

# Effect of a Synthetized Herbal Remineralizing Paste on Enamel Microhardness

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**Objectives** This study aimed to synthesize an herbal remineralizing paste containing the extracts of Miswak, licorice, aloe vera, propolis, and honey and compare its effect with 5% sodium fluoride (NaF) and 1.23% acidulated phosphate fluoride (APF) on demineralized enamel microhardness.

**Methods** This experimental study was conducted on 72 extracted sound premolars. After obtaining the extracts and synthesis of the herbal paste, the baseline microhardness was measured, and the samples underwent a 14-day pH cycling. Next, the teeth were randomly assigned to six groups (N=12) for application of (I) 5% NaF for one minute, (II) 1.23% APF for one minute, (III) herbal paste for one minute, (IV) herbal paste for five minutes, (V) herbal paste for 15 minutes, and (VI) herbal paste for six hours. The microhardness was measured again. Data were analyzed by one-way analysis of variance (ANOVA) ( $\alpha=0.05$ ).

**Results** The mean final microhardness was significantly higher than the primary microhardness in all groups ( $P<0.05$ ). The highest microhardness was noted in group IV, followed by II, VI, I, V and III. One-way ANOVA revealed no significant difference between the final microhardness of study groups ( $P=0.97$ ).

**Conclusion** The effect of synthesized herbal paste on demineralized enamel microhardness was comparable to that of 5% NaF and 1.23% APF. Due to the lack of a significant difference in duration of application, this paste may be applied for one minute to benefit from its remineralizing effects.

**Keywords** Hardness; Tooth Remineralization; Herbal, Medicinal

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## Introduction

Dental caries is the most common oral disease worldwide.<sup>1</sup> According to the World Health Organization (WHO), the prevalence of dental caries in school-aged children is 60% to 90%, and it is also highly prevalent in adults.<sup>2</sup> Dental caries is an epidemic disease often associated with pain that can adversely affect the quality of life and well-being of individuals.<sup>3</sup> It also imposes a financial burden on the healthcare systems.<sup>3</sup>

Caries development is a dynamic process. It develops as a subclinical incipient enamel lesion, which progresses and creates a cavitated lesion.<sup>4</sup> This microbiological infectious disease is characterized by demineralizing tooth structure and local degradation of demineralized lesions.<sup>5</sup> Demineralization occurs through two important mechanisms: (I) Dissolution of enamel apatite crystals and (II) release of calcium, phosphate, and hydrogen ions from enamel microstructure.<sup>6</sup> Clinically, demineralization is manifested in the form of a chalky white opaque lesion in a dry environment. In a normal oral environment, enamel hydroxyapatites interact with the saliva saturated in calcium and phosphate (PO<sub>4</sub>) ions<sup>7</sup>, and remineralization often occurs in the presence of adequate amounts of calcium, phosphate, fluoride, and increased saliva flow and its buffering capacity. The calcium and hydrogen phosphate (HPO<sub>4</sub>) ions present in the saliva can replace the lost ions in the tooth structure and cause remineralization. Also, HPO<sub>4</sub> increases the buffering capacity of saliva and can cause reversal of incipient enamel lesions.<sup>8</sup>

Although in addition to calcium and phosphate, saliva

averagely contains 0.03 ppm fluoride; mouthwash, toothpaste, and other topical fluoride agents can be used to enhance remineralization of incipient enamel lesions.<sup>9</sup> At a  $\text{pH} \leq 5.5$ , hydrogen ions produced by bacterial metabolism react with the phosphate groups of enamel crystals and lead to the conversion of PO<sub>4</sub> ions to HPO<sub>4</sub> ions. Following formation, they cannot form a crystalline network, and the hydrogen ions are buffered. This process leads to enamel dissolution, referred to as demineralization, which is the first phase of incipient enamel caries development.<sup>10</sup> However, if the acidic pH is neutralized, demineralization is reversible, given that sufficient amounts of calcium and phosphate ions are present in the environment. This process results in reformation of some dissolved apatite crystals, referred to as remineralization.

