The effect of brown algae (*Sargassum sp.*) gel on the number of osteoclasts in periodontitis rats

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ABSTRACT

Introduction: Alveolar bone resorption in periodontitis is caused by increased activation of osteoclasts, causing an imbalance of bone remodeling. Periodontitis treatment can be done mechanically with Scaling and Root Planing and accompanied by metronidazole. Antibiotics have long-term drawbacks and can cause allergies, thus requiring an alternative to natural topical drugs such as brown algae gel. This study aimed to to analyzed the effect of brown algae gel (sargassum sp) on the number of osteoclasts of alveolar bone in periodontitis rats. **Methods:** The research method was a True Experimental with a pretest-posttest-only control group design. Rats were divided into three groups; negative control (K-) was not given any treatment, positive control (K+) was given metronidazole ties plus 25% concentration, and the treatment group (KP) was given brown algae gel (*Sargassum sp*) 75% concentration. Data were analyzed by Friedman and Kruskal Wallis test. **Results:** There was a significant difference in the number of osteoclasts on days 8, 10, and 12, the decrease in the number of osteoclasts was higher in the brown algae treatment group than the negative control group (p=0.051). In the positive control group, there was a significant difference in the decrease of the number of osteoclasts compared to the brown algae treatment group (p=0.029). The number of osteoclasts was significantly different on day 12 (p=0.026). **Conclusion:** Adhering brown algae (*Sargassum sp.*) gel decreases osteoclasts in periodontitis rats.

Keywords: bone remodeling; bone resorption; brown algae gel (sargassum sp.); osteoclasts; periodontitis.

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INTRODUCTION

The 2018 RISKESDAS data showed that periodontitis cases in Indonesia were at 74.1%, which was high among dental disorder cases.[1] Periodontitis is an inflammation of the supporting tissues of the teeth that can destroy the periodontal ligament and alveolar bone resorption.[2] The main causes of periodontitis are pathogenic microorganisms that colonize the subgingival plaque, anaerobic gram-negative bacteria namely *Porphyromonas gingivalis* (Pg), *Actinobacillus*

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actinomycetemcomitans, *Prevotella intermedia*, and *Bacteriodes forythus*.[4,5] These bacteria will release endotoxin, namely lipopolysaccharide (LPS), which causes macrophage cells and neutrophils to release pro-inflammatory cytokines such as interleukin-1ß (IL-1ß), Tumor Necrosis Factor (TNF-α), prostaglandins (PGE2).[6,7] These pro-inflammatory cytokines will regulate the expression of the Receptor Activator of Nuclear Factor kB Ligand (RANKL) and Osteoprotegerin (OPG) in osteoblasts. RANKL will bind to Receptor Activator of Nuclear Factor kB (RANK) to stimulate osteoclast differentiation and activation, also OPG acts as an inhibitor of RANKL and RANK binding causing osteoclast cell apoptosis.[8,9] The cytokine TNF- α plays a major role in the inflammatory reaction, loss of periodontal ligament attachment and alveolar bone resorption.[10]

Bone matrix and cells is formed by osteoblasts and resorbed by osteoclasts.[11] Osteoclasts are hematopoietic stem cells derived from the granulocyte-macrophage colony forming unit (CFU-GM), and osteoblasts are mesenchymal stem cells derived from Fibrocyte Colony Forming Unit (CFU-F).[12.13] Bone is composed of organic (collagen) and inorganic (hydroxyapatite and calcium phosphate) matrices. The alveolar bone is the periodontal tissue that forms the tooth socket and the attachment site for the periodontal ligament.[15,16] The structure of the alveolar bone is divided into a compact (cortical) and cancellous (trabecula/spongy) part. The cortical part contains the Haversian canal and the cancellous part contains bone marrow which produces blood cells.[17,18,19]

