

Evaluation of Remineralization Effect of Various Toothpastes on White Spot Enamel Lesions: An in Vitro Study

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Abstract: Despite the high prevalence of tooth-carries and various efforts to prevent it, studies on plant compounds and their anti-carries properties have been largely neglected. Brushing with toothpaste is the most effective way for carries prevention. This study was performed on KREND herbal toothpaste (containing Calendula and Salvia Officinalis), Herbex (containing propolis), and Crest herbal toothpaste. The remineralization effect of these toothpaste brands on carious lesions has not been studied yet. Therefore, we compared the remineralizing effect of Iranian toothpaste and foreign types in this study. Method and materials: 60 caries-free human premolar teeth were collected after extraction for orthodontic purposes. The surface microhardness (SMH) was measured using the Vickers microhardness testing machine at baseline and after four days of immersing in demineralization solution and after treatment. The pH cycle was then performed for 28 days. The teeth were allocated randomly into one of the four groups, group 1 (as the control group and immersed in distilled water), group 2 (treated with KREND herbal solution), group 3 (treated with Crest solution), and group 4 (treated with Herbex solution). Finally, data were analyzed with Repeated Measure ANOVA and SPSS version 18. All study groups' surface micro-hardness measures were decreased after demineralization ($P=0.001$) and were increased after pH cycling ($P<0.001$). There wasn't a statistically significant difference in mean Surface micro-hardness changes between study groups ($P=0.433$). This study showed that the remineralization ratio in study group samples increased after treatment with herbal toothpaste, and the Iranian toothpaste (Krend) and foreign ones had a similar remineralizing effect.

Keywords: White Spot Lesion, Herbal Toothpaste, Krend, Crest, Herbex, Remineralization

1. Introduction

The first clinical sign of tooth caries is the white-spot lesion (WSL), a reversible subsurface enamel porosity caused by carious demineralization. Enhancing remineralization of White-spot lesions is an effective non-invasive treatment. Remineralizing white-spot lesions are essential for minimally invasive dentistry [1-2]. WSLs are commonly clinically characterized by a chalky opaque appearance on tooth surfaces. As the demineralization process progresses, the

intact dental surface ultimately collapses and cavitates [3]. Although several preventive therapies have been studied to prevent WSL formation, they continue to happen [4]. It is of great significance to explore novel agents and strategies to enhance the remineralization process [1].

The forming processes of caries are loops of imbalances between demineralization and remineralization, initiated by acid-producing bacteria in the micro-environment. This balance is affected by the addition of fluoride, calcium, and Phosphate ions and goes toward remineralization [5].

Brushing with toothpaste is the most common form of doing oral hygiene techniques [6]. Among the caries prevention protocols, Fluoride containing toothpaste is well accepted. The ability of the enamel crystal lattice to replace the lost hydroxyapatite is the basis for the use of fluoride as a remineralizer [4].

Due to the disadvantages of chemical compounds, many studies have been done on plant compounds [7]. Herbex herbal toothpaste contains propolis. Propolis, also known as bee glue, is a natural, nontoxic resinous, sticky substance produced by honeybees through mixing the secretions of their hypopharyngeal glands with the digested product of resins collected from leaves, flowers of plants, trees, and certain barks, which is used as a sealant and sterilizer in honeybee nests [8]. Its antibacterial [9], antifungal [10], antiviral [11], anti-inflammatory [12], anti-cancerous effects have been proven in several studies. KREND herbal toothpaste has marigold and salvia. Marigold or *Salvia officinalis* is a Mediterranean plant [15]. Marigold extract has anti-bacterial [7], anti-fungal and antispasmodic [15] and anti-inflammatory effects [16]. Studies have shown the remineralization effect of these materials on white-spot lesions; however, this toothpaste brand has not been compared with other Iranian and non-Iranian herbal toothpaste. So in this study, we assessed the remineralization potential of white spot lesions between Iranian KREND herbal toothpaste (containing *Calendula* and *Salvia Officinalis*), Herbex (containing propolis), and Crest herbal toothpaste.

2. Methods and Materials

Sixty intact (free from any color change, crack, and caries) human premolar teeth, which had been extracted for orthodontic reasons, were collected. Teeth were cleaned with brushes and pumice (using low-speed handpiece) and stored in normal saline.

