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Original Article

Comparison of casein phosphopeptide-amorphous calcium phosphate fluoride paste and a propolis-containing herbal toothpaste in dentinal hypersensitivity: A randomized trial

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ABSTRACT

Background: This study aimed to compare the efficacy of casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) and propolis-based herbal toothpaste in the treatment of dentin hypersensitivity (DH).

Materials and Methods: In this clinical trial, 20 patients (7 men and 13 women) who met the inclusion criteria were randomly divided into two groups. One group received the herbal toothpaste containing propolis (Herbex®), while the other received a paste containing CPP-ACPF (GC® MI-Paste Plus). Two nonadjacent teeth with DH in two quadrants of each patient were assessed. The pain was determined through the visual analog scale (VAS) in cold and airblast tests before the intervention (baseline) as well as at 15 min, 1, 2, 4, and 8 weeks after the first application. Three-level mixed effect model (repeated measurement, tooth, and patients) was used to analyze the VAS score data. Estimation of fixed effect parameters with standard error and the intraclass correlation coefficient that quantifies the degree to which data at the lower level are correlated were reported. The statistical significance level was determined as $P < 0.05$.

Results: The mean pain intensity score after 8 weeks significantly decreased in the propolis-based toothpaste group ($P < 0.001$) and CPP-ACPF paste group ($P < 0.001$) compared with baseline. Betweenn-group comparison in the 8th week showed a significantly lower pain score in the propolis-based toothpaste group compared with the CPP-ACPF-containing paste ($P = 0.02$). However, at other intervals, there were no significant differences between the two groups ($P > 0.05$).

Conclusion: The use of both herbal toothpaste containing propolis and CPP-ACPF-containing paste for 8 weeks effectively reduced DH, with a higher desensitizing effect experienced in the former group.

Key Words: Casein phosphopeptide-amorphous calcium phosphate fluoride, dentin hypersensitivity, propolis, toothpaste

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INTRODUCTION

Dentin hypersensitivity (DH) is a common dental complaint that patients experience when eating, drinking, or brushing their teeth, negatively affecting their quality of life.[1] This sharp, short-term pain is caused by palpable, thermal, chemical, or osmotic stimuli, and there is no dental impairment.[2] DH is found in both sexes and is more common in adults. Usually, the facial surface of the tooth in the cervical region is involved. Periodontal treatments directly increase DH by exposing the dentinal tubules of the root.[3]

Hypersensitivity is mainly related to dentin exposure. Dentinal tubules are normally protected by enamel on the crown or gingiva on the tooth's root surface. When the enamel is removed or the gingiva recesses, the tooth would be susceptible to stimuli. The most accepted theory about this sensitivity is the "hydrodynamic theory" proposed by Branstrom in 1964.[4,5] According to it, when the dentin surface is exposed to stimuli, fluid flow in dentin tubules increases; therefore, it changes the pressure and stimulates pressure-sensitive nerve receptors in dentin.[6] DH has various degrees of pain; however, it can alter patients' daily activities and lead them to the dentist. The basic principle for treatment is to change the fluid flow in the dentinal tubules by blocking or narrowing them.[4] Many treatment methods are now available, such as using adhesives, varnishes, dental bonding materials, periodontal grafts, and restorative procedures with different results. The most common is home remedies.[7] At present, toothpaste containing fluoride seems to have primary and secondary preventive effects against dentinal hypersensitivity, but therapeutic benefits can be increased by adding some ingredients.[8] Chemical compounds available for this purpose are potassium or ferrous oxalates, potassium nitrate, stannous fluoride, sodium fluoride, sodium monofluorophosphate, and strontium chloride.[9]

Propolis is a yellow resin compound of beehives that contains 300 components.[10] It is mainly composed of resin (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds (5%).[11] Propolis blocks the fluid flow by sealing the tubules. It is a source of chemicals, especially active flavonoids. Flavonoids have tissue regenerative activity and are the main stimulants of dentin formation.[9,12] During reactions with dentin, these bioflavonoids form crystals in dentin tubules, thereby reducing fluid flow.[13] Propolis content varies according to its botanical origin and geographical location.[14]

Casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) is a milk nano complex. Casein phosphopeptide allows high concentrations of calcium, phosphate, and fluoride ions to be stabilized in a semi-stable solution that can be used to enhance remineralization. The calcium and phosphate ions released from CPP-ACPF are diffused through the enamel pores and deposited in the enamel crystals. Human studies have successfully shown the CPP-ACPF potential to prevent enamel demineralization and increase the remineralization of enamel defects.[15] Mineral deposition inside dentin tubules increases and blocks the dentinal tubules; therefore, the permeability and diameter of dentinal tubules are decreased by 85% or more, decreasing dentinal hypersensitivity.[16]

Due to the high prevalence of DH in the community, which negatively impacts the quality of life,[17] this study was conducted to compare the effectiveness of two kinds of desensitizing agents: GC® MI-Paste Plus, which contains CPP-ACPF, and Herbex® herbal toothpaste, which contains propolis. The ease of application, availability, and nature of these pastes, as well as contradictory reports in previous studies, encouraged us to do this study. The null hypothesis in this study is that there is no difference between the treatment groups in different times in the reduction of DH.

MATERIALS AND METHODS

Study design and ethical considerations

This randomized, prospective, single-blinded trial was registered with the Iranian Registry of Clinical Trials (IRCT20120901010703N4). The study protocol was approved by the Research Ethics Committee of the Isfahan University of Medical Sciences (IR.MUI.RESEARCH.REC.1399.421). Informed consent was obtained according to the Declaration of Helsinki.[18] The objective, study protocol, and duration of the investigation were explained before a consent form was signed by each participant.

Eligibility criteria

Participants of both genders if aged between 18 and 60 years referred to the Department of Periodontics of Isfahan University of Medical Sciences were enrolled in the study. These patients had cervical dentin sensitivity in at least 2 nonadjacent teeth (in two

separate quadrants). All patients with a history of use of antihistamine, antidepression, and sedative drugs in the past month or antisensitivity toothpaste in the past 6 weeks, smokers, pregnant and nursing mothers, patients allergic to milk protein and benzoate, those with inappropriate acidic diet and unavailable for recalls were not included in this study. Similarly, teeth with erosion, attrition, crack, pulpitis, caries, direct and indirect restoration, orthodontic band, pathologic mobility, fracture, and active periodontal diseases were excluded.

Sample size calculation, randomization and blinding

The sample size calculation was based on comparison of means and the expected difference in means of 1.5 visual analog scale (VAS) score. Considering an alpha of 0.05 and power of 80%, 10 patients (20 sensitive teeth) in each group would be necessary. The initial evaluation was performed using an evaporative test with air jets from a dental syringe (by a single experienced examiner [NN]).

Participants were randomly assigned by a dentist (EA) following simple randomization procedures (computerized random numbers) to the first or second treatment groups. In this single-blind trial, the participants were not aware of the type of treatment they received.

Dentin hypersensitivity assessment

Airblast and cold stimulus tests were used to assess the sensitivity. First, the surface of the teeth was dried and isolated by a cotton roll. An Airblast test was performed using an air-water syringe of the dental unit, applying air from a distance of 10 mm from the tooth surface for 2 s at a pressure of 40 psi (±10 psi). After 5 min, tooth sensitivity to thermal stimulation was measured by using a cold spray on the swab and placing it on the cervical buccal surface of the teeth for 2–3 s.[19] Dental sensitivity was measured individually using the VAS after each test. Patients were asked to rate their pain on a scale of 0–10 (0 for insensitivity and 10 for severe and unbearable pain).[17,20] Patients who had equal or >4 on the VAS were included in the study.

Interventions

A total of 20 Patients, each with 2 nonadjacent teeth in two separate quadrants (40 teeth) were randomly divided into two groups. To standardize oral health conditions, the first phase of periodontal treatment was performed in patients with dental calculus. The appropriate technique of hygiene training was also given to all subjects. All patients were given similar soft toothbrushes.

Herbal toothpaste treated group

This group was given Herbex® herbal toothpaste containing propolis (Dr. Jahangir Health Pharmaceutical Company, Iran). The toothpaste was used twice a day (morning and night) according to the manufacturer's instructions.

GC® MI paste plus treated group

This group was given GC® MI Paste Plus (GC® America Inc. Alsip, USA). The paste was used once a day after brushing with their assigned toothbrush and a dentifrice without desensitizing action, equivalent to the size of a pea grain for each jaw with a clean and dry finger or a cotton ball on the surface of the teeth according to the manufacturer's instructions. This paste should remain on the teeth for at least 3 min and then should be spread in the mouth with the tongue and saliva. After 1–2 min, saliva was spitted out. Eating and drinking for half an hour were avoided.