Fluoride is the most commonly used remineralizing agent. Fluoride can prevent enamel demineralization and enhance remineralization by the formation of fluorapatite. Fluorapatite crystals create a rigid, superficial layer that is more resistant to demineralization than hydroxyapatite.<sup>11,12</sup> The mechanisms of action of fluoride as the gold standard for caries prevention include decreasing acid production by the microorganisms, inhibiting the synthesis of intracellular and extracellular enzymes, and replacing hydroxide ions of hydroxyapatite with fluoride ions.<sup>11,13</sup> Nonetheless, fluoride can cause fluorosis and is toxic in high concentrations.<sup>14,15</sup>

The popularity of herbal medications is rising, and people are becoming more interested in consuming herbal products instead of synthetic chemical products. Many herbal species with cariostatic properties may be used in the formulation of oral hygiene products. Miswak or *Salvadora persica* is

commonly used to prepare Miswak sticks.<sup>16</sup> It contains over ten different natural constituents that are all suitable for oral health, including fluoride and calcium.<sup>17</sup> Repeated use of Miswak can induce remineralization of incipient enamel lesions due to its fluoride and calcium contents.<sup>17,18</sup> It has been confirmed that Miswak contains three important minerals required for remineralization, namely fluoride, calcium, and phosphate.<sup>19</sup>

Propolis is a resinous substance produced by honeybees, which also has cariostatic effects.<sup>20</sup> Depending on its geographical origin, this substance contains different calcium and phosphate concentrations, which can cause enamel remineralization.<sup>21</sup> It inhibits the activity of glycosyltransferase of *Streptococcus mutans*, which plays a role in the formation of insoluble glucans in the biofilm, and exerts its cariostatic effect.<sup>22</sup> It can also increase the availability and reuptake of calcium and phosphate by the hydroxyapatite crystals of demineralized enamel and cause remineralization.<sup>23</sup>

Licorice, or *Glycyrrhiza glabra*, is another medicinal plant with cariostatic activity.<sup>24</sup> The dose-dependent activity of glycyrrhizin present in the root of licorice can inhibit the activity of glycosyltransferase of *Streptococcus mutans*.<sup>25</sup> If added to APF, glycyrrhizin can increase the fluoride uptake and decrease enamel solubility due to its deposition on the surface of porous demineralized enamel.<sup>26</sup>

Honey is another natural substance that can decrease demineralization and enhance remineralization since it can increase saliva flow<sup>27</sup> and has a buffering capacity higher than that of saliva.<sup>28</sup>

Aloe vera or *Aloe barbadensis*, another medicinal plant with 75 active compounds<sup>29</sup>, contains polyphenols, including antrakinons, which can cause enamel remineralization.<sup>30</sup> It also contains necessary amino acids, the most abundant of which is arginine.<sup>31</sup> The enamel surface is porous and may allow passage of ions, such as sodium, potassium, magnesium, and calcium. Thus, the remineralizing capacity of toothpastes containing aloe vera may be attributed to the deposition of calcium-related arginine on the enamel surface.<sup>32</sup>

Considering all the materials mentioned above, this study was designed to synthesize an herbal remineralizing paste containing Miswak, honey, propolis, licorice, and aloe vera and assess its effect, in comparison with NaF and APF, on demineralized enamel microhardness to evaluate the remineralizing capacity of this paste.

## Methods

This in vitro, experimental study was approved by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1399.973). The sample size was randomly calculated to be 12 in each group (a total of 72 assigned to six groups) according to a previous study<sup>33</sup>,

assuming a 95% confidence interval, study power of 80%, expected difference in mean microhardness of six units, and standard deviation of five units.

## Herbal Paste Synthesis

### Extraction Process

In order to obtain Miswak extract, fresh roots and stems of the Miswak plant were first obtained, and after drying, they were ground and powdered in a ball mill. Next, a certain amount of powder, along with 80% ethanol as solvent, was transferred to a Soxhlet extractor (Bakhshi Laboratory Industries, Iran) for extraction in four hours.

