Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.1094

The Influence of Moringa Oleifera Leaf Extract on Periodontitis Cases Through Rankl Expression Analysis

Irene Edith Rieuwpassa[1], Surijana Mappangara[2], Israyani[3], Harun Achmad[4*], Harmawaty[5], Arrang Sesioria[6]

[1]Department of Oral Biology, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
[2]Department of Periodontology, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
[3]Department of Oral Medicine, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
[4]Departement of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
[5,6]Resident of Pediatric Dentistry Program, Faculty of Dentistry, Hasanuddin University
***Corresponding author:** Harun Achmad; Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia, Email: harunachmader@gmail.com

Submitted: 16 November 2022; Accepted: 11 December 2022; Published: 10 January 2023

ABSTRACT

Introduction: 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 and growing on the tooth surface. The "red complex" bacteria consists of Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia. RANKL is a type II homotrimeric transmembrane protein that is expressedas a secretory protein and surrounds the membrane, derived from proteolytic cleavage. RANKL canfunction as an important ligand for the process of osteoclastogenesis.

Objective: to determine the effectiveness of Moringa oleifera leaves in influencing RANKL expression in inflammatory pathways.

Research Methods: The type of research that will be used is a quasi-experimental witha post-test research design with a control group design. This research was conducted in March – November 2022.

Results: On day 0, the average RANKL in the control and treatment groups usingan independent sample t-test showed no significant difference, while on days 7, 14 and 21 the average RANKL in the The control and treatment groups' RANKL scores decreased and the test results showed a significant difference with a $p<0.05$.

Keywords: *Moringa Oleifera, Periodontitis, Porphyromonas gingivalis, RANKL*

.

INTRODUCTION

Chronic periodontitis is an inflammation of the periodontal tissue caused by the accumulation of dental plaque. Periodontitis initially begins as gingivitis that develops into periodontitis.1,2 Periodontitis is characterized by loss of alveolar bone, and the attachment of the periodontal membrane and pocket formation, the incidence of periodontal increases with age. Based on Basic Health Research (RISKESDAS) data from the Ministry of Health in 2018 The percentage of periodontitis cases in Indonesia is 74.1%, with a prevalence between 10% and 60% of the adult population.3

Chronic periodontitis occurs starting with the formation of biofilms from microbes, especially the gram-negative group that affect tooth tissue, causing the body's response thatcauses bone and soft tissue damage.4 Endotoxins produced by bacteria cause osteoclast mediators to become active resulting in the destruction of bone and periodontal ligament. The gram-negative bacteria in question include Porphyromonas gingivalis and Aggregatibacter Actinomycetemcomitans.4,5

Porphyromonas gingivalis secrete extracellular virulence factors and proteases such as lipopolysaccharide, fimbriae, gingipain which result in destruction of the periodontal tissue causing innate immune changes and inflammatory responses.6,7 Porphyromonas gingivalis can induce macrophages and fibroblasts to produce proinflammatory cytokinessuch as interleukin 1-β (IL-1β), interleukin -6 (IL-6), tumor necrosis factor-α (TNF-α) andprostaglandin E2 (PGE-2) which can cause alveolar bone resorption by reducing the number of osteoblasts.5,6

Moringa Oleifera contains higher iron than other vegetables, which is 17.2 mg/100 g.8 In addition, Moringa leaves also contain various kinds of amino acids, including aminoacids in the form of aspartic acid, glutamic acid, alanine, valine, leucine, isoleucine, histidine, lysine, arginine, venylalanine, tryptophan, cysteine and methionine.9 The effectiveness of Moringa oleifera has been extensively researched for many years because of the potential of its natural ingredients to be used medicinally. 9,10

