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Original Article
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Efficacy of Propolis-Based Mouthwash on Generalized Chronic Gingivitis: A Randomized Controlled Clinical Trial

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Abstract

Objectives: This study aimed to investigate the effect of propolis-based mouthwash on the gingival parameters of generalized chronic gingivitis cases in a randomized controlled clinical trial.

Methods: A total number of 69 patients with generalized chronic gingivitis were randomly assigned into three groups (N=23): propolis, chlorhexidine, or placebo mouthwash. The gingival and bleeding indices were evaluated before and after two weeks of mouthwash use. Data analyses were performed using Kruskal-Wallis test, one-way analysis of variance, and paired t-test at p<0 .05.

Results: The average gingival index in the chlorhexidine group was significantly higher than in the propolis group (p=0.005), but there was no significant difference between the placebo and propolis mouthwash groups (p=0.080). Moreover, the average plaque index was significantly higher in the chlorhexidine group than the propolis group (p<0.001). However, no significant difference was observed between the placebo and propolis mouthwash groups (p=0.742). However, the average bleeding index in the chlorhexidine group was significantly lower than the propolis group (p=0.012), with no significant difference between the placebo and propolis mouthwash groups (p=0.134).

Conclusion: The present results showed that scaling and propolis mouthwash consumption significantly improved the bleeding on probing and the gingival indices compared to the placebo group. Therefore, this mouthwash can be useful for treating chronic generalized gingivitis.

Keywords: Gingivitis; Mouthwash; Propolis; Chlorhexidine

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Introduction

Periodontal diseases are a group of chronic inflammatory conditions that affect the periodontal supporting tissues and contribute to tooth loss.[1] Risk factors for periodontal disease include smoking, alcohol consumption, and systemic conditions such as diabetes, obesity, metabolic syndrome, osteoporosis, hypocalcemia, and vitamin D deficiency. Some of these risk factors are modifiable, and their management is integral to periodontal treatment.[2]

The primary etiological factor of periodontal disease is microbial plaque, with local and systemic factors exacerbating plaque accumulation. Gingivitis, characterized by non-destructive and reversible tissue inflammation, occurs without apical migration of connective tissue attachment.[3] Gingivitis includes plaque-induced, drug-induced, hormonal, hyperplastic, acute necrotizing ulcerative, and nutritional types.[4] The most common type is chronic gingivitis induced by plaque.[3]

The prevention of periodontal diseases is centered on controlling supra- or subgingival biofilm through mechanical and/or chemical oral hygiene methods. Chlorhexidine, widely recognized as the gold standard mouthwash for periodontal applications, is effective against a broad spectrum of gram-positive and gram-negative bacteria, fungi such as Candida albicans, and viruses, including HBV and HIV.[5]

Propolis, a natural honeybee resin, is utilized in beehive construction and repair. The biological activity of Brazilian propolis is primarily attributed to prenyl p-coumaric and diterpenic acids.[6] European propolis is noted for its high levels of flavonoids and phenolic acid esters.[7] The antibacterial and anti-inflammatory properties of propolis are due to the presence of flavonoids, aromatic acids, and esters.[8] Propolis exhibits antibacterial activity against a wide range of gram-positive cocci and rods, including Mycobacterium tuberculosis, but shows limited efficacy against gram-negative bacilli. These findings confirm previous reports highlighting the antimicrobial properties attributed to its high flavonoid content.[9] Propolis is utilized in various dental treatments, such as managing stomatitis, halitosis, lichen planus, candida infections, dry mouth, traumatic orthodontic wounds, and wounds caused by dentures. Additionally, it is employed for pulp capping and as a temporary dressing in dental procedures.[10]

The biologically active compound in propolis is caffeic acid phenethyl ester, which exhibits anti-inflammatory properties and modulates the gene expression of LOX and COX enzymes involved in arachidonic acid metabolism.[11,12] The effect of propolis on COX is likely due to its flavonoids, which have been shown to suppress prostaglandin-

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endoperoxide synthase.[12]

While mechanical methods are the most effective for plaque removal, many patients struggle with plaque control. Thus, mouthwashes and chemical methods offer alternative solutions. Despite chlorhexidine being the gold-standard mouthwash, its side effects have prompted pharmaceutical companies to introduce mouthwashes containing natural compounds. Since limited studies have examined the effect of propolis on gingival health, this study aimed to evaluate the impact of propolis mouthwash on gingival parameters of patients with generalized chronic gingivitis.

Methods

This double-blinded randomized controlled clinical trial was performed after approval by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1400.104) and was registered in the Iranian Registry of Clinical Trials (IRCT20180130038558N1). Systemically healthy, non-smoking patients aged ≥18 years who visited the periodontology department of Mashhad Dental School and were candidates for scaling treatment due to generalized chronic gingivitis were included in the study. Exclusion criteria included patients unable to attend follow-up sessions, patients taking systemic or local antibiotics and immunosuppressive or anticoagulant drugs, patients undergoing orthodontic treatment, and pregnant or lactating patients. After completing the informed consent form, examiners and patients were unaware of group allocation. Sealed opaque envelopes and block randomization were used to assign patients to Groups A, B, and C for three different types of mouthwash: chlorhexidine, propolis, and placebo. Seventy-nine patients were enrolled in the study; after accounting for 10 dropouts based on similar research records, 23 patients remained in each group.

