leaves in the 400–4000 cm−1 area; the absorption band at wave number 3383.14 cm−1 shows the OH and NH functional groups.

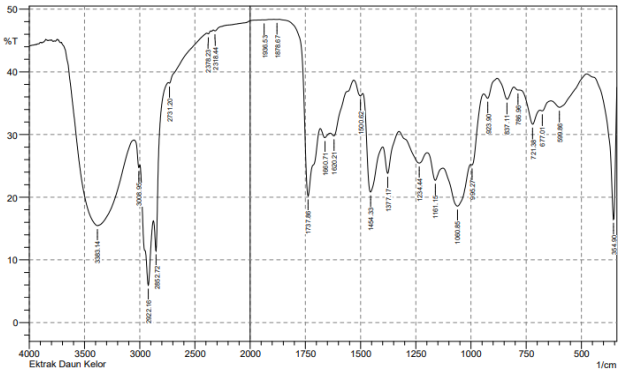

FIGURE 1. FTIR spectrum of flavonoid Moringa oleifera extract

The research data were analyzed descriptively to describe the distribution of the increase in data to clarify the results'presentation. First, the data obtained were tested for normality using the Kolmogorov–Smirnov Test, then the data was then tested for homogeneity using the Levene's Test. Finally, one-way ANOVA was used to analyze the differences between the research groups. The results of the analysis are declared significant, or there is a difference the p-value < 0.05

TABLE 1. Descriptive statistics showing results of NFkB expressions

Group	Sample	Day-0 (mean ± SD)	Day-7 (mean ± SD)	Day-14 (mean ± SD)	Day-21 (mean ± SD)
Control	12	3.00 ± 1.000	13.67 ± 1.528	10.67 ± 2.082	7.00 ± 2.000
Treatment	12	2,00 ± 1.100	4.33 ± 1.528	2.67 ± 2.155	2.00 ± 1.000
P		0,288	0.002*	0.004*	0.018*

*significant using the independent sample t-test (p<0,05)

On day 0, the average NF-kB in the control group was 3.00 ± 1.000; in the treatment group is 2.00 ± 1.100; The results of statistical tests using the independent sample t-test showed that there was no significant difference in the NF-kB values between the control and treatment groups, with a p>0.05. On the 7 days, the average NF-kB in the control group was 13.67 ± 1.528; in the treatment group the NF-kB value decreased, namely 4.33 ± 1.528; The results of statistical tests using the independent sample t-test showed that there was a significant difference in the NF-kB values between the control group and the treatment group, with a p<0.05. On the 14 days, the average NF-kB in the control group was 10.67 ± 2.082; in the treatment group the NF-kB value decreased by 2.67 ± 2.155; The results of statistical tests using the independent sample t-test showed that there was a significant difference in the NF-kB values between the control group and the treatment group, with a p<0.05. On day 21, the average NF-kB in the control group was 7.00 ± 2.000; in the treatment group the NF-kB value decreased by 2.00 ± 1.000; The results of statistical tests using the independent sample t-test showed that there was a significant difference in the NF-kB values between the control group and the treatment group, with a p<0.05.