Osteoclasts are branched motile cells, very large and multinucleated in which theformation and activation of osteoclasts is one of them played by RANKL expressed by osteoblasts.11 Pro-inflammatory cytokines formed in the inflammatory process can stimulate osteoblasts to increase RANKL production and decrease OPG production. OPGas a protein produced by osteoblasts will bind to RANKL to inhibit osteoclast formationand activation, if the inflammation continues OPG will slightly bind to RANKL so that RANKL can easily bind to RANK on osteoclast precursors and activate osteoclasts resulting in bone damage.12 Bone and tissue damage will continue lasts as long as the inflammatory process persists, so to prevent more severe damage and speed up bone repair an anti-inflammatory drug is needed.13,14 Receptor Activator of Nuclear Factor κB Ligand (RANKL) is a type II homotrimeric transmembrane protein that is expressed as a secretory protein and surrounds the membrane, derived from proteolytic cleavage. 15

RANKL can function as an important ligandfor the process of osteoclastogenesis. RANKL expression is stimulated in osteoblasts/stromal cells through several factors known to stimulate osteoclast formation and activity.16,17 RANKL is widely expressed in the lymph nodes, thymus and lungs, and is present in several tissues including the spleen and bone marrow, but at low levels. Alveolarbone resorption is played by osteoclasts. RANKL plays a role in differentiation and activation of osteoclasts.18

Osteoprotegerin associated with bone protection is produced by bone marrow stromalcells, osteoblasts, and periodontal ligament fibroblasts.19 Osteoprotegerin is a membrane thatsurrounds and secretes proteins attached to RANKL to inhibit their role on RANK receptors.16,17 The RANK receptor is expressed on hematopoietic osteoclast progenitors. Osteoprotegerin and RANK are receptors that show the same attraction to RANKL. Osteoprotegerin produced by osteoblasts acts as a RANKL receptor, and prevents RANKLfrom binding to RANK and activating RANK. Osteoprotegerin also inhibits osteoclast development.12,15

RANKL expression is the number of cells that express RANKL by giving a positive reddish or brownish reaction arising from the results of immunohistochemical examination with anti-RANKL polyclonal antibody.20

Biological effects of osteoprotegerns on bone cellsinclude inhibiting the late terminal stages of osteoclast differentiation, suppressing mature osteoclast activation, and inducing apoptosis.19,20 So it can be said that bone remodeling is primarily controlled by the RANKL/OPG balance. The basis of this research is the ability of Moringa leaves (Moringa oleifera) as a herbalplant which has a variety of active substances such as flavonoids, saponins, alkaloids, tannins, and phenols,so that it has the potential to be a periodontitis treatment agent by reducing RANKL expression in periodontitis-inflamed tissues.

MATERIAL AND METHODS

This protocol was approved by the Health Research Ethics Committee for Dental and Oral Hospital, Faculty of Dentistry, University of Hasanuddin, Ministry of Research, Technology and Higher Education, Indonesia (No. 0094/PL.09/KEPK FKG-RSGM UNHAS/2021). This type of research is actual experimental laboratory research. The research subjects were divided into two major groups, namely the group that was given a Moringa oleifera extract, the control group that was given aquades. Porphyromonasgingivalis injected into the treatment group. Then it is left according to the day group, namely 0, 7, 14, and 2nd day.

Research Materials

Moringa leaves come from the Moringa cultivation garden in Blora, Central Java, Indonesia. Moringa leaves are then washed and dried in an oven system to produce dry leaves. The dried moringa leaves are then mashed and processed using a maceration technique to produce a thick moringa extract.

Research Animals

This study used samples of male Wistar rats (Rattus Novergicus) weighing 200 to 250 g) randomly allocated in different groups. Animals were adapted 1 hour before testing and were used only once in each experiment. Animal samples were previously acclimatized (adaptation) for about 1 week in advance in the animal cages to get used to the rats to their new environment and to observe general conditions such as weighing the animals' weight and health. Wistar rats were placed in cages in groups, cycle light/dark 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 lid measuring 40 cm x 60 cm x 25 cm, covered with rice and routinely cleaned three times a week to keep the cage dry and healthy. Sampling was carried out by simple random sampling after fulfilling the inclusion and exclusion criteria. The sample consisted of 24 wistar tails and was divided into 2 groups based on sampling of the periodontal tissue as follows:

The control group consisted of 12 wistar tails irrigated with distilled water after induction of bacteria in the gingival sulcus

The treatment group consisted of 12 wistar tails which were treated with moringa extract and distilled water after induction of Porphyromonas gingivalis bacteria in the gingival sulcus.

