The potential of propolis extract gel Kabanjahe Kelulut bee as an anti-inflammatory agent in periodontal treatment: eksperimental laboratory study

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ABSTRACT

Introduction: Periodontitis treatment includes scaling and root planing, which can be supplemented with additional therapy, such as the administration of propolis. Propolis, a potential local natural resource, contains active metabolites that act as anti-inflammatory agents in periodontal healing. This study aims to analyze the effectiveness of propolis extract gel from Kelulut (stingless bees) as an anti-inflammatory agent in periodontal treatment. **Methods:** This laboratory-based experimental research employed a post-test-only control group design, divided into five groups: 50%; 60%; 70% propolis extract gel; Metronidazole Gel and Placebo Gel. The anti-inflammatory test was conducted using 50 male Wistar rats induced with periodontitis through silk ligature and *P.gingivalis* bacteria. The rats were sacrificed on the third and seventh days after treatment, and macrophage cells count were performed under a microscope at 400x magnification using Haematoxylin-Eosin staining. **Results:** The ANOVA test results showed no significant differences in the macrophage cells counts among the treatment groups on day 3 and day 7 in the periodontitis-induced rat model ($p>0.05$). The anti-inflammatory test results indicated a decrease in the mean macrophage cells count in each group by day 7. **Conclusion:** The use of Kelulut propolis extract gel from Kabanjahe was effective in reducing the number of macrophages in periodontitis-induced rats, with the 70% propolis extract gel demonstrating the best anti-inflammatory effectiveness in periodontal treatment.

Keywords

periodontitis, propolis, anti-inflammatory, macrophage

Potensi gel ekstrak propolis lebah kelulut kabanjahe sebagai agen anti inflamasi pada perawatan periodontal: eksperimental laboratoris

ABSTRAK

Pendahuluan: *Perawatan periodontitis meliputi scaling dan root planing serta dapat didukung dengan terapi tambahan berupa pemberian propolis. Propolis merupakan salah satu sumber daya alam lokal yang potensial, mengandung metabolit aktif yang berperan sebagai zat anti-inflamasi dalam penyembuhan periodontal. Penelitian ini bertujuan untuk menganalisis efektivitas gel ekstrak propolis dari lebah Kelulut (stingless bees) sebagai anti-inflamasi pada perawatan periodontal.* ***Metode:*** *Penelitian eksperimental laboratoris ini memiliki desain post-test-only control group design yang dibagi ke dalam lima kelompok yaitu kelompok gel ekstrak propolis 50%; 60%; 70%, gel Metronidazole dan gel Placebo. Pengujian anti-inflamasi menggunakan 50 ekor tikus wistar jantan yang diinduksi periodontitis dengan ligatur Silk 3/0 dan bakteri P.gingivalis. Tikus dikorbankan pada hari ketiga dan hari ketujuh setelah perlakuan, kemudian dilakukan penghitungan sel makrofag dengan mikroskop pada perbesaran 400x dengan pewarnaan Haematoxylin-Eosin.* ***Hasil:*** *Hasil uji ANOVA, tidak terdapat perbedaan bermakna pada setiap kelompok perlakuan terhadap jumlah makrofag. sel pada hari ke-3 dan hari ke-7 pada model tikus periodontitis ($p<0.05$). Hasil uji anti-inflamasi menunjukkan adanya penurunan rerata jumlah sel makrofag pada masing-masing kelompok pada hari ke-7.* ***Simpulan:*** *Terdapat efektivitas penggunaan gel ekstrak propolis lebah Kelulut Kabanjahe terhadap jumlah makrofag pada tikus yang diinduksi periodontitis dan gel ekstrak propolis 70% memiliki efektivitas terbaik sebagai anti-inflamasi pada perawatan periodontal.*

Kata kunci

periodontitis, propolis, anti-inflamasi, makrofag

INTRODUCTION

Periodontitis is an inflammatory condition that arises from untreated gingivitis, leading to an infection caused by a specific group of microorganisms known as periodontopathogenic bacteria. This condition result in the destruction of the supporting tissues of the teeth.[1] Periodontitis is characterized by apical migration of junctional epithelium, destruction of the periodontal ligament, loss of attachment, and alveolar bone resorption.[2] The severity of periodontitis is closely linked to poor oral hygiene, which facilitates the build-up of microbial plaque.[3] The primary cause of periodontitis is the accumulation of bacteria and pathogenic plaque, which triggers an inflammatory response in the gingiva, leading to tissue damage in several pathogenic organism accumulation areas.[4]

