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Original Article

BENEFITS OF PROPOLIS PERIODONTAL CHIP IN PERIODONTITIS-INDUCED MICE: CLINICAL PARAMETERS AND EXPRESSION OF INTERLEUKIN-1β (IL-1β) EVALUATION

YULIA KAREN[1], RINI OCTAVIA NASUTION[2*], YUANDANI[3], IRMA ERVINA[2], PITU WULANDARI[2]

[1]Periodontics Residency Program, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia. [2]Department of Periodontics, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia. [3]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

*Corresponding author: Rini Octavia Nasution; *Email: rini.octavia@usu.ac.id

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ABSTRACT

Objective: Periodontitis is an infection of the tissues that support the teeth. This infection can cause serious damage to the gums, bone, cementum, and periodontal ligaments that hold the teeth in place. Scaling and root planing (SRP) is the standard non-surgical treatment for periodontal disease. This treatment involves removing plaque and calculus from the surfaces of the teeth and roots. Scaling and root planing are effective in eliminating harmful bacteria that cause infection, but they have limitations in reaching deep periodontal pockets and furcation areas. This may necessitate additional antimicrobial therapy to help control the infection. Propolis is a resinous substance produced by bees that has antibacterial and anti-inflammatory properties that have been shown to be beneficial in the treatment of various health conditions. Previous research has shown that 20% hydroalcoholic propolis extract as an additional therapy decreased probing depth score on periodontal pocket. Therefore, this study investigated the efficacy of propolis with lower concentration in reducing inflammation and *Interleukin 1-β* (IL-1β) levels. This research purposed to analysis the effectiveness of the application of periodontal propolis chip as adjuvant therapy in male Wistar rats on clinical parameters and *Interleukin 1-β* (IL-1β).

Methods: An experimental laboratory study with a posttest control group design was conducted on 45 male Wistar rats induced with periodontitis using *Porphyromonas gingivalis* and ligated with silk. The rats were then divided into five treatment groups: placebo group (application of placebo periodontal propolis chip), positive control group (application of 2.5 mg chlorhexidine chip), treatment group 1 (application of 2.5% periodontal propolis chip), treatment group 2 (application of 5% periodontal propolis chip), and treatment group 3 (application of 10% periodontal propolis chip). Clinical parameter assessments (Gingival Index (GI), Bleeding on Probing (BoP), Pocket Depth (PD), Clinical Attachment Loss (CAL)) and IL-1β expression were performed on days 0, 3, and 7 after treatment in the rats.

Results: The results of this study showed significant improvements and decreases in the clinical parameters GI, BoP, PD, and CAL. Examination of IL-1β expression also showed a significant statistical decrease ($p<0.05$).

Conclusion: The application of a periodontal propolis chip can significantly improve GI, BoP, PD, and CAL and reduce IL-1β expression

Keywords: Interleukin-1β, Periodontal propolis chips, Periodontitis, Adjunctive therapy, Periodontal chip

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INTRODUCTION

Periodontal disease is one of the dental and oral health problems whose prevalence is relatively high worldwide. The Global Burden of Disease Study reports that severe periodontitis ranks 11th among the most common disease conditions in the world, with a global prevalence of about 12.5% or over 1 billion people worldwide had severe periodontitis in 2021 [1, 2]. Basic Health Research (Riskesdas) shows that the prevalence of periodontitis in Indonesia reaches 74.1% [3]. This high number shows the need for more effective prevention and treatment of periodontal disease.

The development of periodontal disease begins with gingivitis, which is limited inflammation to the gingiva that is reversible. However, if left untreated, this condition can develop into periodontitis, characterized by damage to connective tissue and alveolar bones [4]. The inflammatory process in periodontitis involves various pro-inflammatory mediators, including Interleukin-1β (IL-1β), tumor necrosis factor (TNF)-α, IL-6 and IL-17 [5]. IL-1β is a major pro-inflammatory cytokine that plays a major role in the occurrence of periodontal destruction. These cytokines are produced primarily by immune cells such as monocytes, macrophages and dendritic cells in response to microbial stimuli. IL-1β has the ability to promote bone resorption and induce the production of proteinases that can damage periodontal tissue [6].

