

Evaluation of Antiplaque Efficacy of a Propolis-Based Herbal Toothpaste: A Single-Blind Parallel Clinical Trail

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Abstract

Background and Aim: In recent years, the use of herbal oral care products has been taken into consideration for reducing dental plaque accumulation. Propolis has been widely used in medicine due to its multiple biological properties. The aim of this study was to evaluate the antiplaque efficacy of an herbal toothpaste containing propolis in comparison with a control group.

Materials and Methods: This single-blind parallel clinical trial was conducted among 60 dental students. The baseline plaque index was evaluated using a plaque indicator one hour after brushing without toothpaste. Participants were randomly assigned to two groups: (A) herbal toothpaste with propolis, and (B) herbal toothpaste without propolis. After four weeks, the plaque index was re-examined and recorded. Quantitative variables were analyzed by independent t-test, and the mean and standard deviation (SD) were reported. $P < 0.05$ was considered statistically significant.

Results: The mean plaque index differences were not significant between the two groups at the beginning of the study, while there was a significant difference after four weeks ($P < 0.001$). The results signify an approximate 1.7-unit reduction in the mean plaque index of subjects using toothpaste with propolis in comparison with a 0.7-unit reduction in subjects using toothpaste without propolis.

Conclusion: The herbal toothpaste containing propolis was more effective in reducing plaque accumulation in comparison with the control group. Considering the effect of propolis-containing toothpastes on the reduction of dental plaque accumulation, these can be used as an effective oral hygiene product.

Key Words: Dental Plaque, Herbal, Oral Hygiene, Toothpastes, Propolis

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Introduction

Dental caries (particularly during childhood) and periodontal diseases are the most common

oral cavity diseases [1]. A positive correlation has been found between the number of *Streptococcus mutans* (*S. mutans*) in dental

plaque and the development of dental caries [1]. Furthermore, dental plaque bacteria are known as the major etiologic factors of marginal periodontitis [1,2]. Maintenance of proper oral hygiene is one of the most important measures for controlling gingivitis, periodontitis, and dental caries [1]. Self-performed mechanical plaque removal is one of the most accepted methods of controlling plaque and gingivitis but most people experience difficulty in maintaining adequate levels of plaque control; therefore, chemical agents that could supplement patient-dependent mechanical plaque removal have been studied in different essays [3,4].

Over the last several years, a worldwide tendency has been noted towards the use of natural products due to their pharmacological effect on caries prevention [2]. Herbex (Parmoon, Tehran, Iran) is a natural product formulated to maintain oral health, which contains propolis extract, Glycyrrhiza glabra, Satureja, Dianthus, Myrtus communis, and Eucalyptus [5].

Propolis, also known as bee putty [5] or bee glue [2], is a natural resinous mixture produced by honeybees collected from buds and exudates of certain trees and plants and stored inside beehives. This substance has been widely consumed in medicine due to its multidirectional biological properties. Apart from antibacterial activity [6,7], various studies have demonstrated that propolis has other beneficial properties, such as antioxidant, antifungal, antiviral [8,9], anti-inflammatory, cytostatic, and cariostatic properties [2,10]. It also accelerates epithelial repair and controls dentinal hypersensitivity [11]. Additionally, the anti-proliferative action of propolis has been observed in human tumor cell lines [5,12].

Different studies have evaluated the efficacy of propolis-based toothpastes. These studies have shown the effectiveness of herbal toothpastes in the inhibition of oral pathogenic microorganisms [13], reduction of dental plaque [1], improvement of marginal periodontitis [14], and changes in the oral bacterial spectrum [15].

The precise composition of propolis varies with the geographic origin [11] and includes amino

acids, minerals, ethanol, Vitamins A, E, and B complex, and the highly active mixture of compounds known as bioflavonoids. The compounds found in propolis affect the growth and glucosyltransferase activity of *S. mutans* [2]. The antibacterial activity of propolis is reported to be due to flavonoids, aromatic acids, and esters present in resins [10]. Propolis also has an effect on the cytoplasmic membrane, bacterial motility, and enzymatic activity. At low concentrations, it has bacteriostatic activity, while it can be bactericidal at high concentrations [2].

Considering the properties of propolis, the aim of this study was to evaluate the antiplaque efficacy of a propolis-based herbal toothpaste as a clinical study.

Materials and Methods

This study was approved by the Iranian Registry of Clinical Trials (IRCT20111026007910N5). All subjects were given verbal and written information about the study, and after entering the study, they signed a written consent form regarding all information received.