Since the anti-sensitive effect of GC® MI-Paste plus has been proven, in this study, we decided to compare Herbex® herbal toothpaste (an Iranian herbal toothpaste with a lower price) with GC® MI-Paste plus as a standard commercial product. The herbal toothpaste is easier for patients to access and is used at the same time as people brush their teeth. In fact, the main purpose of this study was to test if the Herbex® herbal toothpaste outperforms the GC® MI-Paste plus, or at least provides a similar efficacy to the commercial product when used for 2 months. A single experienced evaluator (NN) performed sensitivity tests at baseline (pretreatment), 15 min after the intervention, and during the follow-up visits including 1, 2, 4, and 8 weeks after the first visit [Figure 1].

Statistical analysis

Continuous variables were described as mean (standard deviation) and categorical variables were described as numbers (percentage). Shapiro–Wilk test was used for normality test. To compare group means, the two-independent samples *t*-test was used. Pearson's Chi-square test was used to compare proportions between groups. Three-level mixed effect model (treating the repeated measurements at level 1 nested within tooth at level 2 nested within patients at level 3) using the unstructured variance-covariance matrix, the maximum likelihood method of estimation was used to analyze the trend of changes in the

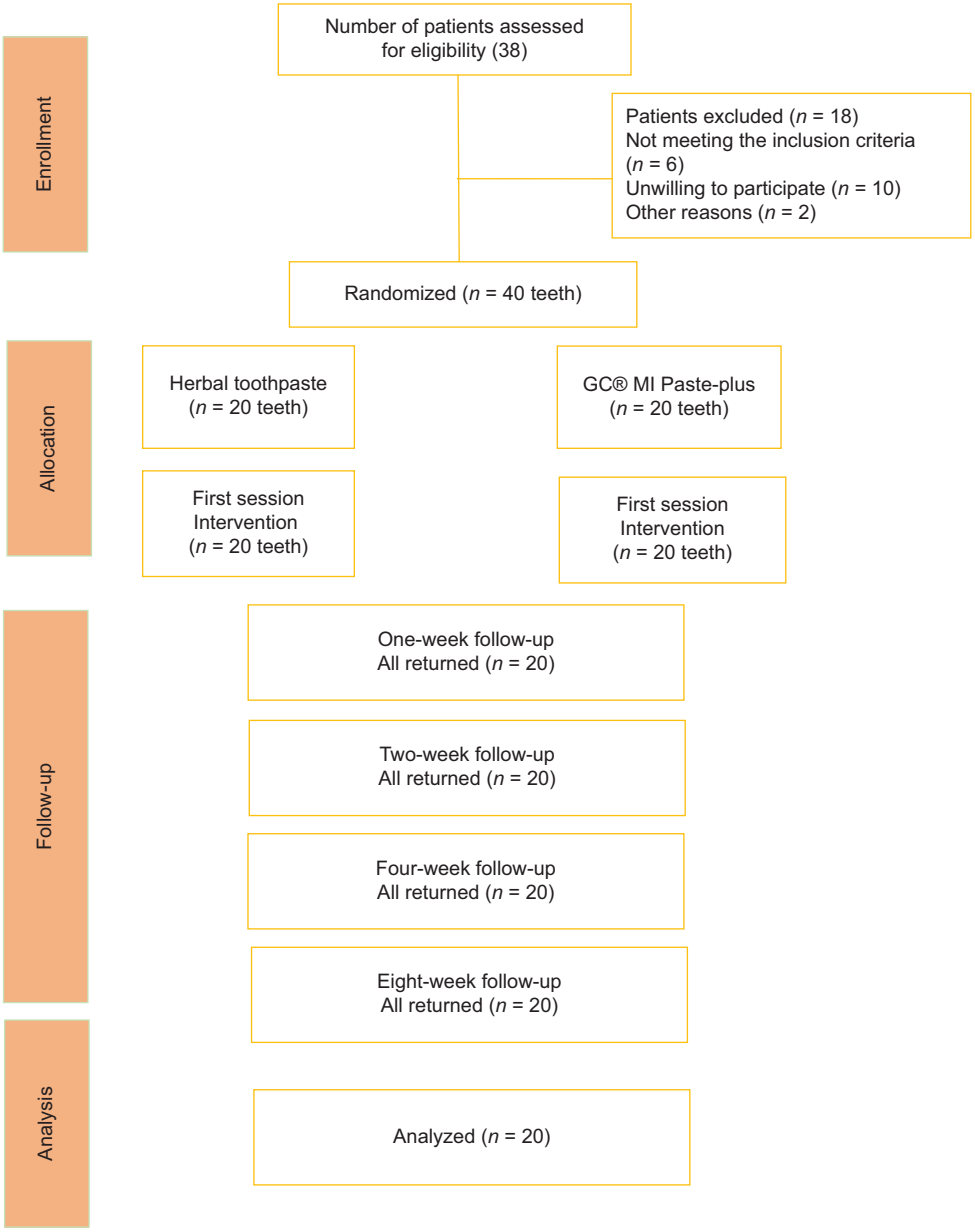

Figure 1: CONSORT flowchart.

VAS score data of tooth sensitivity to cold and air. Estimation of fixed effect parameters with standard error and the intraclass correlation coefficient that quantifies the degree to which data at the lower level are correlated were reported. The statistical significance level was determined as $P < 0.05$. Analyses were carried out in Stata.

RESULTS

A total of 20 patients participated in the 8-week clinical trial, with 10 patients randomized to the Herbex® herbal group (containing propolis) and 10 patients to the GC® MI-Paste Plus group. Baseline demographic characteristics, including age and gender, were statistically similar between the two groups ($P > 0.05$) [Table 1].