The treatment of periodontal disease is generally divided into surgical and non-surgical treatments. Non-surgical treatments include mechanical debridement, either manually or using ultrasonic instruments, which is the standard in periodontal therapy. However, in certain cases such as periodontal pockets depth or furcation involvement, additional therapy is required to improve the effectiveness of treatment [7]. One of the additional therapy options that is often used is the administration of antimicrobials, both systemic and local. Although the use of systemic antibiotics can help manage infections in many areas, it has some drawbacks, such as the risk of side effects, antibiotic resistance, and possible patient non-compliance [8]. The use of local antimicrobial agents is an attractive alternative because it can overcome the limitations of systemic antibiotics. One of the products that has been widely used is PerioChip®, which is a chip that contains 2.5 mg of chlorhexidine gluconate. This product is designed as an adjunctive therapy for scaling and root planing (SRP) in the treatment of chronic periodontitis [9].

Propolis, a natural product produced by honeybees, has long been known to have a variety of health benefits, including antimicrobial and anti-inflammatory effects. This material consists of a complex mixture of bioactive compounds such as polyphenols, flavonoids, and various other organic compounds [10]. Several studies have shown the potential of propolis in the treatment of periodontal disease. A study by Otreba *et al.* demonstrated the antimicrobial activity of propolis against various strains of bacteria that cause periodontal disease [11]. Meanwhile, Kirti *et al.* l's research reported that periodontal pocket irrigation using post-skeletal propolis and root planing gave better results compared to the use of chlorhexidine or saline [12]. One of the main compounds in propolis that plays an important role in its anti-inflammatory effects is Caffeic acid phenethyl ester (CAPE). CAPE is known to inhibit the release of arachidonic acid from cell membranes and reduce the expression of the enzymes cyclooxygenase (COX) and lipoxygenase (LOX) involved in the inflammatory pathway. In addition, CAPE is also able to inhibit

the production of pro-inflammatory cytokines and increase the production of anti-inflammatory cytokines. *In vitro* research conducted by Asgharpour *et al.* showed that ethanol propolis extract was able to inhibit the production of nitric oxide (NO) and reactive oxygen species (ROS) and reduce the expression of COX-2, IL-1β, and IL-6 genes in macrophage cells [13]. Based on the potential of propolis, the development of periodontal chip preparations containing propolis is an interesting idea to be further researched. The use of periodontal chips allows for the controlled and continuous release of active ingredients in the target area, so it is expected to increase the effectiveness of therapy. In addition, the formulation in the form of a chip also facilitates application and can improve patient compliance. However, research on the effectiveness of periodontal propolis chips in the treatment of periodontitis is still limited. Therefore, further studies are needed to evaluate the benefits of the application of periodontal propolis chips as an adjunct therapy to periodontitis, especially in terms of its effect on clinical parameters and expression of inflammatory mediators such as IL-1β.

The study aimed to evaluate the effectiveness of propolis periodontal chips in various concentrations on the cure of periodontitis, focusing on clinical parameters and IL-1β expression as inflammatory markers. This comprehensive approach allows for an in-depth assessment of the therapeutic potential of propolis in the management of periodontal disease, providing valuable insights into anti-inflammatory and regenerative mechanisms at the tissue and molecular levels.