In the next step, the teeth were separated on the buccal surface using a paper label with dimensions 3*4 mm². Other areas were covered with acid-resistant varnish (Colorama nail varnish, Maybelline). Then, the crown part of the specimens was separated from their roots by fissure bur and turbine. Samples were mounted with rose wax and coded from 1 to 60. The labels were then removed, and the windows' surface on the samples' buccal surface was polished with 1000, 800 and 2000 grits silicon carbide abrasive sheets (Starcke, Matador, Wesserfest, made in Germany).

In the next step, before any test, all samples' micro-hardness was measured in three points, using the Vicker's micro-hardness tester machine (Sortorios, AG Gottingen, made in Germany) in the Tehran University of medical science, under a load of 200 newtons during 10 seconds.

Sixty prepared samples were randomly divided into four groups of 15. These groups were: 1: Control group, 2. Herbal KREND group, 3. Herbal Crest group, 4. Herbex group.

During the experiment, two solutions made in the dental

materials laboratory of the University of Tehran, were used for all samples, including:

1. demineralization solution with pH=4.5 and below formulation:

NaCl (2.9 g), CaCl₂ (0.12 g), NaH₂PO₄ (0.13 g), NaF (5cc) (100ppm), NaN₃ (5cc), (2% ww), Acetic acid (1.5cc).

2. remineralization solution with pH= 7 and below formulation: (for 0.5 liter).

NaCl (2.9 g), CaCl₂ (0.12g), NaH₂PO₄ (0.13 g), NaF (5cc) (100ppm), NaN₃ (5cc) (2%ww).

First, a stock solution of the above compounds was prepared. Then, using the $C_1 V_1 = C_2 V_2$ formula, each compound's required volume of solution was determined. All samples were immersed in 150 ml demineralizing solution for four days so that primary enamel carious lesions developed on their surface. Vicker's machine reevaluated the sample's micro-hardness under 200 newtons force for 10 seconds. In the next step, the toothpaste was mixed with distilled water in 1:3 relation, and a complete uniform solution of 150 cc was obtained by stirring. Then pH cycling was performed in samples for 28 days by this protocol:

According to the study group, samples were immersed in the 150 ml toothpaste solution for two 2-minute courses. After the first course was placed in 150 cc demineralization solution for three and half hours, and after the second course was placed in 150 cc remineralization solution for three and half hours. They were immersed in deionized water for 10 minutes at each of these intervals, and for each change of solution, 2 minutes of rest for samples were considered. Samples were kept in an incubator at 37°C at each time, and solutions were changed daily.

The repeated measure ANOVA was used to determine significant differences between microhardness changes between groups and according to the nature of variables. Data analysis was performed using IBM SPSS statistics V.18 statistical software running Windows operating system.

3. Results

This study investigated the effect of KREND herbal toothpaste, Herbex, and Crest herbal toothpaste on the microhardness of 60 permanent premolars. Mean microhardness of teeth was measured before testing (step 1) and after placing in demineralization solution (step 2), and after performing the pH cycle for 28 days and treatment with the desired toothpaste (step 3).

Raw information obtained from the microhardness of the samples in each group in 2 steps is shown in Appendices 1 to 4. This information was stored in the SPSS database version 18, and the standard deviation and mean in each group were calculated.

Despite, the mean microhardness was lower in the control group than the other groups (both before and after treatment), but this difference was not statistically significant. Also, the average microhardness of the crest toothpaste group was higher than other groups, but the difference was not statistically significant.

The minimum microhardness of the sample before the experiment was 247.90 in the control group, 312.70 in the KREND group, 322.5 in the Crest group, and 219 in the Herbex group, and after the test was respectively 309.5, 322.50, 300.30, and 215.30, the difference between the two values was negative for both groups. Also, the maximum

micro-hardness level of samples before testing was 482.4T in the control group, 494.9 in the KREND group, 488.6 in the Crest herbal group, and 488.6 in the Herbex group, While it was 421.30, 453.10, 458.70 and 431.50 for these groups, respectively at the end of the cycle and treating with toothpaste.