As shown in Table 2, the interaction effect of time by group on VAS score was statistically significant. This finding implies that the improvement in VAS score in the two groups was different and the Herbex® group showed a sharper decline in the mean of cold sensitivity score over time compared to the GC® MI-Paste Plus group [Figure 2]. Both groups exhibited considerable improvements in mean VAS score of cold sensitivity during the follow-up period (P value for Time < 0.001) [Table 2].

While the mean VAS score for tooth sensitivity to cold was consistently lower in the Herbex® group compared to the GC® MI-Paste Plus group at all-time points after treatment, a statistically significant difference was only observed at 8 weeks after treatment (2.35 ± 0.408 vs. 3.95 ± 0.507; $P = 0.014$) [Figure 2].

The interaction effect of time by group on VAS score of tooth sensitivity to air was not statistically significant ($P > 0.05$) [Table 2]. Tooth air sensitivity score was significantly improved in both groups 8 weeks after treatment ($P < 0.001$) [Table 2]. However, this decline decelerated over time (polynomial coefficient [se] for time: 0.007 [0.001]; $P < 0.001$), indicating approximately a nonlinear sensitivity to air trend. There was no considerable difference between two groups in the mean VAS score of air sensitivity at any time point ($P > 0.05$) [Figure 3].

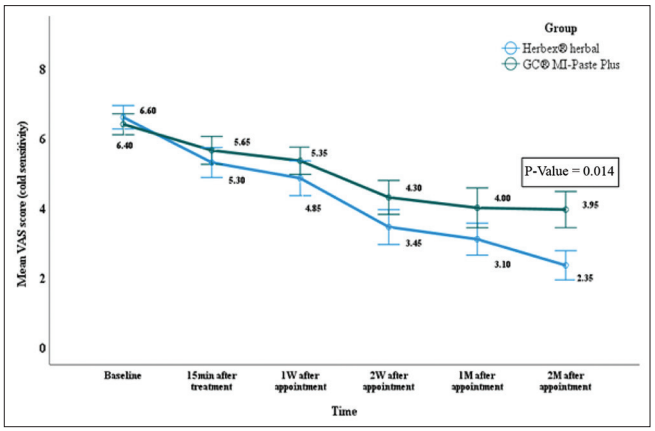

Figure 2: Intergroup analysis Visual Analogue Scale scores in two groups of Herbex® herbal toothpaste containing propolis and GC® MI-Paste plus (Sensitivity to cold). *P-value resulted from multiple comparison test with Bonferroni correction. VAS: Visual Analog Scale.

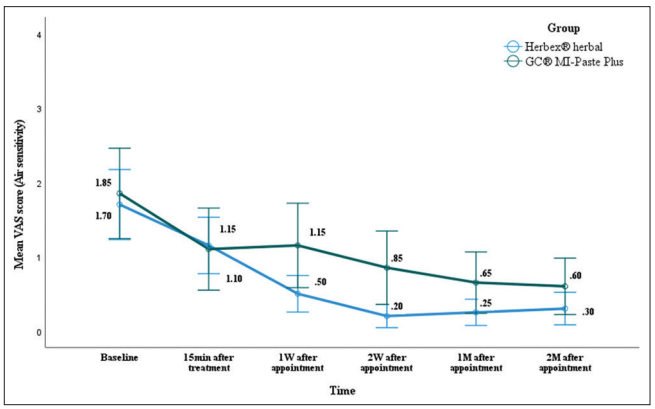

Figure 3: Intergroup analysis Visual Analog Scale scores in two groups of Herbex® herbal toothpaste containing propolis and GC® MI-Paste plus (Sensitivity to Air). VAS: Visual Analog Scale.