MATERIALS AND METHODS

This study is an *in vivo* laboratory experimental study with a pre and posttest control group design for clinical parameters and a posttest only control group design for immunohistochemical expression. This study used propolis chips with propolis content of 2.5%, 5% and 10% where at concentrations of 2.5% and 5% it was non-cytotoxic and at concentrations of 10% it was found to be mildly cytotoxic to gingival cells where CHX was classified as moderately cytotoxic [14]. Male Wistar rats (*Rattus norvegicus*) were utilized as experimental animals by researchers because they could produce more consistent study results than female Wistar rats since they were not impacted by the estrus cycle or pregnancy [15]. The of male Wistar rats aged 2-3 mo weighing 200-250 g are used as animal models. The results of the sample size calculation were calculated using the Federer formula and obtained a total of 5 (4,75) wistar rats each group for 5 treatments, namely placebo chips, 2.5% propolis chips, 5% propolis chips, 10% propolis chips, and 2.5 mg chlorhexidine chips. The total number of experimental animals needed was 25 for each group. To anticipate dead rats, 20% of the samples obtained were added. The total sample in this study was 30 samples on the 3rd day and the 7th day. The total number of samples needed was 60. Induction of periodontitis was performed on the mandibular incisive teeth of mice using a combination of silk thread ligation, junctional epithelial destruction, and Wistar rats injected with 0.05 ml of live *Porphyromonas gingivalis* ATCC 33277 with a population density of 2.59 x 108 CFU/ml. Using a 1cc needle syringe, administer 0.25 ml of bacteria *Porphyromonas gingivalis* intramuscularly to the buccal mandible of the mandibular anterior. A two-week high-carbohydrate diet were given [16]. Confirmation of *Porphyromonas gingivalis* infection is done through microbiological culture of gingival sulcus swabs. After successful induction, periodontal chips are applied to the gingival pockets according to the treatment group. The propolis chips used are made using a mixture of propolis, sodium carboxymethyl cellulose, hydroxypropyl methylcellulose, and propylene glycol formed into a square with a size of 1 x 1.5 mm. The propolis chips used have biodegradability properties. After the propolis chips are inserted, the periodontal pack is attached to keep the periodontal chips in the periodontal pocket. The clinical parameters evaluated included Gingival Index (GI), Bleeding on Probing (BoP), Pocket Depth (PD), and Clinical Attachment Loss (CAL). Measurements were taken on baseline, 3rd and 7thdays after scaling has been done. Clinical parameters was evaluated using Mani® finger plugger size 15, which has been marked with a distance of 1 mm as modified probe. Pocket measurements were carried out directly by researchers who are students of the periodontics specialist education program. In the measurements, there were constraints where the pressure given by the researcher could not be measured. For immunohistochemical analysis, tissue samples were taken from the mandibular segment on 3rd and 7th.

Standard histological procedures are performed, including decalcification, fixation, dehydration, clearing, embedding, and sectioning. Specific immunohistochemical staining for IL-1β polyclonal antibody (Bioss antibodies®) was performed, followed by evaluation using the Immunoreactive Score (IRS). Bioss's IL-1 Beta Polyclonal Antibody (BS-6319R) was dilute at 1:400. Observation of histological preparations using an Olympus C31 microscope (Olympus, Japan) with 10x ocular magnification and 40x objective lens magnification. Immunoreactive score (IRS) is used to see the expression of cell protein staining with a wider spectrum. There are two indicators assessed in IRS, namely the percentage of cell distribution and the intensity of cell staining. Data analysis involves testing the normality of the Shapiro-Wilk and the homogeneity of Levene. Non-parametric data were analysed using the Wilcoxon test for pre-and post-treatment comparisons, Kruskal-Wallis for intergroup effectiveness, and Mann-Whitney for pair comparisons. The statistical significance was set at $p<0.05$. This research has received ethical approval from the Animal Research Ethics Commission, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara ("0676/KEPH-FMIPA/2023").

RESULTS

Evaluation of clinical parameters after application of subgingival periodontal chip propolis in periodontitis-induced male Wistar rats as a support for initial therapy on baseline, 3rd and 7th d

Table 1 showed that on the 3rd day there was a significant difference in IG after application of 10% propolis periodontal chips, and 2.5 mg chlorhexidine periodontal chips when compared to placebo periodontal chips in Wistar rats induced by periodontitis ($p = 0.02$) while on the 7th day of application of 5% propolis periodontal chips ($p = 0.01$), 10% ($p = 0.01$), and 2.5 mg chlorhexidine periodontal chips ($p = 0.00$) showed a significant difference. 2.5% propolis periodontal chips ($p = 0.04$), 5% ($p = 0.01$), and 10% ($p = 0.01$) also showed a significant difference with 2.5 mg chlorhexidine periodontal chips.