In order to obtain propolis extract, a certain amount of propolis was dissolved in 85% ethanol and placed in a shaker incubator (Noorsanattajhizferdos Company, Iran) at 37°C for 48 hours, operating at 150 rpm. The obtained solution was filtered using a #4 Whatman filter paper. The obtained solution was subjected to an extraction process, as explained earlier. The extract was filtered again, stored at 4°C for 24 hours, and filtered through a #1 Whatman filter paper. Then, the solution was transferred to a rotary evaporator (Teifazmateb Company, Iran) for evaporation, and the alcoholic extract of propolis was finally obtained.

In order to obtain aloe vera hydroalcoholic extract, some fresh aloe vera leaves were collected, washed, and their gel was extracted. The obtained gel was added to 95% ethanol in a volume four times that of gel, and the mixture was placed on a shaker for four days. The solution was then filtered, concentrated in a rotary evaporator at 45°C, dried at 40°C, and powdered.

In order to obtain licorice extract, licorice roots were collected, cleaned, and stored at room temperature away from sunlight for a couple of days to completely dry. They were then ground and powdered. For extraction, dried powder and 80% ethanol were placed in the Soxhlet extractor for 90 minutes. The extract was transferred into a Falcon tube and refrigerated for concentration. Then, it was concentrated in a rotary evaporator at 50-60°C for one hour. The concentrated extract was dried in an oven (Parsazma Company, Iran), and a solid extract was obtained.

### Product Synthesis

First, 40 g of guar gum was mixed with deionized water for concentration of paste, and the obtained gel was mixed with aloe vera extract in a mixer at 375 rpm for 10 minutes. Next, honey was added and mixed at 375 rpm for 10 minutes. Finally, propolis, Miswak, and licorice extracts were added and mixed at 375 rpm for 10 minutes. After homogenization, titanium dioxide was added, and final homogenization was performed. After the synthesis of paste, its effect on demineralized enamel microhardness was assessed.

## Evaluation of Synthesized Herbal Paste Effect on Enamel Microhardness

A total of 72 sound human premolars extracted for orthodontic reasons were collected and immersed in 0.5% chloramine T solution. The tissue residues were removed by a #15 surgical scalpel, and the teeth were inspected under a stereomicroscope (Olympus, Tokyo, Japan) to ensure the absence of cracks and caries. The roots were cut at the cervical region, and tooth crowns were split into facial and palatal halves by a diamond saw. Each half was mounted in auto-polymerizing acrylic resin (Acropars, Iran). A 4×3 mm window on the facial or palatal enamel surface remained exposed, while the other parts were coated with transparent nail varnish. The specimens were immersed again in distilled water to prevent dehydration. Before any intervention, a Vickers hardness test was performed to measure the microhardness of specimens using a microhardness tester (Buehler, Lake Bluff, IL, USA). For this purpose, three indentations were created at three random points with 500 g load application within five seconds, and the mean microhardness of the three points was calculated and reported. Teeth with similar primary microhardness were selected for the study. Next, the specimens underwent pH cycling. For this purpose, the specimens were immersed in a demineralizing solution (composed of 2.2 mmol sodium dihydrogen phosphate [NaH<sub>2</sub>PO<sub>4</sub>], 0.5 mmol calcium chloride [CaCl<sub>2</sub>], and 50 mmol acetic acid) synthesized in the Biochemistry Laboratory of Hamadan University of Medical Sciences with a pH of 4.46 for 8 hours. They were then immersed in artificial saliva (composed of 0.4 g/L sodium chloride [NaCl], 0.4 g/L potassium chloride [KCl], 0.795 g/L calcium chloride dihydrate [CaCl<sub>2</sub>.2H<sub>2</sub>O], 0.78 g/L sodium dihydrogen orthophosphate [NaHPO<sub>4</sub>.2H<sub>2</sub>O], 0.005 g/L Na<sub>2</sub>S.2H<sub>2</sub>O, water and urea<sup>11,34</sup> synthesized in the Biochemistry Laboratory of Hamadan University of Medical Sciences for one hour, and subsequently in remineralizing solution [composed of 1.5 mmol Ca<sup>2+</sup> (CaCl<sub>2</sub>), 0.9 mmol phosphate (KH<sub>2</sub>PO<sub>4</sub>), and 1 ppm fluoride (NaF)] with a pH of 7 for 15 hours. The cycle was repeated for 14 days to induce enamel demineralization.