Four bacteria play a critical role in the initiation and progression of periodontal disease, including *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Prevotella intermedia*.[5] *Porphyromonas gingivalis* bacteria is the most dominant bacterium causing chronic periodontitis. This obligate anaerobic Gram-negative bacterium attached to subgingival plaque.[6] Subgingival plaque bacteria can cause damage to periodontal tissues and deepening the gingival sulcus, which increases pockets depth.[7]

The primary treatment for periodontal disease is mechanical therapy, including scaling and root planing, which aims to remove hard and soft deposits as well as bacteria attached to the tooth surface and within subgingival area.[8] However, the removal of periodontal pathogens through scaling and root planing may not yield optimal results in some patients due to limitations in accessing deep pockets, furcation involvement, and challenges in eliminating bacteria from the dentinal tubules. Therefore, alternative treatments are needed to enhance the success of periodontal therapy.[9] Chemical treatment, including the use of anti-inflammatory agents and antimicrobial therapy, can support the treatment of periodontal disease.[10]

Antimicrobial treatments can be administered locally or systemically through antibiotics and antiseptics.[11] The use of antibiotics in antimicrobial therapy is beneficial in preventing and treating periodontal disease. However, antibiotic use carries risks, such as the development of antibiotic resistance due to inconsistent use, the potential for fungal infections, and the possibility of allergic reactions.[12]

In addition to antibiotic treatment, chlorhexidine, an antibacterial agent, can enhance the recovery from periodontal disease. However, chlorhexidine use is associated with several side effects, including the discoloration of restorations, teeth in the gingival third, interproximal teeth, and the tongue, as well as a reduction in taste sensation.[12,13] Consequently, research has explored the use of herbal plants with antibacterial properties.[12]

One of the herbal ingredients with anti-inflammatory and antibacterial effects is propolis, which is recognized for its numerous health benefits and effectiveness in natural medicine. Propolis from stingless bees is produced from a combination of plant resins, saliva secretions, wax, and soil.[14] Studies have shown that propolis extracts exert anti-inflammatory activity.[15] A study by Alanazi et al. (2020) demonstrated that propolis extracts exert an anti-inflammatory effect by the inhibiting pro-inflammatory cytokines and reprogramming the metabolic activity of Lipopolysaccharides (LPS) in macrophages.[16]

The efficacy of propolis extracts from various geographical locations has been shown to reduce the expression of the pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 in macrophage.[17] Propolis contains 10-20% flavonoids, which are among the most potent natural antibiotics, capable of healing and slightly reducing pain.[18] Flavonoids in propolis act as anti-inflammatory agents by inhibiting prostaglandin synthesis, stimulating immune cells, and enhancing the immune system by promoting phagocytosis activity.[19] Propolis also contains alkaloids, saponins, tannins, and triterpenoids, which are known for their antibacterial properties.[18]

A previous study by Gani et al.[12] demonstrated that a 50% propolis gel has inhibitory effect on the growth of *P. gingivalis*.[12] In a pilot study, the effectiveness of propolis extract

on *P. gingivalis* growth was evaluated, revealing that a 70% propolis extract was the minimum bactericidal concentration required to kill the *P. gingivalis*. This study aims to analyze the effectiveness of propolis extract gel from Kelulut (stingless) bees as an anti-inflammatory agent in periodontal treatment.

METHODS

The research design used was a laboratory experimental study with a post-test-only control group design, where measurements were taken only after the treatment was complete. This study utilized stingless bee propolis extract from Kebun Efi, Kacinambun Village, Kabanjahe, North Sumatra. Pure isolates of *Porphyromonas gingivalis* bacteria ATCC 33277®™ were used to induce periodontitis in rat models. The bacteria were cultured using Brucella Sheep Blood Agar (5%) solid media and Brain Heart Infusion Broth (BHIB) liquid media. A solution of 43.1 g of Brucella Sheep Blood Agar (5%) powder was dissolved into 1 liter of distilled water, then heated on a hot plate magnetic stirrer.

The media was then sterilized in an autoclave (Hirayama HVE-50) at 121°C and 15 psi for 15 minutes. After sterilization, the media were cooled to 40-45°C in a refrigerator. Vitamin supplement and 5% sheep blood were added to the media and stirred until homogeneous. The BHIB liquid media was made by dissolving Brain Heart Infusion Broth powder in distilled water and heated on a hot plate magnetic stirrer. *Porphyromonas gingivalis* bacteria ATCC 33277®™ were then cultured and maintained under anaerobic conditions.

