Anti-Osteoclastogenesis effects of gambir extract gel in periodontitis

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Anti-Osteoclastogenesis effects of gambir extract gel in periodontitis

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Abstract

Objective: This study aimed to analyze the anti-osteoclastogenesis effect of gambir extract gel through RANKL and OPG serum levels, the number of osteoblasts and osteoclasts in alveolar bone tissue of Wistar rat.

Material and Methods: Twenty-four male Wistar rats were divided into four equal groups, each receiving a different treatment: Group I received a placebo gel, Group II received Tetracycline gel, Group III received 8% gambir extract gel, and Group IV received 10% gambir extract gel. After 21 days, the serum levels of RANKL and OPG were assessed using ELISA. Hematoxylin-eosin staining of the bone tissue preparation was followed by microscopic counting of osteoblasts and osteoclasts. tial chamfer margin. The crown was made with direct composite resin materials with the aid of silicone key to create palatal enamel wall and then proceed with bilaminar technique until the crown was finished.

Results: Significant differences in RANKL serum levels were seen in Group I (0.70 + 0.020) with Group III (0.031 + 0.014) (p=0.018) and Group I with Group IV (0.038 + 0.012) (p=0.041). Serum levels of OPG differed significantly between Groups I (0.165+0,125) and IV (0.536+0.182) p=(0.015). The number of Osteoblast in Group I (1.16+ 0.40) and IV (1.33+1.03) were significantly different (p=0.023). The number of Osteoclast in Group I (4.33+1.50) and IV (1.5+0.83) were significantly different (p=0.008).

Conclusion: Gambir Extract Gel 10% inhibits osteoclastogenesis by increasing osteoblast and decreasing osteoclast numbers by more than 8%, decreasing RANKL, and elevating OPG serum levels.

Keywords : Gambir extract gel, Osteoclastogenesis, Periodontitis
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Introduction

The prevalence of the periodontal disease is greater in Asia and Africa than in Europe, the United States, or Australia. In the US, 47% of persons over 30 have periodontitis, with 38% having moderate to severe periodontitis.[1] In 2018, basic health research revealed that the prevalence of dental and oral disease in Indonesia was 57.6%, and that of periodontitis was 74%.[2] Compared to other nations, the frequency of periodontitis in the Indonesian community is significant, as seen by these statistics. In addition, periodontitis is the leading cause of tooth extraction, leading to irreversible tooth loss.[3]

Periodontal attachment loss, pocket deepening, and alveolar bone resorption are symptoms of periodontitis, a bacterial infection that causes chronic inflammation of the periodontal tissues. Alveolar bone resorption happens as a result of osteoclastogenesis, which involves inflammatory mediators that may have an impact on osteoclast formation. Periodontitis can trigger osteoclastogenesis via the Receptor Activator of Nuclear Factor Kappa-B Ligand-Osteoprotegerin (RANKL-OPG) signaling pathway.[4-6] Increased expression of the Receptor Activator of Nuclear Factor-Kappa B Ligand (RANKL) is known to have an essential role in the osteoclastogenesis process. Osteoprotegerin (OPG) produced by gingival fibroblasts will block RANKL and RANK binding, which will form osteoclasts. An increased RANKL/OPG ratio in periodontitis will cause an increase in osteoclasts and lead to alveolar bone resorption.[7-9]

The majority of Gambir plant extracts contain the flavonoids catechins and tannic acid. Ethyl acetate, polyphenol, and flavonoid groups are present in catechins. Flavonoids act as anti-inflammatory drugs by protecting cell components. Gambir has a strong anti-inflammatory impact since it is high in catechins. Gambir is a common remedy for treating wounds in Indonesian culture. Traditionally, gambier is used to make areca or betel nuts. In rats with periodontitis, gambier extract has been shown to inhibit Streptococcus mutans bacteria and decrease the number of Actinobacillus actinomycetemcomitans bacteria in the gingival sulcus.[10-12] In addition, it has also been demonstrated that Gambier extract accelerates the healing of gingival wounds.[13] However, no studies on the effectiveness of gambier extract

Table 1. The Mean Value of RANKL, OPG Serum Level and The Number of Osteoblast and Osteoclast