The demineralizing and remineralizing solutions were refreshed every two days.<sup>35</sup>

After 14 days of pH cycling, the teeth were randomly divided into six groups (n=12) for (I) application of 5% NaF (ADS, USA) for one minute, (II) application of 1.23% APF (ADS, USA) for one minute, (III) herbal paste for one minute, (IV) herbal paste for five minutes, (V) herbal paste for 15 minutes, and (VI) herbal paste for 6 hours. The respective material in each group was applied by a micro-brush with fine and soft bristles (such that they could not cause enamel wear) by gentle rubbing motion for 30 seconds. Next, the specimens were rinsed with artificial saliva for 30 seconds and immersed in distilled water for 24 hours. The microhardness of specimens was then measured again.

Finally, two specimens were selected from each group to assess the enamel surface under a scanning electron microscope (Hitachi S-450, Japan) with a 20 kV voltage.<sup>36</sup>

#### Statistical Analysis

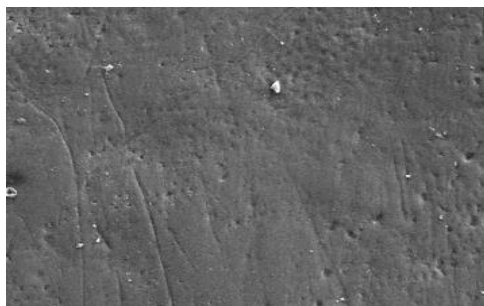
Data were analyzed using SPSS version 21. The Shapiro-Wilk test confirmed the normal distribution of data (P>0.05) at baseline. Thus, comparisons were made using one-way analysis of variance (ANOVA) at a 0.05 level of significance.

### Results

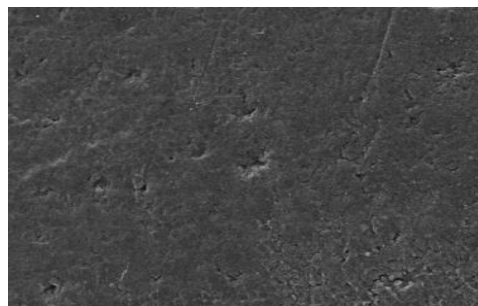
Table 1 presents the mean microhardness of the groups at baseline and after the intervention. As shown, the highest microhardness was noted in herbal paste/ five minutes, followed by 1.23% APF, herbal paste/ six hours, 5% NaF, and herbal paste/15 minutes and /one minute. One-way ANOVA revealed that the final microhardness in all groups was significantly higher than the primary microhardness (P<0.05). However, the final microhardness of the six groups was not significantly different (P=0.97). Figure 1 (A-F) shows the SEM micrographs of the groups.