This study involved 50 male Wistar rats aged 2-3 months, with a body weight ranging from 200-250 grams, all in healthy condition. The rats were housed in suitable conditions to allow for adaptation, with access to food, water, and environmental enrichment to ensure their well-being and minimize stress, which could affect the experimental results. The study comprised five treatment groups: three groups treated with propolis extract gel at concentrations of 50%, 60%, and 70%; one positive control group treated with metronidazole gel; and one negative control group treated with a placebo gel.[20,21]

The negative control group receives a placebo gel, designed to mimic the appearance and application of the treatment but without the active ingredient, to determine if the observed effects were due to the active ingredients or merely the gel application process. The positive control group receives a treatment known to be effective for the condition under study, validating the experimental setup and ensuring that the study could detect treatment effects. Each group consisted of five rats, with sample sizes determined using the Federer formula.[22] The research was conducted using a simple random sampling technique, and data collection took place at the Animal House, Faculty of Mathematics and Sciences, Universitas Sumatera Utara.

In testing the effectiveness of anti-inflammatory drugs, 50 male Wistar rats were induced periodontitis by ligating 3/0 silk ligatures on the cervical part of the incisor teeth on one side of the mandible. After seven days of silk installation, the procedure was followed by the induction of *Porphyromonas gingivalis* bacteria at a concentration of 0.1 ml.

The propolis extract used in the experimental animals was formulated into a gel. After seven days of inducing periodontitis in rats, propolis extract gel, metronidazole gel, and placebo gel were applied. The gel was administered topically to the periodontal pockets or gingival sulcus area using a disposable tip. Fifty rats were sacrificed on the third and seventh days after treatment. The animals were first anesthetized, then euthanized by decapitation, and the anterior segment of the mandible was collected. Histological preparations were made using hematoxylin-eosin staining. Segment observations were conducted using a microscope at 400x magnification, and the number of macrophage cells was counted in four fields of view for each slide. Statistical analysis was performed to assess the effectiveness of each treatment group on macrophage count. A univariate analysis was conducted to observe the mean macrophage count within each group. Normality and homogeneity tests indicated a non-normal data distribution ($p<0.05$). Consequently, bivariate analysis using the non-

parametric Kruskal-Wallis test was employed to analyze differences across all sample groups, followed by the Mann-Whitney test to examine significant differences between specific groups.

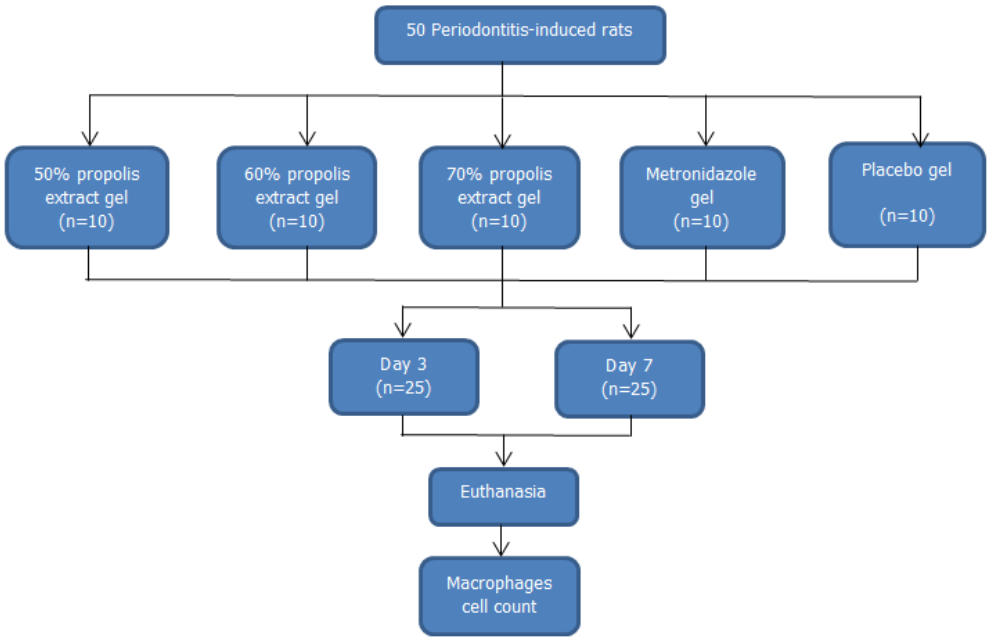

Figure 1. Experimental procedure flowchart

RESULTS

Rats that have been induced periodontitis for seven days show clinical signs of periodontitis, such as a reddish color of the gingiva and changes in the contour of the gingiva. The average number of macrophage cells in each treatment group was found to have abnormally distributed data because the significance value was p=0.27 (p<0.05). Therefore, the Kruskal-Wallis test was continued to determine significant differences in the average number of macrophage cells in each treatment group.