Periodontitis treatment can be done mechanically and supported by systemic pharmacology. [20] Mechanical treatment is Scaling and Root Planing (SRP) which eliminates bacteria by removing plaque on the subgingival, surface, and root of the tooth.[21] Eliminating pathogens with SRP is sometimes not optimal because there are parts that cannot be accessed by SRP tools to maximize treatment. Therefore, it needs to be combined with antibacterial and anti-inflammatory medication.[22] Antibiotics such as metronidazole and nonsteroidal anti-inflammatory drugs (NSAIDs) such as mefenamic acid and ibuprofen are effective for periodontal treatment but these systemic drugs can cause side effects when given in the long term.[23] Systemic use of antibiotics has drawbacks when used in the long term since they can cause allergies, toxicity and bacterial resistance, while systemic administration of NSAIDs in the long-term causes side effects such as digestive disorders and bleeding, inhibition of platelet aggregation, kidney damage, etc. [24, 25]

Ti-eS Metronidazole gel is an antibiotic that is given locally and has the advantage that it can be given to inflammatory areas and is not toxic (no side effects if given for a long time).[26] Brown algae (*sargassum sp)* is widely available in Indonesia waters, especially in the water of Jepara, and contains many minerals, fatty acids, phenols, alginates, fucoxanthin, alkaloids, steroids, saponins, flavonoids, tannins, carbohydrates, proteins, vitamins (A, B, C, D, and E) and contains high levels of iodine that can be used as an antibacterial.[27] Flavonoids are secondary metabolites of polyphenols that can act as anti-inflammatory, antibacterial and antioxidant.[28] The research of Rahmawati proves that the concentration of Sargassum sp 75 % can effectively act as an antibacterial and anti-inflammatory by examining the number of macrophages in the healing process of traumaticn ulcers. Fatimatuzzahro et.al. concluded that the polyphenolic extract of robusta coffee beans can reduce inflammation characterized by a decrease in TNF-α. The decrease in TNF-α cytokines can reduce osteoclast activation so that no further resorption occurs. This study aimed to analyzed the effect of brown algae gel on osteoclasts decreased in periodontitis rats.

METHODS

The extraction of brown algae was carried out by maceration method. Samples of brown algae as much as 1000 grams, were cleaned and then dried. After drying the brown algae, the particles were reduced and soaked in a macerator with ethyl acetate for 3x24 hours. The brown algae extract was filtered through filter paper and then evaporated on a rotary evaporator, resulting in a thick extract. The thick extract of brown algae (*Sargassum sp*.) was dissolved in distilled water and then put into a separating funnel and added with n-hexane and ethyl acetate solvents, shaken, and allowed to form a layer. Then the

ethyl acetate solvent was evaporated to obtain a pure flavonoid fraction. The manufacture of brown algae (*Sargassum sp*) gel was carried out at the Chemistry Laboratory of Sultan Agung Islamic University.

This study used male Sprague Dawley rats aged 2-3 months weighing 200-300 grams. Rats were obtained at the Research Center for Food and Nutrition Studies, Gadjah Mada University. Before being induced with periodontitis,Sprague Dawley rats were anesthetized with ketamine HCL intramuscularly in the hamstrings at a dose of 0.2 ml/200-gram body weight. Induction of periodontitis was performed with a 3.0 size ligature thread forming a figure 8 pattern in the subgingival area of the lower incisor cervical area. Induction was carried out for 14 days until periodontitis appeared, namely the formation of redness and gingival recession, as well as a periodontal pocket with a depth of 3-4 mm established from the bottom of the pocket as measured by a probe. Then the ligature thread was removed, the plaque was cleaned, and a curettage was performed. Rats were divided into 3 groups; negative control (A) was not given any treatment, positive control (B) was given metronidazole ties plus a concentration of 2,5 %, and the treatment group (C) was given brown algae gel (*Sargassum sp.*) with a concentration of 7 5 %. The gel was applied twice a day with a 7 hour difference. Rats were decapitated on days 0, 8, 10, and 12 with ketamine 75mg/kg BW, and then the mandibular alveolar bone was taken for histological preparation.