Table 1: Gingival index (GI) differences after subgingival application between periodontal chips

Gingiva index (GI) measurement				
Group		**p-value**		
		Day 0 (p-value)	**Day 3 (p-value)**	**Day 7 (p-value)**
Periodontal placebo chips	Periodontal chlorhexidine chips 2.5 mg	1,00	0,01*	0,00*
	Periodontal propolis chips 2,5%	1,00	0,05	0,05
	Periodontal propolis chips 5%	1,00	0,05	0,01*
	Periodontal propolis chips 10%	1,00	0,02*	0,01*
Periodontal chlorhexidine chips 2.5 mg	Periodontal propolis chips 2,5%	1,00	0,27	0,04*
	Periodontal propolis chips 5%	1,00	0,50	0,01*
	Periodontal propolis chips 10%	1,00	1,00	0,01*
Periodontal propolis chips 2,5%	Periodontal propolis chips 5%	1,00	0,74	0,50
	Periodontal propolis chips 10%	1,00	0,27	0,31
Periodontal propolis chips 5%	Periodontal propolis chips 10%	1,00	0,50	0,55

*significance $p<0.05$, GI gingiva index

Table 2 showed that on the 3rd day there was a significant difference in BoP after application of 10% propolis periodontal chips, and 2.5 mg chlorhexidine periodontal chips when compared to placebo periodontal chips in Wistar rats induced by periodontitis (p = 0.02) while on the 7th day of application of 5% propolis periodontal chips (p = 0.01), 10% (p = 0.01), and 2.5 mg chlorhexidine periodontal chips (p = 0.00) showed a significant difference. 2.5% propolis periodontal chips (p = 0.04), 5% (p = 0.01), and 10% (p = 0.01) also showed a significant difference with 2.5 mg chlorhexidine periodontal chip.

Table 2: Bleeding on probing (BoP) differences after subgingival application between periodontal chips

Bleeding on probing (BoP) measurement				
Group		**p-value**		
		Day 0 (p-value)	**Day 3 (p-value)**	**Day 7 (p-value)**
Periodontal placebo chips	Periodontal Chlorhexidine Chips 2.5 mg	1,00	0,01*	0,00*
	Periodontal propolis chips 2,5%	1,00	0,05	0,05
	Periodontal propolis chips 5%	1,00	0,05	0,01*
	Periodontal propolis chips 10%	1,00	0,02*	0,01*
Periodontal Chlorhexidine Chips 2.5 mg	Periodontal propolis chips 2,5%	1,00	0,27	0,04*
	Periodontal propolis chips 5%	1,00	0,50	0,01*
	Periodontal propolis chips 10%	1,00	1,00	0,01*
Periodontal propolis chips 2,5%	Periodontal propolis chips 5%	1,00	0,74	0,50
	Periodontal propolis chips 10%	1,00	0,27	0,31
Periodontal propolis chips 5%	Periodontal propolis chips 10%	1,00	0,50	0,55

*significance p<0.05, BoP Bleeding on probing table 3 shows that there is a significant difference in PD after subgingival application of propolis periodontal chips 2.5% (p=0.01), 5% (p=0.01), 10% (p=0.01), and chlorhexidine 2.5 mg (p=0.01), with placebo periodontal chips in male Wistar rats induced with periodontitis on the 3rd day. On the 7th day, significant differences in PD were also seen after subgingival application of 2.5% propolis periodontal chips (p=0.02), 5% (p=0.03), 10% (p=0.01), and 2.5 mg chlorhexidine (p=0.01), with placebo periodontal chips

Table 3 above also shows that there are significant differences in PD after subgingival application of 2.5 mg chlorhexidine periodontal chips with 2.5% propolis periodontal chips in male wistar rats induced by periodontitis on the 3rd day (p=0.01) and 7th day (p=0.02), while between 2.5 mg chlorhexidine periodontal chips and 5% and 10% propolis periodontal chips there was no significant difference (p>0.05), thus indicating that 5% and 10% propolis periodontal chips are comparable to 2.5 mg chlorhexidine periodontal chips.

Table 3: Pocket depth (PD) differences after subgingival application between periodontal chips

Pocket depth (PD) measurement				
Group		**p-value**		
		Day 0 (p-value)	**Day 3 (p-value)**	**Day 7 (p-value)**
Periodontal placebo chips	Periodontal Chlorhexidine Chips 2.5 mg	0,24	0,01*	0,01*
	Periodontal propolis chips 2,5%	0,74	0,01*	0,02*
	Periodontal propolis chips 5%	0,12	0,01*	0,03*
	Periodontal propolis chips 10%	0,33	0,01*	0,01*
Periodontal Chlorhexidine Chips 2.5 mg	Periodontal propolis chips 2,5%	0,15	0,01*	0,02*
	Periodontal propolis chips 5%	0,74	0,14	0,16
	Periodontal propolis chips 10%	0,75	0,50	0,11
Periodontal propolis chips 2,5%	Periodontal propolis chips 5%	0,08	0,13	0,36
	Periodontal propolis chips 10%	0,29	0,11	0,08
Periodontal propolis chips 5%	Periodontal propolis chips 10%	0,92	0,59	0,65