Tissue samples were collected on days 0, 7, 14, 21. Analysis of serum cytokine levels (pg/mL) was quantified using a commercial IL-6 ELISA kit following the manufacturer's instructions (Elabscience).

Porphyromonas gingivalis Bacterial Culture Media

Preparation of agar media for Porphyromonas gingivalis bacterial culture. Initially, agar media was prepared for bacterial culture, namely BHI-A enriched with hemin and vitamin K. To make 100 ml of BHI-A, 50 µl of hemin solution was needed, 10 µl of vitamin K, 37 grams of BHI-A in 100 ml of sterile distilled water and extract. yeast 500µl. The media was divided into four, then put into the petridish @ 25 ml and waited until it was solid. One ose of pure Porphyromonas gingivalis strain ATCC 33277 (F0) was inoculated on each petridish then incubated for 2x24 hours.

Porphyromonas gingivalis Bacterial Suspension

The preparation of the Porphyromonas gingivalis bacterial suspension was previously carried out by preparing 10 ml of liquid media, namely from 0.37 grams of BHI-B, 1 µl of vitamin K, 5 µl of hemin and 50 µl of yeast extract. Next, a suspension of Porphyromonas gingivalis bacteria was made. Liquid media that has been made is divided into 2 parts @ 5cc.

In each liquid medium, one ose of bacteria originating from the culture on BHI-A agar media was given. The bacterial suspension obtained was then put in a desiccator and incubated for 2x24 hours. After incubation, the concentration of the bacterial suspension was measured until 1.5x106 was obtained. Furthermore, preparations were made with gram staining to determine the bacteria were in good condition and not contaminated.

RESULTS

The distribution of RANKL was observed using immunohistochemical techniques, with anti-ranklspecific antibodies (santarcruz biotech). By using the technique of calculating immunohistochemical results as in Soini, Y., Paakko, P. andLehto, V-P.1997; Pizem, J. and Cor,A., 2003) modified for bone tissue, this study has finished counting the number of bone tissue osteoclast cells, which express RANKL (brown color in cell cytoplasm).

On day 0, the mean RANKL in the control group was 2.00 ± 1.000; in the treatment group was 2.67 ± 1.155; The results of statistical tests using the independent sample t-test showed that therewas no significant difference in the RANKL scores between the control and treatment groups, witha $p>0.05$.

On the 7th day, the mean RANKL in the control group was 13.00 ± 3.000; in the treatment group,the RANKL score decreased, namely 3.33 ± 1.528; The results of statistical tests using the independent sample t-test showed that there was a significant difference in the RANKL values between the control group and the treatment group, with a $p<0.05$.

TABLE 1. Descriptive statistics showing results of RANKL expressions

Group	Day 0 (mean ± SD)	Day 7 (mean ± SD)	Day 14 (mean ± SD)	Day 21 (mean ± SD)
Control	2.00 ± 1.000	13.00 ± 3.000	14.00 ± 1.000	14.67 ± 0.577
Intervention	2,67 ± 1.155	3.33 ± 1.528	3.00 ± 1.000	2.67 ± 1.155
P	0,492	0.008*	0.000*	0.000*

*significant using independent sample t-test ($p<0.05$)

On the 14th day, the mean RANKL in the control group was 14.00 ± 1.000; in the treatment groupthe RANKL score decreased by 3.00 ± 1.000; The results of statistical tests using the independentsample t-test showed that there was a significant difference in the RANKL values between the control group and the treatment group, with a $p<0.05$.