Bone histology preparation was stained with HE, observed under a light microscope, and then the number of osteoclasts was manually counted of HE-positive multinucleated (≥3 nuclei) cells visualized . Observations was done from distal to the lingual or buccal part of the right central incisor. The number of osteoclasts was counted per mm of the bone surface. Data were analyzed by Friedman ($p<0.05$) and Kruskal Wallis ($p<0.05$) tests. This type of research is a true experimental design using a pretest-posttest-only control group design. Health Research Ethics Commission, Faculty of Dentistry, Sultan Agung Islamic University Semarang No. 291/B.1-KEPK/SA-FKG/VII/2021.

RESULTS

This study showed that the mean osteoclasts decreased the most in Group B, Group C, and Group A consecutively on day 12th.

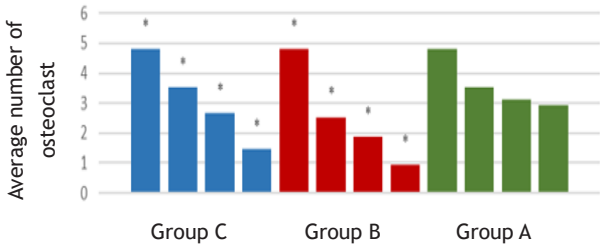

Figure 1. Graph of the average number of osteoclasts in each group; *p<0.05 (significant)

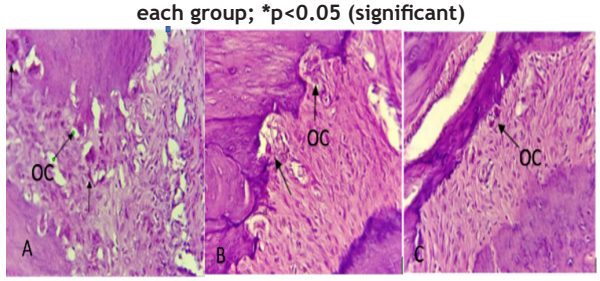

Figure 2. Histological analysis of the area around the distolingual or buccal pressure side of the tooth right first central incisor of the mandible, showing the presence of osteoclasts (OC) day 8 (A), day 10 (B) and day 12 (C)

Figure 1 shows that the most decreased osteoclasts were on day 12th,and the highest was on day 0.Histological features of osteoclasts are large, multinucleated blue or dark purple cell nucleus, reddish cytoplasm, and is located in shallow depressions (Howship lacuna) on the surface of the alveolar bone.

The result of the Friedman test showed the p-value for each group in the brown algae treatment group, and the positive control was 0.029 ($p<0.05$), indicating a significant difference in the decrease of the mean number of osteoclasts for each group. The negative group obtained a p-value of 0.051 ($p> 0.05$), indicating no significant difference in the decrease in the number of osteoclasts in the negative control group. With the Kruskal Wallis test, it was found that the p-value was 0.026 ($p<0.05$), which can be showed that there was a significant difference in the decrease in the mean number of osteoclasts between groups.

This study showed that the treatment group with brown algae had an average decrease in the number of osteoclasts, comparable to the positive control (metronidazole) every day, namely on the 8th, 10th, and 12th days. This is in line with the research of Fatimatuzzahro et al. The study results showed that the 7th and 14th-day treatment groups had significant differences with the negative control group. The decrease in osteoclasts occurred in the treatment group on the 7th day and further decreased on the 14th day.

The decrease in osteoclasts was due to the flavonoids contained in brown algae gel (*Sargassum sp.*), which can reduce the number of osteoclasts and prevent the expansion of inflammation by inhibiting arachidonic acid synthesis, thereby suppressing the enzymatic activation of cyclooxygenase 2 (COX-2) and lipoxygenase (LOX).[28,31,32] Inhibition of enzymatic lipoxygenase (LOX), namely leukotrienes, can reduce chemotaxis and adhesion of neutrophils and macrophages, resulting in a decrease in TNF- cytokines α and activation of osteoclasts.[33,3] TNF-α cytokine can upregulate RANKL, neutrophil activation and fibroblast cell apoptosis.