Research Group	RANKL Serum Level	OPG Serum Level	The Number of Osteoblast	The Number of Osteoclast
Group I	0.070±0.020	0.165±0.125	1.16±0.40	4.33+1.50
Group II	0.030±0.009	0.353±0.139	2.33±1.21	1.83+0.98
Group III	0.031±0.014	0.452±0.192	0.33±0.51	3.33+1.63
Group IV	0.038±0.012	0.536±0.182	1.33±1.03	1.50+0.83

Table 2. The Significant Differences in RANKL's Mean Between Two Groups

Group	Mean Differences	p
Group 1 and Group 2	0,040	0,013*
Group 1 and Group 3	0,038	0,018*
Group 1 and Group 4	0,032	0,041*
Group 2 and Group 3	-0,013	0,997
Group 2 and Group 4	-0,008	0,619
Group 3 and Group 4	-0,006	0,828

*significant differences

Table 3. The Significant Differences in OPG Mean's Between Two Groups

Group	Mean Differences	p
Group 1 and Group 2	-0,187	0,075
Group 1 and Group 3	-0,286	0,053
Group 1 and Group 4	-0,370	0,015*
Group 2 and Group 3	-0,986	0,746
Group 2 and Group 4	-0,182	0,274
Group 3 and Group 4	-0,084	0,864

*significant differences

Table 4. The Significant Differences in Osteoblast Mean's Between Two Groups

Group	Mean Differences	p
Group I and Group II	-1,17	0,004*
Group I and Group III	0,83	0,523
Group I and Group IV	-0,17	0,023*
Group II and Group III	2,00	0,008*
Group II and Group IV	1,00	0,154
Group III and Group IV	-1,00	0,058

*significant differences

Table 5. The Significant Differences in Osteoblast Mean's Between Two Groups

Group	Mean Differences	p
Group I and Group II	2.50	0.017*
Group I and Group III	1.00	0.243
Group I and Group IV	2.83	0.008*
Group II and Group III	-1.50	0.099
Group II and Group IV	1.33	0.527
Group III and Group IV	1.83	0.045*

*significant differences

have been done by observing biomolecular markers linked to the generation of osteoclasts, a factor in alveolar bone resorption and tooth loss in periodontitis.

Through RANKL and OPG blood levels, the number of osteoblasts and osteoclasts in the alveolar bone tissue of Wistar rat periodontitis, and gambir extract gel, this study seeks to examine the anti-osteoclastogenesis impact of gambir extract gel.

Material and Methods

This research received a certificate of animal ethics approval at the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, on June 7, 2021, with Number: B/118/UN14.2.9/PT.01.04/202. The research design was an experimental randomized posttest control group design. There were four research groups with each group consisting of 6 Wistar rats, group 1 negative control with topical administration of placebo gel, group 2 with positive control giving topical antibiotics, group 3 giving 8% gambier extract gel topically, and treatment group 4 administration of 10% gambier extract gel topically.

We are obtaining and producing gambir extract from the Laboratorium Kimia Bahan Alam dan Sintesis Departemen Kimia Fakultas MIPA Universitas Padjajaran. The manufacture of gambir gel extract and phytochemical tests at the laboratorium Farmakologi dan Bahan Alam Fakultas Farmasi Universitas Mahasaraswati Denpasar. The treatment of experimental animals and analyze the number of osteoblast osteoclast at the Laboratorium Histologi Universitas Udayana, and analyzed RANKL, OPG levels at Laboratorium Patologi Fakultas Kedokteran Hewan Udayana. The process of making gambir extract gel begins with the maceration process. First, Gambir leaves were cut into small pieces and then mashed, then extracted by maceration using 8L redest ethanol solvent for 1x24 hours. Then the maceration results are filtered through a filter funnel, and the filtrate is collected. The filtrate is then concentrated by evaporation using a rotatory evaporator at a temperature of ± 40°C to obtain a concentrated extract. Finally, the results of the methanol extract were obtained, and then made general gel preparations.

Induction of periodontitis and application gambir gel extract

Prior to induction, ketamine and xylazine were used to sedate the mice. Ketamine and xylazine dosages were administered subcutaneously and intramuscularly, respectively. Periodontitis is brought on by the silk ligature and LPS activation of Porphyromonas gingivalis (pg). First, the cervical lower incisor's subgingival area was ligatured in a figure-eight pattern with a 3.0 non-resorbable silk ligature to cause periodontitis. Then, to strengthen the floss on the ligated rat teeth, composite resin material was put to the rat teeth. Replace any ligatures that are found to be loose or missing. After that, a 30 G insulin needle was used to inject LPS Pg intrasulcularly into the

gingival sulcus of the labial right mandibular first incisor at a dosage of 5 g/0.05 ml PBS, three times a week. The samples were divided arbitrary into four groups after the initial induction.