In this single-blind parallel clinical trial, 60 dental students (30 females and 30 males) aged 24-30 years old, who volunteered to participate in the study and agreed to continue oral hygiene using the prescribed toothpaste, were included. The sample size was calculated to be 52 considering type one error of 5% and type two error of 20% ($n=26$ for each of the groups). For more accurate results, 60 samples were considered. Participants who met the exclusion criteria, such as having periodontal pockets with a depth of more than 3 mm, having orthodontic appliances, having a history of smoking, xerostomia, and systemic diseases, having untreated dental caries, and having a history of using Herbex mouthwash or toothpaste, were excluded from the study.

Random assignment was performed, and participants were randomly allocated to each experimental group using a randomized permuted block design: group (A) Herbex containing propolis (Parmoon, Tehran, Iran), and group (B) Herbex without propolis (Parmoon, Tehran, Iran).

In the first phase of the study, the plaque index of all participants was examined by a dental student using a plaque indicator (Visuplac Pastilhas, Maquira, São Paulo, Brazil) one hour after brushing without toothpaste. Oral hygiene was scored on all surfaces according to the criteria of the plaque index system. Participants were trained to chew the plaque indicator for 30 seconds. The disclosing solution stained the dental plaque on buccal and lingual/palatal surfaces, and the presence or absence of dental plaque was determined using the modified Turesky index. The modified Turesky index scoring is described below [16]:

0: No plaque,

1: Separate flecks of plaque at the cervical margin of the tooth,

2: A thin continuous band of plaque (up to 1 mm) at the cervical margin of the tooth,

3: A band of plaque wider than 1 mm but covering less than one-third of the crown of the tooth,

4: Plaque covering at least one-third but less than two-thirds of the crown of the tooth,

5: Plaque covering two-thirds or more of the crown of the tooth.

An average plaque score was derived for each subject at each examination by summing the individual plaque scores of each surface and dividing that sum by the number of sites graded for that subject.

All participants were instructed to use medium-bristle toothbrushes (Oral-B, Procter & Gamble Co., Cincinnati, OH, USA) and fluoride-free dental floss (Orkid, Tehran, Iran). They were asked to brush twice daily (after breakfast and before bedtime) using the bass technique, floss once a day, and avoid using other oral hygiene items, such as mouthwashes or chewing gums containing propolis, during the study course (four weeks).

The manufacturer designed the toothpastes with a similar packing so that the subjects were unaware of each tube contents, while the examiner was aware of the type of toothpaste.

The components of the two toothpastes were exactly the same, except for propolis. Both of them contained hydrated silica gels, sodium lauryl sulfate, water, xanthan gum, sorbitol,

glycerol, saccharine, sodium fluoride, zinc citrate, sodium pyrophosphate, menthol, flavors, abrasives, clove extract, succulent extract, and licorice extract, with and without propolis.

After four weeks, the plaque index was re-examined by the dental student who performed the first phase of the study.

Data were analyzed using SPSS 18 software (SPSS Inc., Chicago, IL, USA). The normal distribution of data was analyzed using Shapiro-Wilk test. Due to in all groups, quantitative variables were analyzed by parametric tests, such as independent t-test, and the mean and standard deviation (SD) were reported. $P > 0.05$ was considered statistically significant.

Results

The participants were 30 females and 30 males in the age range of 24-30 years old. Shapiro-Wilk test showed the normal distribution of data at the two time points and in the two groups ($P > 0.05$); therefore, parametric tests were used.

At the two time points (at the beginning of the study and after four weeks of using the toothpaste), the mean plaque index and the differences were evaluated. The results signified an approximate 1.7-unit reduction in the mean plaque index of subjects that used Herbex toothpaste with propolis compared to 0.7-unit reduction in subjects that used Herbex toothpaste without propolis (Table 1). Paired t-test confirmed that the mean plaque index differences were not significant between the two groups at the beginning of the study ($P = 0.104$) but after 4 weeks, the plaque index evaluation proved significant reduction in both groups ($P < 0.001$); the group that used Herbex with propolis had a lower plaque index compared to the group that used Herbex without propolis ($P < 0.001$).

Although the differences in the plaque index were not significant at the baseline, for more accurate results, the plaque index of the groups was assimilated using analysis of covariance (ANCOVA). The results showed that after matching the plaque index at the baseline, the

Table 1. Mean plaque index and standard deviation (SD) at the beginning of the study and at four weeks after the intervention

Subjects	Mean plaque index		At the beginning of the study		After four weeks		differences	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Using Herbex with propolis (N=30)	4.0038	1.09947	2.3420	.90929	-1.6618	.72773		
Using Herbex without propolis (N=30)	4.5372	1.38593	3.9067	1.50151	-.6304	.60012		
Total (N=60)	4.2705	1.26910	3.1243	1.46187	-1.1461	.84130		

plaque index in the group that used the propolis contained-toothpaste was still lower than that in the other group ($P<0.001$; Table 1).