Table 1. Differences in the mean number of macrophage cells on day 3 and day 7 in the treatment groups

Observation	Groups	Mean±SD	*p-value*
Day 3	Placebo gel	0.15±0.13	0.741
	50% propolis gel	0.75±1.40	
	60% propolis gel	0.50±0.11	
	70% propolis gel	1.50±2.54	
	Metronidazole gel	0.90±1.24	
Day 7	Placebo gel	0.30±0.67	0.476
	50% propolis gel	0.00±0.00	
	60% propolis gel	0.15±0.13	
	70% propolis gel	0.55±0.79	
	Metronidazole gel	0.55±0.75	

Kruskal-Wallis test;*p significant p<0.05

Based on the results of Kruskal-Wallis testing, table 1 shows that each group experienced a decrease in the number of macrophage cells on day seven except the placebo gel group. However, there was no significant difference in each treatment group on the

number of macrophage cells on day 3 and day 7 in the periodontitis rat model, as seen from the p-value ($p<0.05$).

Table 2. The mean difference in macrophage cell counts between treatment groups on days 3 and 7

Groups		p-value	
		Day 3	Day 7
Placebo Gel	50% propolis gel	0.729	0.317
	60% propolis gel	0.221	0.403
	70% propolis gel	0.910	0.521
	Metronidazole gel	0.910	0.606
50% propolis gel	60% propolis gel	0.189	0.050
	70% propolis gel	0.737	0.136
	Metronidazole gel	0.737	0.136
60% propolis gel	70% propolis gel	0.368	0.910
	Metronidazole gel	0.368	0.910
70% propolis gel	Metronidazole gel	0.638	1.000

Mann-whitney test; *p significant $p<0.05$

Table 2 shows that the number of macrophage cells in all comparisons between the two groups has no significant difference. Based on the results obtained, day 3 and 7 showed that the average number of macrophage cells in the 70% propolis gel group was greater than the metronidazole gel group. The results also showed that the average number of macrophage cells in the placebo gel group did not decrease. The results of histopathological anatomy examination using a binocular microscope at 400X magnification in the graticule box with four fields of view in the preparation showed no difference in the number of macrophages. This is supported by the results of the Kruskal-Wallis test, which states that there is no significant difference in propolis gel on the number of macrophage cells in periodontitis rats ($p>0.05$).

DISCUSSION

Based on the results of the anti-inflammatory effectiveness test, there was no significant difference in the number of macrophage cells across the groups. The Mann-Whitney test results showed no significant difference in the mean number of macrophage cells on day 3 and day 7 among the propolis gel concentrations of 50, 60, and 70%. However, the 70% propolis gel has the greatest decrease in the average number of macrophage cells compared to other propolis gel concentration groups (Table 1). Macrophages play a role in defending against bacterial infection, antigen presentation, mobilization, and regulation of the immune defense response, maintaining the balance between host and microorganisms. They are also vital in the termination of inflammation and tissue repair processes.[23] Studies have shown that macrophage activity is associated with an increased prevalence of periodontitis with age. Clark et al.[24] found that the expressions of M1-related markers, pro-inflammatory cytokines, and chemokines significantly increased in elderly mice in their study of periodontal tissue. However, while there was no significant difference in the number of macrophages between young and elderly mice, the elderly mice exhibited faster macrophage depletion during the recovery stage.[24,25] Although this early depletion of macrophages may halt inflammation sooner, it remains unclear whether the tissue rebuilding and healing process also concludes permaturely.

In this study, no significant differences were observed in the number of macrophage cells between treatment groups on day 3 and day 7 in the periodontitis rat model, as indicated by the p-value (<0.05) (Table 2). A p-value below 0.05 typically indicates a

significant difference in means between groups[26] Propolis suppresses the formation of prostaglandins and leukotrienes by inhibiting the expression and activity of cyclooxygenase and lipoxygenase.