*significance p<0.05, PD pocket depth

Table 4 showed that there is a significant difference in CAL after subgingival application of placebo periodontal chips with propolis 2.5% (p=0.01), 5% (p=0.01), 10% (p=0.01), and chlorhexidine 2.5 mg (p=0.01) in male wistar rats induced periodontitis on day 3. On day 7, a significant difference in CAL was also seen after subgingival application of propolis periodontal chips 2.5% (p=0.02), 5% (p=0.03), 10% (p=0.01), and chlorhexidine 2.5 mg (p=0.01), with placebo periodontal chips. Table 16 above shows that there is a significant difference in CAL after subgingival application of 2.5 mg chlorhexidine periodontal chip with 2.5% propolis (p = 0.01) on day 3 and (p = 0.1) on day 7, on day 3 there is a difference in CAL but not significant chlorhexidine periodontal chip 2.5 mg with placebo periodontal chip, 5% and 10% propolis. 2.5% propolis periodontal chip shows a significant difference in CAL on day 0 and day 7 with 10% propolis.

Table 4: Clinical attachment loss (CAL) differences after subgingival application between periodontal chips

Clinical attachment loss (CAL) measurement				
Group		**p-value**		
		Day 0 (p-value)	**Day 3 (p-value)**	**Day 7 (p-value)**
Periodontal placebo chips	Periodontal Chlorhexidine Chips 2.5 mg	0,37	0,01*	0,01*
	Periodontal propolis chips 2,5%	0,61	0,01*	0,02*
	Periodontal propolis chips 5%	0,16	0,01*	0,03*
	Periodontal propolis chips 10%	0,05	0,01*	0,01*
Periodontal Chlorhexidine Chips 2.5 mg	Periodontal propolis chips 2,5%	0,24	0,01*	0,012*
	Periodontal propolis chips 5%	0,91	0,08	0,10
	Periodontal propolis chips 10%	0,66	0,83	0,07
Periodontal propolis chips 2,5%	Periodontal propolis chips 5%	0,12	0,18	0,36
	Periodontal propolis chips 10%	0,05	0,16	0,04*
Periodontal propolis chips 5%	Periodontal propolis chips 10%	0,74	0,40	0,40

*significance p<0.05, CAL Clinical attachment loss

Evaluation of IL-1β expression after application of subgingival periodontal propolis chips

This study evaluated the expression of interleukin-1β (IL-1β) after the application of subgingival periodontal propolis chips in periodontitis-induced Wistar rats. Immunohistochemical analysis (IHC) was performed to assess the Immunoreactive Score (IRS) of IL-1β in epithelium and gingival connective tissue on 3rd and 7th d after periodontal chip application. The results of IRS analysis of IL-1β on epithelium (table 5) showed no significant difference between days 3 and 7 for all treatment groups (p>0.05). However, there was a significant difference between groups on day 7 (p<0.05). Periodontal propolis chips of 2.5%, 5%, and 10% showed better decreased IL-1β expression compared to the placebo group. Periodontal chips of chlorhexidine 2.5 mg showed the highest effectiveness in reducing IL-1β expression in the epithelium. Table 5 shows the differences in IRS IL-1β epithelium after subgingival application between periodontal chips on days 3 and 7. On day 7, there was a significant difference between the groups (p=0.01), indicating different effects of different concentrations of propolis and chlorhexidine on IL-1β expression.