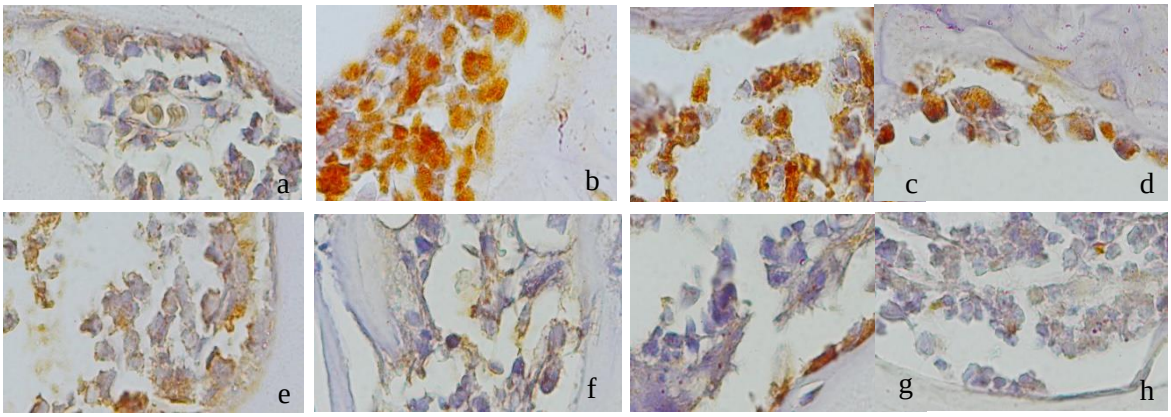

FIGURE 2. The distribution of NFkB was observed using immunohistochemical techniques, with specific anti-NFkB antibodies, counting the number of bone tissue osteoclast cells, which display NFkB (brown color in cytoplasmic cells). Expression of NFkB with 1000 times magnification, (a) expression of NFkB on the baseline in the control group (aquades), (b) expression of NFkB on the 7th day in the control group, (c) expression of NFkB on the 14th day in the control group, (d) expression of NFkB on the 21th day in the control group, (e) expression of NFkB on the baseline in the moringa group, (f) expression of NFkB on the 7th day in the moringa group, (g) expression of NFkB on the 14th day in the moringa group, (h) expression of NFkB on the 21th day in the moringa group

DISCUSSION

Moringa leaves contain several chemical compounds in various forms several bioactive compounds, namely vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, oxalates [24,25]. The main flavonoids found in *MO* leaves are myrecytin, quercetin and kaempferol, in concentrations of 5.8, 0.207 and 7.57 mg/g, respectively. Quercetin is found in dried *MO* leaves, at concentrations of 100 mg/100 g, as quercetin-3-*O*-β-d-glucoside (iso-quercetin or isotrifolin) [26,27]. The content of flavonoids is known to have the effect of capturing free radicals by inhibiting lipid oxidation and antibacterial activity [21,28]. Flavanoids also have antioxidants, anti-inflammatory, anti-allergic, antiplatelet, and anti-tumor activity. Flavonoids also inhibit the action of collagenase bacteria. The main flavonoids from Moringa leaves are myricetin, quercetin, and kaempferol [29]. Bioactive compounds involved in the anti-inflammatory properties of Moringa leaves, such as quercetin, can inhibit NF-κB activation, which is a crucial step to release the chain of inflammatory processes [3,30]. In rat studies, quercetin regulates the expression of iNOS, IFN-g, and C-reactive protein and reduces the release of TNF-a and IL-6 [26].

Flavonoids are known to have a mechanism similar to NSAIDs. In addition, flavonoids inhibit the activity of proinflammatory gene expression mediators other than COX [28]. Flavanoids can up and down-regulate transcription factors in inflammatory and antioxidant pathways, such as NF-κB and Nrf-2 [21,31]. In the activated NF-κB pathway, especially the p50 and p65 heterodimers, enter the nucleus and bind with NF-κB-responsive elements to regulate the expression of the genes involved in the regulation of immune and inflammatory responses, cell proliferation, tumorigenesis, and antiapoptotic [31,33]. This research was conducted to find an alternative solution for healing chronic periodontitis. The data from previous research indicate that oral hygiene is the main cause of pathogen bacteria accumulation [28]. This accumulation of bacteria causes periodontal tissue damage characterized by loss of attachment and migration of gingival junctional epithelium to apical [22,28,34].

The results of the analysis in Table 1 and Figure 3 show that between the control group and NF-κB expression test in each group on days 7, 14, and 21 showed a significant decrease ($p < 0.05$) in NFkB expression from day 7 to day 21.

The distribution of NF-κB p65 was observed using immunohistochemical techniques, with specific anti-NF-κB antibodies (santarcruz biotech) in Figure 2 using immunohistochemical results calculation techniques modified for bone tissue, this study has finished counting the number of bone tissue osteoclast cells, which express NF-κB (brown color in the cell cytoplasm. In the control group it shows that most of p65 is present in the nucleus cells (active). Significantly different from the treatment group that was given Moringa leaf extract, there was a decrease in the amount of p65 in the cell nucleus, on the contrary it was found in the cytoplasm. These data indicate that Moringa leaves can inhibit p65 transfer (NF-κB activation).