Propolis also slows the expression of nitric oxide synthase genes and the NF-κB activation mediated by TNF-α. This inhibition of NF-κB activation may be the molecular basis for the anti-inflammatory properties of propolis.[27] TThe flavonoids in propolis further contribute to its anti-inflammatory effects by inhibiting COX-2, thereby reducing the formation of prostaglandins and, consequently, the number of inflammatory cells in inflamed tissues. Eyarefe et al. observed that propolis induced a higher level of inflammatory infiltrates, such as monocytes, macrophages, eosinophils, mast cells, and platelets. However, this inflammatory response regressed by day 8, resulting in significantly better wound healing compared to the untreated control group.[29]

In dentistry, propolis is recognized for its antimicrobial and anti-inflammatory activities, making it applicable in various specialties, including periodontics. Propolis has been reported as a potent anti-inflammatory agent, with recent in vitro and in vivo studies further investigating its effects on inflammation. Caffeic acid phenethyl ester (CAPE), a primary component of propolis derived from honeybee hives, is particularly noted for its anti-inflammatory properties.[31] In periodontal tissues, macrophages play a critical role in mainting tissue homeostasis and defense. However, excessive aggregation and activation of macrophages can also lead to periodontal tissue damage. Bacteria and their products, such as endotoxin, can activate the monocyte/macrophage system, leading to the production of a large number of pro-inflammatory factors that can trigger inflammation or an immune response.[32,33] Macrophages (Møs, Macs) are innate immune cells located on the surface of the epithelium that quickly respond to infection. The epithelium of periodontal tissue is highly permeable, facilitating interactions between macrophages and the oral environment.[25]

Macrophages exhibit distinct responses to stimuli from pathogen and symbiotic bacteria. They are key cells in the pathology of chronic inflammation, characterized by their ability to adapt to different tissue microenvironments and responses to various pathogenic injuries. This adaptability aligns with the diverse nature of chronic inflammatory responses.[33,34]

In addition to defending against bacterial infections, macrophages are involved in antigen presentation, mobilization and regulation of the immune defense response, and maintaining the balance between the host and microorganisms. As efficient antigen-presenting cells, macrophages stimulate T cells, phagocytose pathogens, secrete cytokines to amplify the immune system, induce and expand inflammation, catalyze the local inflammatory response, and stimulate tissue destruction.[35,36]

Polyphenols demonstrate notable immunostimulatory effects on macrophages, including enhancing the proliferation of T and B cells.[37] Propolis has been shown to promote the recruitment of leukocytes, including macrophages, without triggering ROS release. Additionally, the tissue repair induced by propolis is linked to macrophage polarization (from M1 to M2) and the downregulation of IGF1 expression, independent of Nrf2. Propolis also reduces the migration of immune cells, such as macrophages and neutrophils, possibly by downregulating chemokines CXCL9 and CXCL10.[38]

Based on the results obtained from previous research, there was a decrease in the number of *Porphyromonas gingivalis* bacterial colonies as the concentration of stingless bee propolis extract increased. This is likely due to the higher concentration of active substance in propolis extract, which have antibacterial properties. This study utilized pure isolates of *Porphyromonas gingivalis* bacteria ATCC 33277®TM. *Porphyromonas gingivalis* bacteria are Gram-negative pathogenic bacterium responsible for periodontal disease. The cell wall structure of Gram-positive bacteria is composed of 90% peptidoglycan and polysaccharides (teichoic acid), whereas Gram-negative bacteria have only 5-20% peptidoglycan and a significant amount of lipopolysaccharides and lipoproteins. This structural difference makes Gram-positive bacteria more susceptible to inhibition by antibacterial compounds compared to Gram-negative bacteria.[24]

Research conducted by Amanda et al.[39] showed that the flavonoid extract of *Trigona Sp*. Propolis has an antibacterial effect, as evidenced by a reduction in the number of *Porphyromonas gingivalis* bacterial colonies on solid media. The decrease in the number of colonies is attributed to the presence of antibacterial compounds, particularly flavonoids, which are derived from phenol compounds.

Phenol-derived compounds interact with bacterial cells through an adsorption process involving hydrogen bonds. At low concentrations, phenol-protein complexes form weak bonds that rapidly decompose, allowing phenol to penetrate the cells, leading to precipitation and protein denaturation. At high concentrations, phenol causes coagulation of cell proteins and lysis of the cytoplasmic membrane. However, when collecting clinical data in mice, such as pocket probing, accuracy may be limited due to the lack of precise measurement tools during the research process.

CONCLUSIONS

The application of stingless bee propolis extract gel has shown effectiveness in reducing the number of macrophages in periodontitis-induced rats, with the 70% propolis extract gel from Kelulut Kabanjahe bees demonstrating the most significant anti-inflammatory effects in periodontal treatment. However, there was no statistically significant difference in macrophage reduction among the intervention groups. The findings suggest that this treatment could potentially promote faster healing in periodontal patients, contributing to improved periodontal health.

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