DISCUSSION

This study showed that using both GC® MI-Paste plus containing CPP-ACPF and Herbex® herbal toothpaste containing propolis reduced symptoms and pain intensity up to 8 weeks after starting treatment. Furthermore, Herbex® herbal toothpaste was significantly more effective in the reduction of pain intensity in the 8th week. Based on the comparison of the results of the cold test, the immediate response (15 min after the intervention) was not significantly different between the two groups, but the delayed response (2 months after the intervention) showed a significant decrease in the Herbal Toothpaste group. Furthermore, there was no significant difference in any of the immediate and delayed responses between the two groups in the airblast test. However, in each group, an immediate and delayed effect was observed in reducing tooth sensitivity pain. The average intensity of tooth sensitivity in all periods in both groups and by both tests was significantly lower than before treatment.

Tactile, cold, and evaporative stimuli have been recommended as reliable tests to measure DH, and it is recommended to use at least two hydrodynamic stimuli. The interval between tests should be long enough to minimize interaction between the stimuli. In the present study, the air blast test and the cold test were performed to measure DH. The air blast test was performed 5 min before the cold test to minimize interaction between stimuli.[19]

Madhavan *et al*.[21] compared CPP-ACPF, sodium fluoride, propolis, and placebo in treating DH. All three study groups showed a decrease in dentin sensitivity over 3 months compared to the control group. The propolis group showed the lowest mean of DH. The delayed response was seen in all three groups, especially in the propolis group. However, in the present study, only two groups were compared, and the results showed that the herbal toothpaste containing propolis was more effective than the toothpaste containing CPP-ACPF only in the 8th week. Still, in Madhavan's study in all periods (7, 15, 28, and 60 days), the propolis was more effective than CPP-ACPF.[21] However, that study did not indicate the consistency and application method of propolis in the form of gel or toothpaste. On the other hand, Torwane *et al*. compared the 30% ethanolic extract of

Table 1: Demographic characteristics data according to the study groups

Characteristics	Group		*P*
	Herbex® herbal toothpaste containing propolis (*n*=10)	GC® MI-paste Plus (*n*=10)	
Age (years), mean±SD	35.50±12.3	34.30±9.03	0.653
Gender			
Female	6 (60)	7 (70)	0.507[a]
Male	4 (40)	3 (30)	

[a]*P*-value results from Chi-square test. Data are shown as mean±SD for continuous variables, and as frequency (%) for categorical variables. SD: Standard deviation

Table 2: Results from multilevel mixed effect model for visual analog scale score

Fixed effect	Cold sensitivity			Air sensitivity		
	Coefficient	SE	*P*	Coefficient	SE	*P*
Time	−1.078	0.157	<0.001*	−0.572	0.094	<0.001*
Time (polynomial)	0.006	0.003	0.051	0.007	0.001	<0.001*
Group (GC® MI-paste plus)	−0.433	0.624	0.487	0.140	0.828	0.866
Group time*	0.314	0.135	0.020*	0.060	0.105	0.568
Intercept	7.622	0.491	0.000	2.182	0.594	<0.001*
Random effect						
$ICC_{Individuals}$ (SE)	0.30 (0.149)			0.405 (0.104)		
$ICC_{Tooth\ within\ individual}$ (SE)	0.532 (0.115)			0.935 (0.014)		

**P*<0.05 was considered as statistically significant. *P* values are resulted from three-level mixed effect model. Reference group is shown in the parenthesis.
ICC: Intraclass correlation; SE: Standard error

Indian propolis with Recaldent™ as positive control group containing CPP-ACP and sterile distilled water as negative control. In the airblast test, all three groups showed a significant reduction in dentin sensitivity, but unlike our study, CPP-ACP was significantly more effective and acted faster than propolis.[16] It seems that fluoride in the CPP-ACPF-containing toothpaste (900 ppm) can react with ACP in the casein complex, causing the deposition of calcium fluoride and neutralizing both mineral components of this paste.[22] This hypothesis may be considered the etiology for the lower effect of this paste in the 8th week in the present study.