Table 5: IRS IL-1β epithelial differences after subgingival application between periodontal chips

IL-1β Expression Measurement			
Group	**Day 3 (Mean±Elementary)**	**Day 7 (Mean±Elementary)**	**p-value**
Periodontal propolis chips 2.5%	2.40±1.517	2.25±1.258	0.47
Periodontal propolis chips 5%	2.40±1.517	2.00±1.414	0.47
Periodontal propolis chips 10%	2.00±1.225	1.40±0.548	0.01*
Periodontal Chlorhexidine Chips 2.5 mg	1.60±0.548	1.00±0.000	0.01*
Placebo periodontal chip	5.00±3.367	5.50±1,000	0.01*

*significance p<0.05, IL-1β Interleukin 1-β

Table 6 showed that IRS analysis of IL-1β in connective tissue has a similar pattern. There was no significant difference between days 3 and 7 for all treatment groups (p>0.05). However, on the 7th day, there was a significant difference between the groups (p<0.05). Periodontal chips of 2.5%, 5%, and 10% propolis showed comparable effectiveness to chlorhexidine 2.5 mg in reducing IL-1β expression in connective tissue. Table 6 shows the difference in IRS IL-1β connective tissue after subgingival application between periodontal chips on days 3 and 7. These results confirmed that on day 7, there was a significant difference between the groups (p=0.01), which showed different effects of different concentrations of propolis and chlorhexidine on IL-1β expression in connective tissue.

Table 6: Differences in IRS IL-1β connective tissue after application of subgingival between periodontal chips

IL-1β Expression Measurement			
Group	**Day 3 (Mean±Elementary)**	**Day 7 (Mean±Elementary)**	**p-value**
Periodontal propolis chips 2.5%	2.20±1.643	2.00±1.414	0.13
Periodontal propolis chips 5%	2.00±1.414	1.50±0.577	0.01*
Periodontal propolis chips 10%	1.60±1.342	1.20±0.447	0.01*
Periodontal Chlorhexidine Chips 2.5 mg	1.60±1.342	1.00±0.000	0.01*
Placebo periodontal chip	4.00±1.633	4.50±1.000	0.01*

*significance p<0.05, IL-1β Interleukin 1-β

DISCUSSION

This study evaluated the benefits of the application of periodontal propolis chips in periodontitis-induced mouse models, focusing on the clinical parameters and expression of Interleukin-1β (IL-1β). The results showed that propolis had significant potential as an adjuvant therapy in the management of periodontitis. The clinical parameters evaluated included gingival index (GI), bleeding on probing (BoP), pocket depth (PD), and clinical attachment loss (CAL). GI analysis using the modified index showed a significant decrease on day 7 after the application of propolis periodontal chips, with a concentration of 5% indicating optimal effectiveness. These findings are consistent with previous studies that reported significant improvements in GI after the use of topical propolis [17]. The BoP evaluation showed a significant decrease on day 7, with the 5% propolis periodontal chip providing optimal effect. These results are consistent with previous studies that reported a significant reduction in BoP after the application of propolis as an adjuvant therapy for periodontitis [18]. Pocket depth analysis showed a significant reduction on day 3 for all propolis concentrations (2.5%, 5%, and 10%), but on day 7, only a 10% concentration retained a significant effect. These findings are consistent with clinical studies that reported a significant reduction in pocket depth after subgingival irrigation with propolis extract [19].

Clinical evaluation of attachment loss showed a similar pattern with pocket depth, with significant improvement on day 3 for all concentrations, and sustained effects on day 7 for only 10% concentration. These results support the potential of propolis in improving periodontal tissue adhesion, as reported in previous clinical studies [20]. Immunohistochemical analysis of IL-1β expression showed a decrease in epithelium and connective tissue after the application of propolis periodontal chips. Although this decrease was not statistically significant between days 3 and 7, comparisons between groups showed that all concentrations of propolis significantly decreased IL-1β expression compared to placebo. Periodontal chips of 5% and 10% propolis showed comparable effectiveness to chlorhexidine 2.5 mg in decreasing IL-1β expression in epithelium, while all concentrations of propolis showed effectiveness equivalent to chlorhexidine in connective tissue. These findings are consistent with previous studies that reported significant reductions in IL-1β levels after propolis application in periodontitis models [21, 22].

Further analysis revealed that periodontal propolis chips at all concentrations (2.5%, 5%, and 10%) showed significant differences compared to the placebo group (p<0.05) in reducing IL-1β expression in epithelium and connective tissue. This indicates the therapeutic potential of propolis in the management of periodontitis. Interestingly, periodontal chips of 5% and 10% propolis showed comparable effectiveness to chlorhexidine 2.5 mg, which is the standard antimicrobial agent in periodontal therapy. The decrease in IL-1β expression observed after the application of propolis periodontal chips indicates the anti-inflammatory potential of propolis. IL-1β is a pro-inflammatory cytokine that plays an important role in the pathogenesis of periodontitis. A decrease in its expression indicates a reduction in the inflammatory response, which is a crucial aspect in the management of periodontitis. The results of this study highlight the potential use of propolis as an

alternative or adjuvant in periodontal therapy. The anti-inflammatory effects shown by propolis, especially at concentrations of 5% and 10%, comparable to chlorhexidine 2.5 mg, suggest that propolis can be a promising therapeutic option in the management of periodontitis. However, more research is needed to confirm these findings in the human population and to evaluate the long-term effects of the use of periodontal propolis chips.