A topical substance was administered on the seventh day following the development of clinical signs, including redness, loss of attachment, and deepening of the periodontal pocket as determined by a dental probe (eryzol). A 0.05 mL syringe was used to inject the substance into the gingival sulcus once every day for 21 days.

Blood and Alveolar Bone Tissue Collection

the mice were sacrificed for blood collection on day 28 following periodontitis induction. Ketamine was used as the agent of anesthesia. Blood was drawn through the orbital sinus, centrifuged to remove the serum, and then put in a tube filled with phosphate buffered saline (PBS), alufo, and stored at -20 degrees Celsius in a freezer. To count the osteoblasts and osteoclasts, take a sample of rat mandibular alveolar bone. Because the material was in the form of alveolar bone, it was first softened by soaking in HCL for a day or two before being placed in formalin. After washing the alveolar bone with running water, it is fixed with a 10% formalin solution for an hour.

Calculation RANKL, OPG Serum Levels and The Number of Osteoblast and Osteoclast

RANKL, OPG examination using the quantitative sandwich enzyme immunoassay (ELIZA) technique. RANKL, OPG-specific antibodies were coated on the microplate. Pipetted sample and standard into the well and an immobilized antibody will sandwich the presence of RANKL in the well. Remove unbound substances after washing, and an enzyme-linked polyclonal antibody specific to RANKL or OPG is added. Then, the substrate was added to the well after washing again to remove the unbound enzyme antibody reagent. Then a color will be formed, proportional to the amount of RANKL bound. The color formation was stopped, and then the color intensity was measured. Observation of osteoblast and osteoclast using the Olympus BX-51 microscope with 400x magnification on four tissue sections in five selected fields of view.

Data Analysis

The normality test (Shapiro-Wilk) revealed that the mean of RANKL and OPG in each group were normally distributed ($p>0.05$), whereas the homogeneity test (Levene) revealed that RANKL was homogeneous ($p>0.05$) and OPG was not homogeneous ($p<0.05$). The mean RANKL and OPG data were analyzed using the One-Way Anova Welch Test, which was observed in the Robust Test of Equality of Means, to discover differences between groups. To determine between which groups differences existed, the Gomes-Hewell test was conducted. The mean of osteoblasts and osteoclasts in one group was not normally distributed ($p>0.05$), the Kruskal-Wallis test was used to assess differences between groups based on the number of osteoblasts and osteoclast. In the meantime, the Post-Hoc Test with the Mann-Whitney U Test is administered to determine whether groups exhibit differences.

Results

The RANKL and OPG serum levels and the number of osteoclasts and osteoblasts were shown in table 1.

RANKL

The One Way Anova Welch test's significance analysis revealed that the $H = 5.911$ and p value $p=0.012$. This indicates that the mean RANKL differs across the four groups ($p < 0.05$). The Gomes-Hewell test was used to identify the groups where there are differences (table 2). The RANKL serum level in groups 2, 3, and 4 is lower and has significant differences than in group I.

OPG

The significance analysis of the One Way Anova Welch test results showed $H = 13.792$ and $p = 0.001$. This suggests that there are differences in the four groups' means of RANKL ($p < 0.05$). The groups with differences were found using the Gomes-Hewell test (table 3). The OPG serum level in group 4 higher and has significant differences than in group 1.

Osteoblast

The significance analysis of Kruskal-Wallis data on the number of osteoblasts showed $p= 0.004$. This indicates that the mean number of osteoblasts in the four groups differs ($p < 0.05$). The Mann-Whitney U Test was employed as a post hoc analysis (table 4). The number of osteoblast in group I was significantly less than in group 4, whereas the number of osteoblast in group 2 was significantly more than in group 3.

Osteoclast

The significance analysis of Kruskal-Wallis data on the number of osteobcasts showed $p= 0.013$. This indicates that the mean number of osteoclasts in the four groups differs ($p < 0.05$). The Mann-Whitney U Test was employed as a post hoc analysis (table 5). The number of osteoclast in group I was significantly less than in group 4, whereas the number of osteoblast in group 3 was significantly more than in group 4.