Discussion

Dental caries is a multifactorial disease caused by the interaction of the host, microorganisms, substrate, and time. Oral microorganisms of dental biofilms are critical for the initiation of dental caries and for the onset and development of inflammatory reactions in tissues surrounding the teeth [15,17]. To prevent oral diseases, biofilm is controlled through regular mechanical measures. In addition, biofilm formation can be limited by using antimicrobial agents [17].

In recent years, the use of herbal oral care products has been taken into consideration. Properties of an ideal oral medicine include the ability to interfere with caries development, non-toxicity to the host cells, long-lasting effects, and also not causing a significant imbalance in the oral microbial flora. Libério and colleagues [17] have demonstrated that propolis fulfills these requirements.

Propolis is a resinous substance, which is effective against cariogenic microbiota [18]. *S. mutans* initiates plaque accumulation on the tooth surface by the synthesis of extracellular polysaccharides (mainly glucan), which are produced by glucosyltransferase. Moreover, the glucosyltransferase helps the adhesive interaction of *S. mutans* [2]. Two different mechanisms have been suggested for the anti-caries effect of propolis, including antimicrobial activity against *S. mutans*, and

inhibiting the activity of glucosyltransferase enzymes, consequently interfering with bacterial adherence [18]. The antimicrobial activities are usually attributed to flavonoids [17,19]. The proven anti-inflammatory action of propolis seems to be particularly advantageous for preventive services in patients with an increased risk of periodontal inflammatory problems [15].

The present study was conducted among 60 dental students to compare the efficacy of an herbal dentifrice containing propolis in controlling plaque formation. Based on the results, there was a significant reduction in the plaque index after the course of the study (four weeks) in both groups (Herbex with propolis and Herbex without propolis; $P<0.001$), which shows the impact of using propolis-containing dentifrices. Furthermore, after four weeks, the results demonstrated statistically significant differences between the two groups, which indicate the higher anti-plaque efficacy of propolis-based herbal toothpaste in comparison with the herbal toothpaste without propolis ($P<0.001$).

Moumen Beyt Elahi and colleagues [13] compared the antimicrobial effect of 30% ethanol extract of propolis with Chlorhexidine (CHX) solution and concluded that 30% ethanol extract of propolis is effective against oral pathogenic microorganisms such as *S. mutans*, *Candida albicans*, and *Actinobacillus actinomycetam comitans*. Tanasiewicz et al [1] and Skaba and colleagues [14] also supported the reduction of dental plaque and the local therapeutic effect of gels and toothpastes

containing propolis on the marginal periodontium, which concur with the results of this study.

Morawiec et al [15] found propolis-containing toothpaste to be distinctively effective in improving oral health and inhibiting gingivitis triggered by dental plaque. Also, qualitative and quantitative changes in the oral bacterial spectrum were observed in the cited study [15]. Fereidooni et al [20] investigated the effect of toothpastes containing propolis on plaque control and stated that the propolis toothpaste caused more reduction in dental plaque compared to the regular toothpaste; the results are in line with those of the present study.

Akca et al [19] conducted a study in order to compare the antimicrobial effectiveness of ethanolic extract of propolis (EEP) with CHX gluconate in planktonic and biofilm states of oral microorganisms. The results of the study revealed that propolis was more effective in inhibiting Gram-positive bacteria in comparison with Gram-negative bacteria in their planktonic state [19]. Also, it was indicated that propolis was as effective as CHX in the biofilm state, which is an indicator of the antiplaque efficacy of propolis and supports the results of the present study.

In all aforementioned studies, the use of propolis in oral hygiene products led to effective plaque control and improvement of oral health. These results are consistent with the findings of the present study. One of the points that distinguish this article is that the cultural and social levels of the participants are distinct from regular research on this field, which can be considered as an advantage. Also, the fact that the company manufactured the toothpastes especially for the present study with the exact similar components, except for propolis, is another discriminating factor. In this study, interfering factors, such as the presence of caries in participants, which can lead to plaque accumulation, sampling time, and patient awareness of the type of toothpaste (whether it contains propolis or not), were omitted. In addition, in this study, the plaque index was determined using a plaque indicator

and the Turesky plaque index, which increase the accuracy of the results.

Conclusion

This study indicated the positive biological activity of toothpastes containing propolis in reducing plaque accumulation and suggests that propolis can be used as an effective substance to improve oral diseases caused mainly by plaque biofilm.

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