Table 1. Microhardness of teeth before and after placing in demineralization solution.

group		N	Minimum	Maximum	Mean	Std. Deviation
Control	Hardness before	45	247.90	482.40	376.3178	57.36510
	Hardness.demin	45	12.20	289.80	84.2476	69.96953
	Hardness after	45	309.50	421.30	367.3133	33.12567
	Valid N (listwise)	45				
Krend	Hardness before	45	312.70	494.90	385.7178	39.72248
	Hardness.demin	45	17.10	302.90	69.0667	61.20601
	Hardness after	45	322.50	453.10	375.1044	31.39448
	Valid N (listwise)	45				
Crest	Hardness before	45	322050	488.60	405.9444	33.36736
	Hardness.demin	45	13.40	192.20	77.2956	39.42549
	Hardness after	45	300.30	458.70	390.08667	40.65864
	Valid N (listwise)	45				
Herbex	Hardness before	45	219.00	488.60	379.6933	58.68915
	Hardness.demin	45	12.30	284.20	77.2400	64.33930
	Hardness after	45	215.30	431.50	370.7733	46.49677
	Valid N (listwise)	45				

The microhardness of all samples decreased similarly after demineralization in the first stage ($P=0.001$). Also, the microhardness of the samples after being placed in the 28-day pH cycles and treatment with the desired toothpaste in all groups increased ($P<0.001$). The rate of remineralization ups was similar in all groups ($P=0.433$).

4. Discussion

Due to the high prevalence of tooth caries and considering that White-spot lesions (WSL) are a primary and preventable form of this disease and due to the general popularity of herbal products and their therapeutic properties; this study assessed the effectiveness of three types of herbal toothpaste including Crest, Krend, and Herbex on WSLs, to introduce the most effective substance. This study's other purpose was to compare Iranian products with their similar foreign brands.

Marigold (*Salvia officinalis*, sage), which has been used in the formulation of Krend toothpaste, is one of the most widely used herbal medicines used in traditional medicine [7]. It has anti-bacterial, anti-oxidant [7], anti-fungal and antispasmodic [15] and anti-inflammatory effects [16]. *Salvia officinalis* is an aromatic plant that had been thoroughly evaluated. Although there are no restrictions on its use in food, the European pharmacopoeia Agency considers 5 mg daily to be acceptable [15]. This substance can be used as an additive to conventional plaque control techniques [7]. It can also be used as a cleanser to prevent *Candida Albicans* seedling stomatitis [15]. Marigold is rich in Quercetin, Carotenoids, Lutein, Lycopene, Rutin, Ubiquinone, Xanthophyll, and other anti-oxidants. Quercetin inhibits the recombinant matrix of human matrix metalloproteinases that are related to periodontal disease. So it can be very beneficial as adjunctive therapy in periodontal disease prevention. It

can also speed up wound healing [16].

Herbex toothpaste contains propolis, which has a strong antimicrobial effect [23] that Justifies its use in reducing the population of cariogenic bacteria and perio-pathogens and even as a rinsing agent in endodontic treatment. It also has antifungal effects [10], which can treat chronic erythematous candidiasis in patients using dentures. Propolis also has a stimulating effect on bone formation [54], which can be used in oro-maxillo-facial surgery and periodontology to accelerate the process of ossification. It contains various chemical components such as phenolic acid, trepans, caffeic acid, and esters. Most of these compounds are lipophilic, which is why propolis's ethanolic extracts are more popular than water-soluble ones [55]. The composition of propolis varies in different regions and seasons and in other studies, which cause different therapeutic effects. The bio-activity of propolis is primarily related to its phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives [54].

Despite all the favorable properties mentioned for this natural substance, propolis is known as an allergen. So far, 180 compounds have been identified in propolis; the most sensitive of which is caffeic acid esters. Other compounds such as free aromatic acids, isoflourides, and flavonoids also play an essential role in allergenicity [56]. The proper dose of propolis remains unknown and given that most of the published findings of propolis in dentistry have been in vitro or on animal models, generalizing the results to human clinical conditions may not be correct.