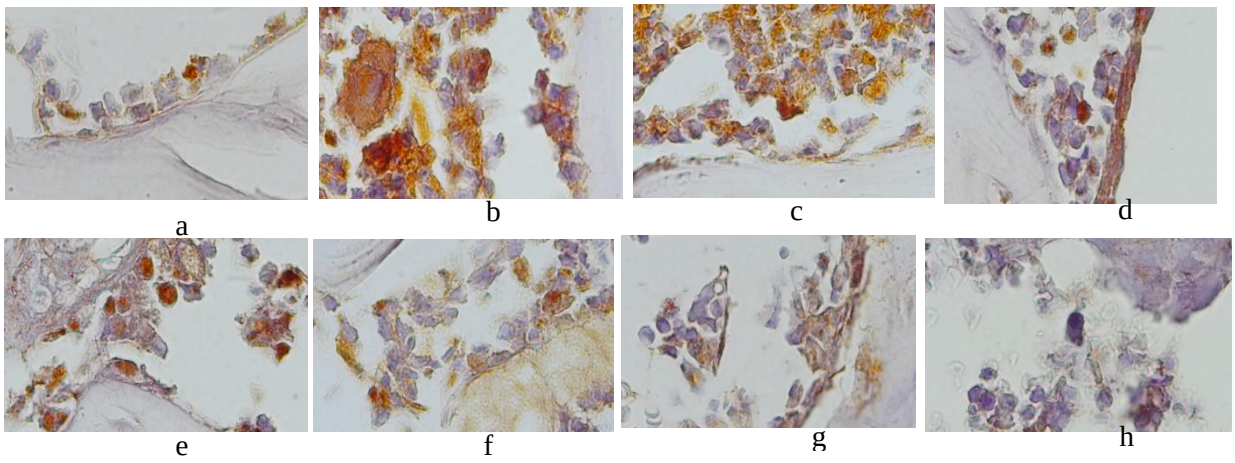

FIGURE 1. The distribution of RANKL was observed using immunohistochemical techniques, with specific anti-NFkB antibodies, counting the number of bone tissue osteoclast cells, which display RANKL (brown color in cytoplasmic cells). Expression of RANKL with 1000 times magnification, (a) expression of RANKL on the baseline in the control group (aquades), (b) expression of RANKL on the 7th day in the control group, (c) expression of RANKL on the 14th day in the control group, (d) expression of RANKL on the 21th day in the control group, (e) expression of RANKL on the baseline in the moringa group, (f) expression of RANKL on the 7th day in the moringa group, (g) expression of RANKL on the 14th day in the moringa group, (h) expression of RANKL on the 21th day in the moringa group

On the 21st day, the mean RANKL in the control group was 14.67 ± 0.577; in the treatment group the RANKL score decreased 2.67 ± 1.155; The results of statistical tests using the independent sample t-test showed that there was a significant difference in the RANKL values between the control group and the treatment group, with a $p<0.05$

.

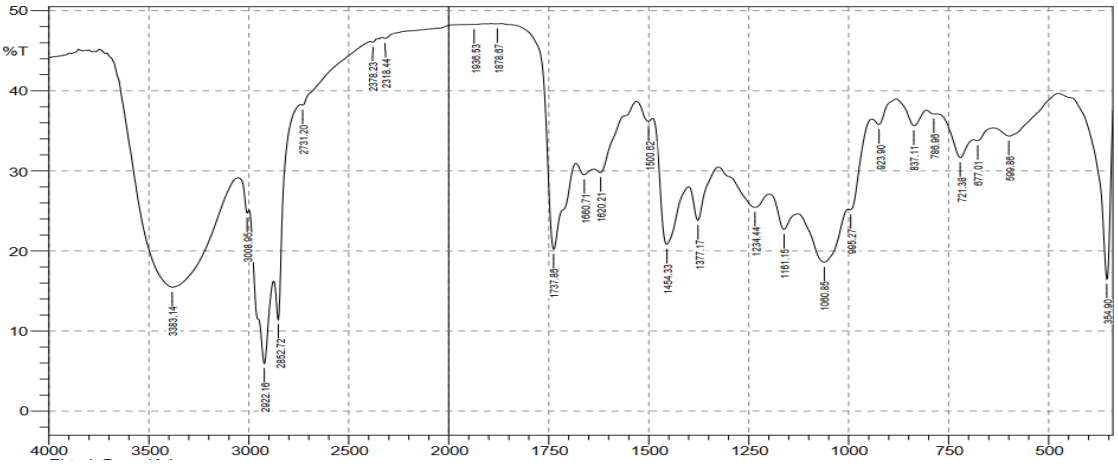

FIGURE 2. FTIR spectrum of flavonoid Moringa oleifera extract

After getting the results of the moringa extract. Examination of the content of this research material so that it is known for its natural content that is still awake. This shows the FTIR spectrumof moringa extract in the 400–4000 cm−1 area; the absorption band at wave number 3383.14 cm−1shows the OH and NH functional groups.