**Table 1-** Mean microhardness (kg/mm<sup>2</sup>) of the groups at baseline and after the intervention

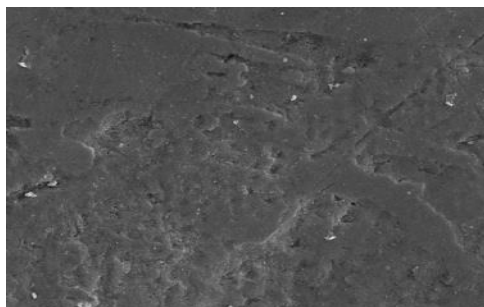
Groups	Baseline microhardness	Final microhardness	Difference	P value
	Mean ± SD	Mean ± SD	Mean ± SD	
5% NaF	299.86 ± 9.77	359.19 ± 13.14	59.33 ± 16.47	0.97
1.23% APF	298.33 ± 11.13	362.09 ± 10.97	63.75 ± 18.76	
Herbal paste/1 min	302.52 ± 12.37	358.56 ± 12.97	56.4 ± 12.17	
Herbal paste/5 min	298.56 ± 10.34	368.73 ± 11.33	70.16 ± 19.11	
Herbal paste/15 min	310.30 ± 6.36	367.45 ± 11.70	57.15 ± 10.28	
Herbal paste/6 h	308.65 ± 6.45	370.24 ± 6.32	61.59 ± 10.74	



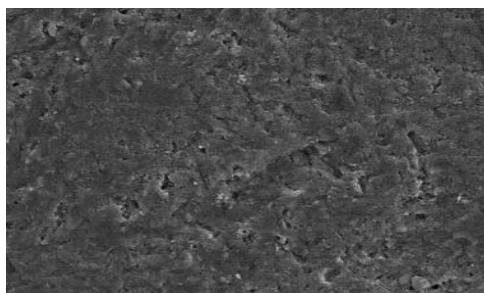
**Figure 1 A:** SEM micrograph of the enamel surface in 5% NaF group



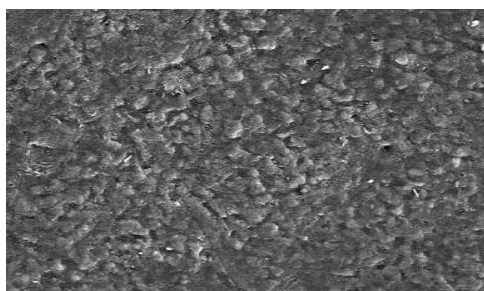
**Figure 1 F:** SEM micrograph of the enamel surface in herbal paste/6 hours group



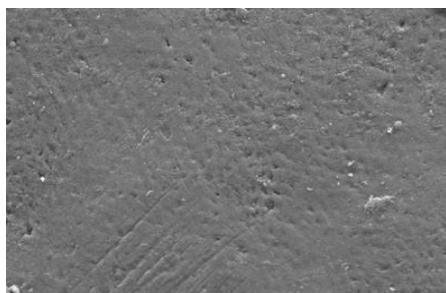
**Figure 1 B:** SEM micrograph of the enamel surface in 1.23% APF group



**Figure 1 C:** SEM micrograph of the enamel surface in herbal paste/1 minute group



**Figure 1 D:** SEM micrograph of the enamel surface in herbal paste/5 minutes group



**Figure 1 E:** SEM micrograph of the enamel surface in herbal paste/15 minutes group

## Discussion

This study was designed to synthesize an herbal paste containing Miswak, honey, propolis, licorice, and aloe vera and assess its effect, in comparison with NaF and APF, on demineralized enamel microhardness to evaluate the remineralizing capacity of this paste. The results showed that the mean final microhardness was significantly higher than the primary microhardness in all groups. No significant difference was found in the final microhardness of the groups. In other words, the extracts used in the herbal paste had synergistic effects and yielded a remineralizing capacity comparable to NaF varnish and APF gel.

In the present study, pH cycling was performed to induce enamel demineralization artificially.<sup>37</sup> Also, the Vickers microhardness test was conducted to assess enamel remineralization since this test can reveal a reduction or increase in mineral content by assessment of the hardness of enamel and dentin.<sup>38</sup> An increase in indentation depth indicates mineral loss, while a reduction in depth indicates mineral gain. Thus, lower values indicate demineralization and vice versa.<sup>39</sup> This test is fast and reliable for assessment of enamel remineralization/demineralization.<sup>40,41</sup>