In the present study, samples were immersed in a demineralizing solution with pH = 4.5 for four days before starting pH cycling to cause primary enamel decay lesions on them artificially. In general, the main component of these solutions used in various studies includes calcium and lactic acid. In each study, according to the desired pH and the

duration of exposure of the samples to create lesions, the final composition of the solution is determined. The pH of the solution used in different studies varies from 3.5 to 5 [57]. The study of Lata *et al.* was done by immersing the samples for three days in a solution with pH = 5.4 [52]. Hussam *et al.* Immersed the samples in a demineralizing solution with pH = 6.4 for 14 days to induce artificial caries, but then they did not apply pH cycling and were satisfied with exposing the samples to the mineralizing solution for seven days [45]. In the study of Shishir Shetty *et al.*, The samples were placed in a demineralizing solution with pH = 5 for four days [43].

In this study, we used the pH cycling approach to stimulate the oral environment. We performed this cycle for 28 days so that samples were immersed in the remineralizing solution for 17 hours and then in demineralizing solution for 3 hours [43]. Hung *et al.* performed this cycle for 12 days, based on the protocol presented in picture 17, so that samples had been immersed in the remineralizing solution for Twenty-one hours and 2 hours in demineralizing solution and four 3-minutes periods in the vicinity of toothpaste [51]. Lata *et al.* placed the samples in demineralizing solution for 3 hours daily and in remineralizing solution and artificial saliva for 21 hours for five consecutive days [52].

Various methods have been used to evaluate the remineralization of samples in studies. For example, the use of electron microscopy (SEM) is a common method in studies. In one study, the surface morphology of the samples was investigated by SEM (Scanning Electron Microscope) and mineral changes using EDAX (Energy Dispersion X-ray Spectrophotometer) [59]. In the study by Elkassas *et al.*, SEM was used to evaluate microhardness and surface roughness [42]. Huang *et al.* used XRD (X-Ray Diffraction) and PLM (Polarized Light Microscopy) in addition to SMH and SEM [52]. In a study that assessed the appearance of the samples, they used standard photography for evaluation [2]. In other studies, a precise method for evaluating the mineral content of the samples, namely Micro CT, has been mentioned [57], which could not be accessed in our country.

In a significant number of articles, a microhardness evaluating device has been used to check the surface hardness of the samples [38, 39, 42, 43, 45, 52]. In this study, we used the Vickers microhardness device to evaluate SMH (Surface Micro Hardness) among the available evaluation methods. In such a way, the surface hardness of each sample was measured at three points of it, under the force of 200 N and within 10 seconds, once before the test and once after demineralization, and once after the end of the test.

It should be noted that in many studies, the therapeutic effect of herbal compounds has been compared with chemical compounds. In a study by Pannuti *et al.* to compare the efficacy of Paradontax herbal toothpaste with conventional chemical fluoride toothpaste, It was found that this herbal toothpaste has a similar effect to the chemical sample [5]. Some studies also show that using plant extracts in combination with common chemicals is more effective than using each of them alone. Hung and colleagues conducted a study to investigate the effect of a combination

of Galla extract, a Chinese plant, and apatite nanohydroxide; In this study, it was found that due to the application of the combination of these two materials, WSL remineralization occurs in a greater extent [51]. Propolis has received more attention from the studies conducted to compare plant compounds with chemical types than others. An example was found in a 2013 study by Hongal *et al.*, that Propolis extract can be as effective as GC Tooth Mousse in closing dentinal tubules and reducing sensitivity [11].

In the present study, it was found that the remineralizing effect of herbal KREND toothpaste and Herbex was similar to herbal crest toothpaste as the selected standard, and The hardness of the samples increased after being placed in the pH cycle and treated with the herbal toothpaste tested. But what seems necessary is to do more studies and compare these samples with different types of chemicals and also examine them in the oral environment to achieve more reliable results.

5. Conclusion

In the present study, it was found that even though the mean microhardness was lower in the control group, the remineralizing effect of herbal KREND toothpaste and Herbex on primary enamel decay lesions was similar to the effect of herbal crest toothpaste as the selected non-local standard sample. In other words, there is no statistically significant difference in terms of the ability to remineralize primary enamel decay lesions between herbal KREND and Herbex toothpaste, with crest herbal toothpaste, but it seems necessary to do more studies and compare these samples with different types of chemicals and examine them in the oral environment to achieve more reliable results.

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