Mahesuti *et al*. compared a paste containing CPP-ACPF and a gel containing potassium nitrate to treat dentinal hypersensitivity. Both significantly reduced dentinal hypersensitivity, but there was no difference between these agents. Although potassium nitrate acted faster than MI Paste, but MI Paste had a longer performance after treatment than potassium nitrate.[23] In the present study, the immediate effect of CPP-ACPF was determined in addition to the delayed effect which is consistent with the above study. MI Paste® Plus contains glycerol which is a humectant that keeps moisture on the tooth's surface by absorbing water. It seems that glycerol plug formation can be the primary mechanism of prevention of dental hypersensitivity in bleaching treatments. This was confirmed by the immediate effect observed in the present study. This process is later completed by calcium and phosphate ions sedimentation that maintains its effects for longer.[24] Purra *et al*. compared saturated ethanolic propolis solution, 5% potassium nitrate, and distilled water. The results showed no significant difference between propolis and potassium nitrate in immediate, 1-month, and 3-month responses, and propolis was more effective than potassium nitrate only in 1- and 2-week assessments. According to this study, propolis is as effective as potassium nitrate in the immediate and delayed response.[9]

Davari *et al*. showed that using Nd: YAG laser and propolis over three sessions progressively reduced the pain due to dentinal sensitivity. Pain reduction in each treatment session, relative to the previous session, was significant. Propolis was as effective as the Nd: YAG laser, which is the standard method for treating DH.[25] The authors attributed this property of propolis to its high content of flavonoids, which block the tubules. Flavonoids can neutralize free radicals by binding to heavy metal ions, known as accelerators of radical production reactions. The propolis used in the mentioned study was a layer of 40% propolis gel with an overall 8000 ppm polyphenol content. It was applied to the tooth surface by microbrushes in three sessions. Nevertheless, in Davari's study, noncommercial

propolis gel and, consequently, different thicknesses were used on the teeth. In our previous study, propolis gel prepared by Green Plants Company positively but insignificantly reduced postinflammation after periodontal surgeries. However, the low viscosity was a limitation of this gel.[26] One of the strengths of the present study is the use of commercially available toothpaste containing propolis, which facilitated its easier and constant use by all people.

Demydova's study compared the effects of the diode laser, propolis ethanolic extract, fluoride varnish, and a combination of the laser and propolis treatments. The results showed that all agents were similar in the immediate effect. Furthermore, all four groups showed a significant decrease in the mean severity of dentinal sensitivity over 6 months. These findings on the immediate and delayed effects of propolis are in line with the present study.[27]

Although our study demonstrated the individual effects of each paste in relieving dentin sensitivity compared to the baseline, it is recommended to explore these agents with sodium fluoride varnish as a positive control in future clinical studies. In our previous research,[28] which was done on the percentage of open, semi-closed, and closed dentinal tubules, it was shown that the effect of Herbex® herbal toothpaste containing propolis was more than sodium fluoride varnish in reducing the percentage of open dentinal tubules.

This study encountered numerous limitations as a result of the COVID-19 pandemic and associated issues, which made it impossible to conduct additional follow-ups and assess treatment outcomes over the long term. According to the above limitation and the difficulties in clinical trials such as patients' cooperation, it seems that both Herbex® herbal toothpaste containing propolis and GC® MI-Paste Plus were effective in reducing DH after 2 months. Moreover, on one hand, GC® MI-Paste Plus has a higher cost for patients, and on the other hand, Herbex® herbal toothpaste is easier to use, more beneficial, and more cost-effective. Therefore, it seems that Herbex® herbal toothpaste can be used as an alternative to MI paste to treat DH. However, more studies are needed to be more conclusive.

CONCLUSION

Both herbal toothpaste containing propolis and CPP-ACPF-containing paste for 2 months effectively reduced DH. However, after 2 months Herbex® herbal toothpaste seemed to be more beneficial.

Ethics

This study was approved by the Research Committee of Isfahan University of Medical Sciences, Isfahan, Iran (No. 399414).

Authors contributions

NN developed the theoretical framework of this manuscript, performed and supervised the project, and contributed to the final version of the manuscript. AM and AS wrote and prepared the final manuscript. EA randomly divided the participants and registered the clinical parameters.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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