Flavonoids accelerate the process of bone remodeling by increasing the migration of fibroblasts and osteoblasts so that the process of bone formation and periodontal tissue healing can occur.[29] Flavonoids can act as antibacterials. Three mechanisms of flavonoids act as antibacterial, including inhibiting energy metabolism, inhibiting nucleic acid synthesis, and inhibiting cytoplasmic membrane function.[35.36] Flavonoids as antibacterial inhibit bacterial growth by forming complexes with bacterial proteins membrane and lysing bacterial membranes.[37] Flavonoids can act as antioxidants by slowing the formation of ROS and breaking down ROS. [38]

The process of resorption of alveolar bone by osteoclasts, when the activation of osteoclasts to osteoclast tissue will form a sealing zone, cells will bind to the bone matrix by integrins, then osteoclasts create an acidic environment.[12,13] Osteoclasts resorption in two ways; namely, osteoclasts will secrete collagenase such as MMP causing the digestion of collagen proteins in the bone matrix.[5,18] Osteoclasts will secrete HCL (hydrochloric acid), which will dissolve hydroxyapatite into dissolved calcium (ca^{2+} and PO_4^{3-}). These ions will be released into the bloodstream then osteocytes in the bloodstream will be phagocytized by osteoclasts.[39]

The decrease in osteoclasts was greater in the negative treatment group with metronidazole ties plus gel. Ties metronidazole gel plus contains 25% metronidazole and mefenamic acid. Since Metronidazole is a bactericidal antibiotic widely used to treat periodontitis to eliminate all anaerobic cocci and anaerobic gram-negative bacilli, including *Porphyromonas gingivalis* and *Prevotella intermedia*.[23]

Mefenamic acid is an NSAID drug that can act as an anti-inflammatory by inhibiting the cyclooxygenase (COX) enzyme so that the conversion of arachidonic acid to PGE2 is disrupted. PGE2 is a proinflammatory cytokine that plays a role in inducing osteoblasts to produce RANKL and reducing OPG production resulting in RANKL binding to RANK, which causes increased formation of osteoclasts. The use of this combination is widely used to increase the effectiveness of the gel. [24] This situation can occur because metronidazole is a bactericidal antibiotic that is widely used as a treatment for periodontitis.[25] Metronidazole is effective against anaerobic bacteria that cause periodontal disease, it acts as an antibacterial by disrupting the helical structure of DNA, then inhibiting nucleic acid synthesis and resulting in cell death. This process is the most effective in eliminating anaerobic microorganisms.[40]

Metronidazole gel, after 8 hours of application, has a very high concentration of 128 µg/ml, which is about 100 times the minimum inhibitory concentration (MIC) of anaerobic bacteria, and can inhibit > 90% subgingival bacteria.[41] Abdurrohman et al. examined the effect of metronidazole in rats induced by bacteria that cause periodontitis. The results showed that the administration of the antibiotic metronidazole could increase the tissue inhibitor of matrix metalloproteinase-1(TIMP-1), which is an MMP inhibitor. Matrix metalloproteinase (MMP) proteolytic enzymes play a role in tissue damage in periodontitis, especially causing the degradation of collagen fibers.[2,4]

MMP-8 in periodontitis is a collagenase secreted by neutrophils that actively breaks down collagen types I and III, which are the main interstitial collagenases in the periodontal

ligament and gingiva, allowing it to work more to reduce the number of osteoclasts.[6,4 0]

The process of bone resorption usually occurs on days 3 to 5, followed by days 5 to 7 of the healing phase, and the final stage of bone resorption on days 7-14.[19] The bone remodeling process normally takes about 2-8 weeks.[13] This is the reason why the decrease in the number of osteoclasts in this study was most significant on day 12 because, at this time, we have entered the final stage of resorption, where the activity of the osteoclasts themselves has decreased. The osteoblasts have begun to be active in the remodeling process.

CONCLUSION

Adhering brown algae (*Sargassum sp.*) gel decreases osteoclasts in periodontitis rats.

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