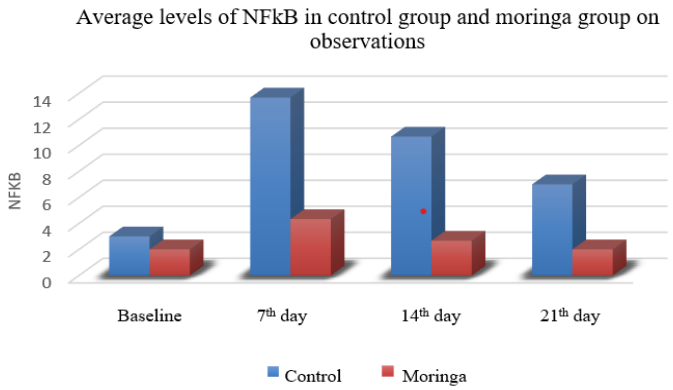

FIGURE 3. Bar chart of NFkB expression between groups

Nuclear Factor-κB is a transcription factor and essential for the detection of activation of the innate immune response because their activation is associated with the TLR2/TLR4 receptors in the innate immune system [13]. In periodontitis bacteria that play a role are the P. gingivalis and A. actinomycetemcomitan in which LPS activates germ cells using the TLR2 pathway and/or TLR4 [5,11]. The interaction between LPS/TLR will activate NF-κB which plays a role in activating inflammatory mediators' transcription [13,32,35].

Moringa oleifera extract is rich in flavonoids, saponins, alkaloids, and tannins. Flavonoids contained in Moringa oleifera extract can help osteoblast differentiation, leading to bone formation [22,30,36]. Meanwhile, saponin affects osteogenic activity that promotes the proliferation and differentiation of osteoblasts [34,35]. Flavonoids have an important role in inhibiting the biological activity of another protein, such as Akt and NF-κB [30,32]. The antioxidant content of flavonoids have the ability to modulate cellular transmissions of important processes such as cell growth, cell differentiation and actiovation of NF-κB [24,34]. Different types of flavonoids can disrupt the flow of signal transduction that regulates cell growth, cell cycle and apoptosis [37].

According to research conducted by Guo et al. (2017), kaempferol content in moringa may inhibit osteoclastic resorption and stimulate both the occurrence of osteoblast differentiation and the mineralization of cells [34]. In general, kaempferol inhibits the cyclooxygenase-2 enzyme, leading to the inhibition of prostaglandin synthesis, and in turn, a decrease in PGE-2 and macrophage infiltration [32,34]. PGE-2 plays a role in osteoclast formation either directly or through the receptor activator of nuclear factor kappa-B, resulting in the differentiation of osteoclast precursors into mature osteoclasts [13,38]. On the other hand, a decrease in the number of macrophage cells will be followed by a decrease in inflammatory mediators, such as histamine, serotonin, and pro-inflammatory cytokines (tumor necrosis factor-α, interleukin IL-1, and IL-6). As a result, the number of osteoclasts will decrease, with a result that bone resorption decreases[9,28,39].

Based on research Kim et al. (2022) [29], Moringa leaf extract can decreases in the alveolar bone loss scores, receptor activator of nuclear factor kappa-B ligand/osteoprotegerin mRNA expressions, osteoclast cell numbers and activations, increased osteoblast cell numbers and activities, and increased alveolar bone volumes according to this research can reduce the number of Nf-κB expressions observed on the 7th, 14th, and 21st days. Among all parts of Moringa oleifera leaves and extracts its active is the best source as a natural antioxidant due to the presence of ascorbic acid, β-carotene, flavonoids, phenolics, and carotenoids [14,24,40]. In the study of the effect of the Moringa Oleifera fraction on the mechanism of action of macrophages due to lipopolysaccharide (LPS) showed that the ethyl acetate fraction of Moringa Oleifera significantly inhibited the NFkB signaling pathway through proinflammatory mediators and regulated the expression of inhibitors of κB (IκB)α [18,19,38].

The results of this study are supported by research by Martínez-González et al.(2017) [19] which states that the bioactive compounds involved in the anti-inflammatory properties of Moringa leaves can inhibit NFkB activation and the chain of inflammatory processes.

CONCLUSION

According to the results of this study, it can be concluded that the Moringa oleifera extract can make decrease NF-κB levels in the treatment group lower than that in the control group. This means that the degree of inflammation in the periodontal tissues can reduce, especially gingival junctional epithelium, by decreasing the NF-κB level.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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