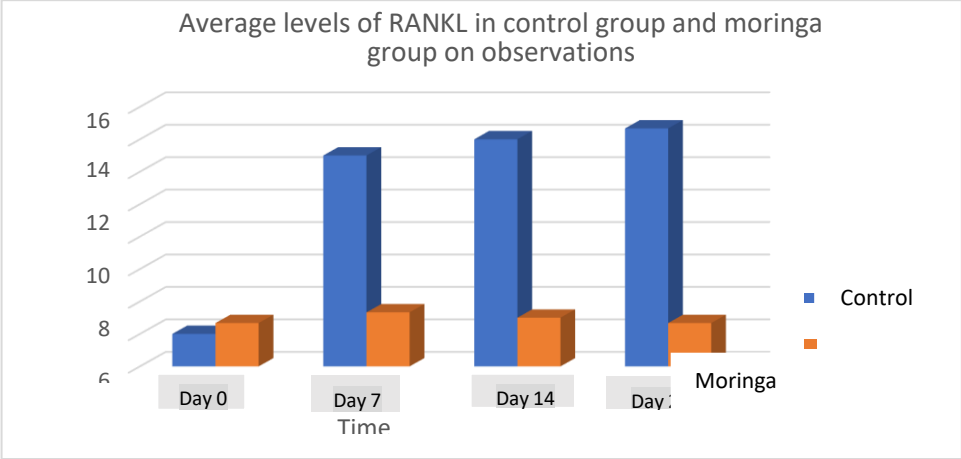

FIGURE 3. Bar chart of RANKL expression between groups

This study aims to determine the effectiveness of Moringa oleifera leaves in influencing RANKL expression in inflammatory pathways. Benefits Research 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 analysis of RANKL expression. The type of researchthat will be used is a quasi-experimental with a posttest research design with a controlgroup design. This study used a sample consisting of 30 Wistar (Rattus Novergicus) and divided into 2 groups based on sampling of the periodontal tissue as follows: the treatment group treated with moringa extract and the control group with aquadest irrigation after induction of bacteria in the gingival sulcus.

DISCUSSION

This research by inducing Wistar gingiva with Porphyromonas gingivalis bacteria forms periodontitis. The inflammatory response is a non-specific and multifaceted chemical response of the immune system to various disorders or stimuli in the body.14 These different stimuli trigger a cascade of cytokines and inflammatory mediators that lead to vasodilatation, vascular leakage, infiltration and recruitment of immune cell networks, and stimulation of mucous membranes.19 These molecular and microscopic tissue changes are clearly visible as redness, heat, swelling, and pain.14,19 Periodontal tissue damage is mainly caused by the interaction of bacterial antigens and inflammatory cells which results in the production of cytokines.4,5 IL-6 is secreted by macrophages in response to inflammation and is involved in leukocyte recruitment and apoptosis and T-cell activation. IL-6 and its receptors induce bone resorption by increasing nuclear factor receptor activator nuclear ligand (RANKL) or by directly inducing osteoclast formation. 5,15

In this study reported that administration of Moringa oleifera leaf extract data analysis using the ANOVA test showed that there were significant differences in OPG expression, the number of osteoblasts and osteoclasts between the control and treatment groups ($p<0.05$). Meanwhile, the RANKL expression did not show any difference between the control and treatment groups ($p>0.05$)

In this study there was no significant difference in Rankl expression on day O while on Days 7, 14 and 21 there was a significant difference with a p value of 0.000 ($p <0.05$). RANKL is a pleiotropic cytokine that is produced in response to tissue damage and infection.21 RANKL also increases expression of the zinc importer ZIP14 in hepatocytes and induces the hypozincemia seen in inflammation. 22

CONCLUSION

There was no significant difference in the Rankl expression on day 0 while on days 7, 14 and 21 there was a significant difference with a p value of 0.000 ($p < 0.05$). Moringa leaf extract can reduce RANKL cells, it was seen after being treated with moringa extract on observation days from day 7, day 14, and day 21 in experimental animals of Wistar rats and induced using Porphyromonas gingivalis bacteria.