Discussions

Many studies have demonstrated that the bioactive component of gambir extract as a medical plant has been considered to have anti-inflammatory, antioxidant, and antibacterial properties. Gambir leaf extract contains catechin as the main compound as well as several other compounds such as catechu tannic acid, quercetin, red catechu, fluorescent gambir, fat and wax14,15,16. The phytochemical screening test of the gambir extract used in this study showed that it contains alkaloids, saponins, flavonoids, tannins, quinones, and steroids. Catechin is secondary metabolite compound derived from flavonoids which has anti-inflammatory17, antioxidant, and antibacterial14,15,16 and osteoprotective activity18. The content of catechins in gambier plants is higher (73.3%) than the content of catechins in tea leaves16.

In this study, administration of 10% gambir extract gel was shown to decrease RANKL serum levels, increase OPG serum levels, increase the number of osteoblasts and decrease the number of osteoblasts in the alveolar bone of Wistar rats with periodontitis due to LPS P.gingivalis-induced. According to Nakamura et.al. stated that catechins inhibited NF-kB activation induced by LPS stimulation19. Bacteria do not cause the destruction of periodontal tissues by themselves, they also generate an inflammatory immune response that contributes to the destruction20. The results of this study are in accordance with the research of Nurhayati et al., stated that Gambier toothpaste containing catechins was effective in reducing dental plaque. In periodontal diseases, the accumulation of bacterial plaque is commonly found in the periodontal pocket with the tooth surface located in the junction between gingiva and tooth14.

Gambir extract contains quercetin affects several inflammatory processes and immunological reactions. Quercetin has been demonstrated to be able to block LPS-induced IL-8 and TNF-alfa production. Additionally, quercetin prevents the production of histamine, tryptase, and inflammatory cytokines like IL-6, IL-8, and TNF from human cultured mast cells. Through altering NFk-B in human peripheral blood mononuclear cells, quercetin also prevents the synthesis and gene expression of TNF. Quercetin exhibited in vitro antibacterial action. In a rat model, quercetin also decreased the development of osteoclasts brought on by LPS21.

RANK is a cell surface receptor that functions as an activator of the NFkB receptor, a transmembrane protein that induces osteoclast precursor cells to develop into osteoclasts when activated by RANKL. If RANK and RANKL bind, osteoclast production is stimulated and the bone resorption process continues20. Osteoclastogenesis is predominantly regulated by macrophage-colony stimulating factor (M-CSF) and the receptor activation of nuclear factor kB (RANK)/RANK ligand (RANK-L)/osteoprotegerin (OPG) system. Mature osteoclasts with many nuclei initiate bone resorption and remodeling. In old stromal/osteoblastic cells, age-dependent augmentation in osteoblast-regulated osteoclastogenesis has been demonstrated by decreased OPG expression and increased expression of RANKL and M-CSF. Inflammatory cytokines, including as IL-1, TNF, IL-6, and IL-17, promote osteoclastogenesis at the expense of osteoblast development and function. In addition, interferon (IFN)-γ, interleukin (IL)-10, interleukin (IL)-4, and interleukin (IL)-12 decrease osteoclastogenesis, whereas IL-4 promotes osteoblast migration and proliferation and inhibits osteoblast differentiation18.

Catechin effects may be exerted directly on pre-osteoclasts/osteoclasts or indirectly by modification of mesenchymal stem cells (MSCs)/stromal cell control of pre-osteoclasts via activation of the nuclear factor kB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) system. Catechins can also promote osteoblastogenesis by promoting osteogenic differentiation of MSCs and osteoblastic survival, proliferation, differentiation, and mineralization. In vitro, catechin can increase osteoclast apoptosis, reduce osteoclastogenesis, and prevent bone resorption. Pre-osteoclasts and osteoclasts can be affected directly or indirectly by altering how MSCs and stromal cells control pre-osteoclasts. Through a Fenton reaction, EGCG treatments have been found to enhance the death of multinucleated cells that resemble osteoclasts while sparing osteoblasts. By preventing MMP-9 production in osteoblasts and osteoclastogenesis, catechin may reduce alveolar bone resorption in periodontal diseases18,22,23.

Conclusion

Gambir Extract gel 10% has anti-osteoclastogenesis effects via decreased RANKL and raised OPG serum levels, as well as increased osteoblast and decreased osteoclast number.

Acknowledgment

None.

Conflict of Interest

The authors declare no conflicts of interest

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