The anti-inflammatory mechanism of propolis can be attributed to its main content, especially caffeic acid phenethyl ester (CAPE) and flavonoids. CAPE has been shown to have a strong anti-inflammatory effect, while flavonoids suppress the activation of COX-1, COX-2, and the genes responsible for COX-2 expression [23]. Propolis's ability to inhibit the production of proinflammatory cytokines and inflammatory mediators contributes to the reduction of redness and swelling of the gingiva, as well as the prevention of the formation of periodontal pockets. The results of this study show that periodontal propolis chips, especially at concentrations of 5% and 10%, have the potential to be an effective adjuvant therapy in periodontitis management. Zulhendri *et al.* stated various mechanisms of propolis in modulating inflammation towards an anti-inflammatory environmental balance and faster repair. In general, propolis acts as an anti-inflammatory agent by inhibiting and downregulating inflammation TLR4, MyD88, IRAK4, TRIF, NLRP, and related pro-inflammatory cytokines, such as IL-1β, IL-6, IFN-γ, and TNF-α. Propolis has been shown, not only in *in vitro*, ex vivo, and *in vivo* studies, but also in various human clinical trials with consistent results, such as reducing serum and tissue inflammation markers: IL-1β, IL-6, TNF-α, and hs-CRP, and reducing immune cell infiltration at the site of inflammation [24].

Caffeic acid phenethyl ester has also been shown to inhibit the production of inflammatory cytokines and increase the production of anti-inflammatory cytokines, including IL-10 and IL-4. Furthermore, it stimulates T cells, inhibits IL-2 synthesis and also IL-2 gene transcription. In addition, CAPE reduces the infiltration of monocytes and neutrophils, which are inflammatory cells. Another study showed that CAPE interferes with the interaction of ligand (LPS) with its receptor complex (TLR4/MD2) so that it can inhibit the activation of Toll-like receptor 4 (TLR4). TLR4 receptors are dysregulated in chronic inflammatory diseases. Therefore, CAPE may be effective in inflammatory diseases [25]. The anti-inflammatory and antibacterial effects of propolis contribute to the improvement of clinical parameters and the decrease in IL-1β expression. However, it should be noted that propolis cannot replace gold standard treatments such as scaling and root planing, but rather serves as an adjunct therapy that can improve treatment outcomes. The limitations of this study include a relatively small sample size and difficulties in measuring clinical parameters in mouse models. Further research with larger sample sizes and longer observation periods is needed to confirm these findings and evaluate the long-term effects of the application of propolis periodontal chips on periodontitis management.

This research suggest that propolis may be a promising natural alternative to chlorhexidine, with minimal potential for side effects in long-term use. Although these findings are very positive, it should be noted that the study was conducted on animal models with relatively short observation periods. Therefore, further studies with larger sample sizes, longer observation periods, and in human subjects are needed to confirm these results and evaluate the safety and long-term effectiveness of the application of periodontal propolis chips in periodontitis management. Overall, this study provides a solid basis for the further development of propolis periodontal chips as an innovative therapeutic approach in the treatment of periodontitis.

CONCLUSION

Based on the results of this study, periodontal propolis chips show significant potential as an adjuvant therapy in periodontitis management. Periodontal propolis chips, especially at concentrations of 5% and 10%, effectively improved the clinical parameters of periodontitis, including gingiva index, bleeding on probing, pocket depth, and clinical attachment loss. Increasing the sample size and managing the pressure given while probing for further researches is recommended to increase the validity of results before it is applied to human subjects. Long-term tissue regeneration may not be captured by the brief seven-day observation period, and there are immunological variations when translating a rat model to human clinical practice.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declare that there is no conflict of interest regarding the publication of this paper.

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