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.

ACKNOWLEDGMENTS

The authors are thankful to all the staff members of the Department of Oral Medicine, Pediatric Dentistry, Periodontology and Public Health of Hasanuddin University, for their valuable suggestions and encouragement during this research. This study was supported by the Hasanuddin University Research and Community Service Institute (LPPM)

REFERENCE

1. Shaddox LM, Walker CB. Treating chronic periodontitis: current status, challenges,and future directions. Clinical, Cosmetic and Investigative Dentistry. 2010;2:79–91
2. Heitz-Mayfield LJ, Schatzle M, Loe H, Bürgin W, Anerud A, Boysen H, et al. Clinical course of chronic periodontitis. II. Incidence, characteristics and time of occurrence of the initial periodontal lesion. J Clin Periodontol. 2003:30(10):902–8. DOIs:10.1034/j. 1600051X.
3. Health Research and Development Agency (Balitbangkes). RISKESDAS 2017-2018. Jakarta:Ministry of Health of the Republic of Indonesia. 2018;h207.
4. Eberhard J, Jepsen S, Jervøe-Storm PM, Needleman I, Worthington HV. Full-mouth treatment modalities (within 24 hours) for chronic periodontitis in adults. Cochrane Database System Rev. 2015 Apr 17;(4):CD004622. DOIs:10.1002/14651858. CD004622.
5. Sumintarti, Ramadany S, Handayani H, Achmad H, Mutmainah N, Inayah NH et al. Assessment of the Anti-inflammatory Activities of the Moringa Leaf Extracts in Periodontitis Cases through IL-6 Cytokine Analysis in Wistar (Rattus Copernicus). Macedonian Journal of Medical Sciences. 2022:10(D):124-30.
6. Rafiei M, Kiani F, Sayehmiri F, Sayehmiri K, Sheikhi A, Azodi MZ. Study of Pophyromonas gingivalis in periodontal diseases: A systematic review and meta- analysis. Med J Islamic Republic of Iran. 2017; 31: 62. DOI: 10.18869/mjiri.31.62
7. Mysak J, Podzimek S, Sommerova P, Lyuya-Mi Y, Bartova J, Janatova T, et al. Porphyromonas gingivalis: Major periodontopathic pathogen overview. J Immunol Res. 2014; 2014: 476068. DOI: 10.1155/2014/476068
8. Dwika, Pratama PIW, Oka, Dharmayudha AAG, Sudiamartini, LM, 2016 Identification of Chemical Compounds of Moringa Leaf Ethanol Extract (Moringa oleifera L) in Bali, Indonesia Medicus Veterinus, October 2016, p. 466.
9. Aminah, Syarifah., Tezar, Ramdhan & Muflihani Yanis. Nutritional Contentand Functional Properties of Moringa (Moringa oleifera) Scientific Article Research Results from the Jakarta Agricultural Technology Study Center. 2015;36.
10. Rieuwpassa IE, Ramadany S, Sumintarti, Israyani, Achmad H, et al. Journal of International Dental and Medical Research; Diyarbakir. 2022;15(2):611-617.
11. Mescher, AL. Junqueira's Basic Histology 13th Edition. 2013. United States: McGraw Hill Education.
12. Tang JH, H Meng, Y Zhao, et al. Downregulation of RANKL and RANKL/Osteoprotegrin Ratio in Human Periodontal Ligament Cells During their Osteogenic Differentiation. Journal of Periodontal Research. 2016;51: 125-132.
13. Wisdom, N. et al. The role of Rankl in the process of alveolar bone resorption in diabetic conditions'. 2013;10(3):105–109.
14. Achmad H, Supriatno, Singgih MF, Hendrastuti H. Akt Signal Transduction Pathways and Nuclear Factor-kappa B (NF-κB) Transcription as a Molecular Target of Oral Tongue Squamous Cell Carcinoma (SP-C1) Using Papua's Anthill Plant (Myrmecodia pendans). Pak J Biol Sci. 2016;19(8-9):323-330.
15. Grimaud, et al. Receptor activator of nuclear factor kappaB ligand (RANKL)/osteoprotegerin (OPG) ratio is increased in severe osteolysis. National library of Medicine. 2013;163(5):2021-31.
16. Fard MT, Arulselvan P, Karthivashan G, Adam SK, Fakurazi S. Bioactive Extract from Moringa oleifera Inhibits the Pro-inflammatory Mediators in Lipopolysaccharide Stimulated Macrophages. Pharmacogn Mag. 2015 Oct; 11(Suppl 4): 556–S563.