To extract propolis, brown propolis was obtained from the Faculty of Pharmacy of Iranian Medicine, crushed in a mill, and placed into filter paper. Half of the Soxhlet volumetric flask was filled with 70% ethanol (Khorasan Distilling Company, Iran), and the Heating Mantle (SCI FINETECH, South Korea) was turned on to heat the ethanol. The ethanol circulation process began in the Soxhlet set (DURAN, Germany), purifying the propolis extract. When the solvent in the Soxhlet became clear and the solvent in the volumetric flask became colored, the liquid extracts were removed from the flask and placed in the incubator (SCI FINETECH, South Korea) for ethanol evaporation. Once the extracts reached a honey-like consistency, they were removed from the machine, and the final product was stored at -20°C. The extract was standardized using the Folin-C reagent method based on the amount of the total phenolic content.

To prepare the propolis-based mouthwash, 1% katira was thoroughly mixed with 15% glycerin (Dr. Mojallali Industrial Chemical Complex Co., Iran) in a mortar. Propolis (5%) and 5% Tween 80 (Sigma Aldrich Co.) were added and mixed again until the mixture became homogeneous. Sodium benzoate (0.1%, Sigma Aldrich Co.), saccharin (Sigma Aldrich Co.), and pectin (Sigma Aldrich Co.) were then added to the solution, followed by the addition of distilled water (Zarattar Novin Pharmed Iranian, Iran) to reach the desired volume.

To prepare the placebo mouthwash, 0.5% Carbopol (Sigma Aldrich Co.) was dissolved in water to form a gel. Then, 5% Tween and 15% glycerin were mixed and added to the Carbopol gel. Sodium benzoate (0.1%) and saccharin were also added to the mixture. The mouthwash solutions were poured into similar dark plastic bottles, each encoded with one of the labels A, B, or C.

An experienced periodontist trained a dental student to blindly measure the baseline plaque index (Silness & Loe, 1964), gingival index (Loe & Silness), and bleeding on probing (Ainamo & Bay) of all patients, and provide scaling and oral hygiene instructions. Each patient was given one of the three mouthwashes based on the group: chlorhexidine (0.2% SHD, Iran), propolis, or placebo. The patients were instructed to use 10 ml of mouthwash, swish for 30 seconds twice a day for 14 days, and refrain from eating and drinking for 30 minutes afterward. The patients were re-examined after two weeks, and gingival parameters were recorded again by the same blinded examiner.

Data analyses were performed using SPSS software (version 12). All data were reported as mean ± standard deviation (SD) in the descriptive statistics section. The Kruskal-Wallis test, one-way analysis of variance, and paired t-test were used. A p-value $< .05$ was considered statistically significant in all tests.

Results

Sixty-nine patients, including 39 women (56.5%) and 30 men (43.5%), with an average age of 36.26 ± 10.59 years and ages ranging from 18 to 59 years, participated in this study. There was no significant difference between the groups regarding the average age (p=0.641). The average number of teeth was 27.74, and there was no significant difference between the groups in terms of the average number of teeth (p=0.896) (Table 1). The number of men and women in the groups also showed no statistically significant difference (p=0.616) (Table 2).

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Table 1- Comparison of age and number of teeth between chlorhexidine, propolis and placebo groups

variable	Groups	Number	Mean(min-max)	The result of the Kruskal-Wallis test
age	Chlorhexidine	20	35.95(22-57)	χ2 = 0.89 P=0.641
	Propolis	25	35.12(18-54)	
	Placebo	24	37.71(19-59)	
number of teeth	Chlorhexidine	20	27.55(20-32)	χ2 =0.22 P=0.896
	Propolis	25	27.88(24-32)	
	Placebo	24	27.75(24-32)	

Table 2- Frequency distribution of gender in the studied groups

		Female	Male	Total
Group	Chlorhexidine	10(50%)	10(50%)	20(100%)
	Propolis	16(64.09%)	9(36.0%)	25(100%)
	Placebo	13(54.2%)	11(45.8)	24(100%)

Chi-square test result: χ2 =0.97 P=0.616

Table 3 shows the variables before and after treatment separately for the chlorhexidine, placebo, and propolis groups. Before treatment, there was no significant difference between the groups regarding the average gingival index (p=0.059). However, the groups were significantly different in terms of the average plaque index (p<0.001), with the lowest mean plaque index in the chlorhexidine group and the highest in the propolis group. The groups also showed a significant difference in terms of the mean gingival bleeding index (p=0.026), with the lowest mean gingival bleeding index in the placebo group and the highest in the propolis group.