In the present study, 5% NaF increased the microhardness by 34%, which was not significantly different from other groups. Lata et al.<sup>42</sup> evaluated the effect of fluoride varnish, casein-phosphopeptide amorphous calcium phosphate (CPP-ACP), and a combination of both on enamel microhardness and remineralization and found that fluoride varnish had a greater efficacy for remineralization of superficial enamel, while CPP-ACP and a combination of fluoride varnish and CPP-ACP were not as effective as fluoride varnish alone. None of the products effectively remineralized deep enamel.<sup>42</sup> The difference between their results and the present findings regarding the efficacy of NaF varnish may be due to different methodologies since the pH cycling period was 14 days in the present study versus three days in their study. Also, NaF was applied only once on the specimen surface after pH cycling while they applied it twice, which may explain the difference in the results.

Consistent with the present results, Lee et al.<sup>43</sup> compared the

effects of 2% and 5% NaF and 1.23% APF on enamel microhardness and found no significant difference among them. Their results were similar to the present findings despite some methodological differences between the two studies, such as the use of bovine teeth, different assessment time points, and application of APF for four minutes and 5% NaF for 12 hours in their study. In the present study, both 5% NaF and APF gel were applied for one minute for standardization, although according to the manufacturer, APF gel can be applied for 1 to 4 minutes. APF is composed of a combination of hydrofluoric acid, phosphoric acid, and NaF. Phosphoric acid in its composition has a pH of 3.5 and can cause enamel porosities for better penetration of fluoride. However, the concentration of phosphoric acid in its composition has not been disclosed by the manufacturer. Thus, its effect on microhardness should be assessed after both 1-minute and 4-minute applications. NaF varnish (5%) has 22500 ppm fluoride, while 1.23% APF with 2.09% NaF has 12300 ppm fluoride. Despite the difference in fluoride content, the difference in the remineralizing effects of 5% NaF and 1.23% APF was not significant in the present study.

Zhang et al.<sup>44</sup> evaluated the effect of Chinese Galla on demineralized enamel remineralization and concluded that it could cause superficial enamel remineralization with an unknown mechanism. Ranjana et al.<sup>45</sup> compared the remineralizing effects of a non-fluoridated commercial toothpaste (Mee Mee) and an herbal toothpaste (Mamaearth) containing xylitol, Stevia, and aloe vera and found insignificant remineralizing effects of herbal toothpaste, which was different from the present results, which can be due to different methodology, and use of different products. Gocmen et al.<sup>46</sup> evaluated the remineralizing capacity of a combination of honey and ginger, compared with NaF varnish and some other herbal compounds (honey, ginger, chocolate, and rosemary oil mixture) by the microhardness test and a fluorescence camera. They demonstrated that the mixture of honey and ginger had the highest remineralizing effect on demineralized enamel; however, the quantitative light-induced fluorescence method, which was applied for quantitative assessment of demineralized enamel lesions, revealed no significant difference between NaF and herbal groups. Their results regarding significantly higher remineralization by honey and ginger, compared with NaF, were different from the present findings. This difference may be attributed to their different methodologies, and use of different compounds. Thus, different methods employed for qualitative and quantitative assessments of enamel mineralization can affect the final results. Basiony et al.<sup>47</sup> showed that Himalaya herbal toothpaste had the greatest effect on the remineralization of demineralized enamel after three months compared to toothpastes with fluoride, and the

cause of this increase was the fluoride contained in the Miswak plant. They used the Diagnodent laser in their study to evaluate the white spot lesions. The use of a Diagnodent laser in detecting white spots on patients has high sensitivity, which can lead to false positive diagnoses.<sup>48</sup> However, the three-month recall period can evaluate the effects on patients' oral health. Asgartooran et al.<sup>49</sup> showed that herbal toothpaste had the least effect on remineralization of demineralized enamel compared to fluoride toothpaste and Sensodyne toothpaste.<sup>49</sup> According to their study, Novamin in Sensodyne toothpaste, which is a type of bioactive glass, increased remineralization compared to the other two types of toothpaste.