17. Owen J, Punt J, Stranford S. Kuby Immunology, 7th ed. Freeman, W. H. & Company ;2013.
18. Omodanisi E, Aboua Y, Oguntibeju O. Assessment of the Anti-Hyperglycaemic, Anti-Inflammatory and Antioxidant Activities of the Methanol Extract of Moringa Oleifera in Diabetes-Induced Nephrotoxic Male Wistar Rats. Molecules. 2017; 22(4): 439.
19. Tobeiha M, Moghadasian MH, Amin N, Jafarnejad S. RANKL/RANK/OPG Pathway: A Mechanism Involved in Exercise-Induced Bone Remodeling. BioMed Research International. 2020;2020: 1–11. doi:10.1155/2020/6910312
20. J-Y Kim, H-J Kim, D-H Park, Y-A Shin, S-K Min, C-S Kim. Effects of acute exercise on RANKL/RANK/ OPG pathway and bone metabolic markers in healthy college female. Exercise Science. 2018;27(3):225–231.
21. Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harbor Perspectives in Biology.2014; 6(10): a016295–a016295.
22. Liuzzi JP, Lichten LA, Rivera S, Blanchard RK, Aydemir TB, Knutson MD, Cousins RJ. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proceedings of the National Academy of Sciences. 2005; 102(19) :6843–6848.
23. Achmad H, Djais AI, Hatta M, Thahir H. Effect of Moringa Leaf Extract (Moringa Oleifera) on Increasing the Number of Osteoblas as a Marker of Bone Remodeling. Indian Journal of Public Health Research and Development 2019;10(9):1394.
24. Newman M, Takei H, Klokkevold P, Carranza F. Newman and Carranza's clinical periodontology. 13th ed. Philadelphia: Elsevier Saunders; 2018. p. 346–7.
25. Quamilla N. Stres dan kejadian periodontitis (kajian literatur). J Syiah Kuala Dent Soc 2016; 1(2): 161–8.
26. Putri CF, Bachtiar EW. Porphyromonas gingivalis and pathogenesis of cognitive disfunction: role of neuroinf lammation cytokine (Literature review). Cakradonya Dent J 2020; 12(1): 15–23.
27. Jamal M, Tasneem U, Hussain T, Andleeb S. Bacterial biofilm: Its composition, formation and role in human infections. Res Rev J Microbiol Biotechnol 2015; 4(3): 1–14.
28. Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm. Virulence. 2011; 2(5): 435–44.
29. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. Trop. J. Pharm. Res 2008;7:1019–1024.
30. Davinelli S, Bertoglio JC, Zarrelli A, Pina R, Scapagnini G. A randomized clinical trial evaluating the efficacy of an anthocyanin-maqui berry extract (Delphinol®) on oxidative stress biomarkers. J. Am. Coll. Nutr. 2015;34(Suppl. 1):28–33.
doi: 10.1080/07315724.2015.1080108.
31. Murillo AG, Fernandez ML. The relevance of dietary polyphenols in cardiovascular protection. Curr. Pharmacol. Rev. 2017;23:2444–2452.
doi: 10.2174/1381612823666170329144307.