Table 3- Comparison of variables between groups before treatment

Variable	Group	Number	Mean±Sd	Min-Max	One-Way Analysis Of Variance
Gingival index	Chlorhexidine	20	1.37±0.31	1-2	F=2.96 P=0.059
	Propolis	25	1.63±0.43	1-3	
	Placebo	24	1.41±0.42	1-2	
Plaque index	Chlorhexidine	20	1.12±0.31	1-2	F=9.25 P<0.001
	Propolis	25	1.68±0.47	1-3	
	Placebo	24	1.40±0.49	1-3	
Bleeding index	Chlorhexidine	20	0.54±0.20	0-1	F=3.86 P=0.026
	Propolis	25	0.60±0.16	0-1	
	Placebo	24	0.46±0.17	0-1	

After treatment, the groups showed significant differences in all three indices (p<0.001). The lowest and highest average gingival indices were observed in the chlorhexidine and placebo groups, respectively. The lowest and highest average plaque indices were observed in the chlorhexidine and propolis groups, respectively. The lowest and highest average gingival bleeding indices were observed in the chlorhexidine and placebo groups, respectively.

Table 4 shows the changes in the variables before and after treatment in each group. The groups showed significantly different changes in all three indices (p<0.001). Gingival index changes in the placebo group were found to be significantly lower than those in the chlorhexidine and propolis groups (p<0.001 for both). However, there was no significant difference between the chlorhexidine and propolis groups (p=0.821). The plaque index changes in the chlorhexidine group were significantly higher than those in the placebo and propolis groups (p<0.001 and p=0.004, respectively). However, there was no significant difference between the placebo and propolis groups (p=0.073). The average changes in gingival bleeding were significantly lower in the placebo group than in the chlorhexidine and propolis groups (p<0.001 for each). However, there was no significant difference between the chlorhexidine and propolis groups (p=0.860).

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Table 4 -Comparison of variables between groups after treatment

Variable	Group	Number	Mean±SD	Min-max	Kruskal-Wallis test
Gingival index	Chlorhexidine	20	0.47±0.20	0-1	$\chi2$ =27.34 P<0.001
	Propolis	25	0.77±0.30	0-1	
	Placebo	24	1.05±0.38	0-2	
Plaque index	Chlorhexidine	20	0.42±0.14		$\chi2$ =39.66 P<0.001
	Propolis	25	1.24±0.37	1-2	
	Placebo	24	1.10±0.45	0-2	
Bleeding index	Chlorhexidine	20	0.17±0.08	0-0	F=10.03 P<0.001
	Propolis	25	0.25±0.09	0-0	
	Placebo	24	0.32±0.13	0-1	

Discussion

The present study aimed to evaluate the effect of a propolis-based mouthwash on gingival parameters in patients with generalized chronic gingivitis. The results indicated that for plaque index changes, chlorhexidine was more effective than propolis, and propolis outperformed the placebo. For both bleeding and gingival indices, chlorhexidine and propolis were similarly effective, with both being superior to the placebo. Additionally, no patients reported any side effects from the mouthwashes during the study period.

Pereira et al. investigated the clinical effectiveness of alcohol-free Brazilian propolis mouthwash for gingivitis. They reported a 24% improvement in the plaque index and a 40% improvement in the gingival index compared to the baseline, that was attributed to the antimicrobial properties of this type of propolis.[13] Kukreja and Dodwad showed that propolis improves gingival conditions in patients with gingivitis.[10] Dehghani et al. examined the effect of propolis mouthwash on orthodontic patients and reported that it has antimicrobial properties and significantly reduces the gingival index.[14] The results of the present study are not aligned with those of Pereira et al., who reported a 24% decrease in the plaque index, and Dehghani et al., who showed a significant decrease in the plaque index at the end of the study.[13,14] The present results were similar to those of Yueh-Juen et al., who conducted a meta-analysis and found that propolis significantly reduces the gingival index. However, the reduction in the plaque index was not statistically significant.[15]

The study by Kripal et al. stated that propolis mouthwash reduced both plaque and gingival indices.[16]

All these studies demonstrated that propolis mouthwash significantly reduces the gingival index, likely due to its anti-inflammatory properties. However, more studies are needed to confirm the effect of propolis mouthwash on the plaque index. The differences in findings may be due to variations in protocols, durations, or frequencies of use, as well as the concentration of the mouthwash.

One limitation of this study was the small sample size. Future studies with larger sample size are suggested. Another limitation was the relatively short follow-up period, as the American Dental Association recommends a six-month evaluation period for anti-plaque and anti-gingivitis agents.[17] It is also recommended that microbiological studies be conducted in this field.

Conclusion

Based on the findings of this double-blind clinical trial involving 78 patients, after two weeks of mouthwash use, chlorhexidine was more effective than propolis in reducing the plaque index, while propolis was more effective than the placebo. Both chlorhexidine and propolis significantly improved bleeding and gingival indices, outperforming the placebo.

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Informed Consent Statement: Registered in the IRCT system (IRCT20180130038558N1). Informed consent was obtained from the patients.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Conflict of Interest: No Conflict of Interest Declared. ■

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