The constituents of the paste synthesized in the present study have antimicrobial effects on cariogenic bacteria by affecting the bacterial enzymes. The fluoride and calcium contents of Miswak can enhance enamel remineralization.<sup>17,18,50</sup> Also, Miswak extract releases small amounts of fluoride but considerable amounts of calcium and phosphorous in water.<sup>51</sup> Moreover, glycyrrhizin present in licorice root decreases the solubility of porous demineralized enamel by coating the surface and thus enhances fluoride uptake.<sup>26</sup> It also inhibits the glycosyltransferase of *Streptococcus mutans*.<sup>25</sup> Gedalia et al.<sup>26</sup> indicated that adding glycyrrhizin to APF enhanced fluoride uptake and decreased enamel solubility. Glycyrrhizinic acid inhibits acid production by dental plaque and decreases enamel solubility *in vitro*.<sup>52</sup>

Gocmen et al.<sup>46</sup> and Celik et al.<sup>53</sup> demonstrated that a combination of several plants with cariostatic effects had significant remineralizing effects on demineralized enamel compared with chemical compounds containing fluoride. Celik et al.<sup>53</sup> revealed that organic honey caused demineralized enamel remineralization according to the microhardness test results and the absence of erosion on the enamel surface after applying honey. This result was attributed to the presence of calcium, phosphorous, and fluoride in honey.<sup>54</sup> Propolis has different calcium and phosphorous levels depending on its geographical origin<sup>21</sup> and enhances the availability and uptake of calcium and phosphate.<sup>55</sup> Ali et al.<sup>23</sup> demonstrated that toothbrushing with propolis enhanced calcium and phosphate uptake by the enamel and increased remineralization. Although they simulated toothbrushing in their study, their results regarding the enhancement of microhardness were in agreement with the present results. Moreover, Aprillia et al.<sup>56</sup> indicated comparable efficacy of propolis fluoride and NaF varnish in the prevention of enamel demineralization.

It should be noted that in the clinical setting, many other factors are present that can affect enamel remineralization. For instance, saliva composition and its flow rate play fundamental roles in the occurrence of remineralization, which can affect the efficacy of external products applied to

enhance remineralization. Also, the present results were obtained under in vitro conditions in the absence of cariogenic bacteria. Since the herbal ingredients of the synthetic paste all have antibacterial activities, even better results may be obtained in the clinical setting. Thus, clinical studies are required to assess the efficacy of this paste. Also, further studies are recommended to assess the substantivity of the effects of this paste under both in vitro and in vivo conditions. Future studies are also recommended to compare the efficacy of applying 1.23% APF gel for 1 and 4 minutes for enamel remineralization. Moreover, it is recommended that an enamel wear test be performed after applying the herbal paste. Furthermore, it is important to find out which herbal extract has the highest efficacy for the enhancement of microhardness of demineralized enamel. One of the limitations of this study is the absence of an intermediary between the industry related to herbal paste production and the university.

## Conclusion

The effect of synthesized herbal paste on demineralized enamel microhardness was comparable to that of 5% NaF varnish and 1.23% APF gel. Due to the lack of a significant difference in duration of application, this paste may be applied for one minute to benefit from its remineralizing

effect, given that its safety is confirmed in the clinical setting.

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**Author Contributions:** M.S. Conceptualization and Data curation; S.K. Formal analysis and Investigation; S.K. and M.S. Methodology; M.S. Project administration; S.K. Resources; M.S. Supervision; S.K. Validation; M.S. Visualization; S.K. Writing – original draft and editing

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**Institutional Review Board Statement:** This in vitro, experimental study was approved by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1399.973).

**Informed Consent Statement:** This article is in\_vitro study and it has no informed consent.

**Data Availability Statement:** The data are original and we cannot share them for ethical reasons. Upon request from the corresponding author, we can share the data with reasons.

**Conflict of Interest:** No Conflict of Interest Declared ■

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