

# Evaluation of Remineralization Potential of Preventive Regimen Containing Herbal-Based Compared to Fluoride-Based Toothpastes in High Caries Risk Patients with Initial Carious Lesions: A Randomized Clinical Trial

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## ABSTRACT

**Objective:** This study was conducted to assess the effect of preventive regimens using herbal in comparison fluoride toothpastes on remineralization of initial carious lesions in high risk patients.

**Materials and methods:** Two parallel armed group, randomized, controlled trial was conducted on 32 participants with high caries risk, having initial carious lesions at their smooth surfaces. Participants were randomly allocated into two groups, 16 participants each. Assessment of remineralization of initial lesions were evaluated by diagnodent, visual examination of initial lesions was evaluated by International Caries Detection and Assessment System (ICDAS) scoring system.

**Results:** Assessment using diagnodent there was no statistically difference throughout the follow-up phases; baseline and 1 week ( $P=0.6669$ ), while at 3 months' intergroup comparison revealed statistically difference between herbal and fluoride toothpaste ( $P=0.0155$ ), intergroup comparison within Himalaya or Colgate have proven that there is a statistically difference between the various follow-up times ( $P<0.0001$ ). Regarding ICDAs, there was no statistical difference ( $P=0.1506$  and  $P=0.7273$ ), intragroup comparison within Himalaya have shown statistically difference between different periods ( $P=0.0159^*$ ). Within Colgate, intragroup comparisons revealed no significant differences between different periods ( $P=0.1506$ ).

**Conclusion:** Preventive regimens containing different toothpastes are positively influencing remineralization potential of initial lesions in high caries risk patients, while herbal toothpaste was superior along three months follow up. Different regimens didn't produce significant effect in altering visual appearance of initial lesions.

**Keywords:** Remineralization; Herbal toothpaste; Fluoride toothpaste; Preventive regimen; High caries risk patients

## INTRODUCTION

Caries of the teeth is one of the most frequent diseases on the planet. It works by transforming carbohydrates to acid, which then mixes with food debris and saliva to form biofilms on the tooth's surface, lowering the mouth's pH to 5.5 and beginning the demineralization process [1]. The timing is quite specific due to their dynamic nature; initial carious lesions' remineralization is essential for caries management. The first carious enamel lesions start out as white spots, which are a warning sign that the lesions are developing and will grow into cavities after that, remineralization is the process that prevents an active initial carious lesion from advancing to the cavitated stage [2].

A paradigm shift in caries management has occurred, moving away from restorative procedures and toward non-restorative management of caries, containing methods of early prevention. The implementation of regimens to control early carious lesions enables the dentist to

revert beginning lesions with initial carious lesions, achieving the goal of modern dentistry; less interventions [3], enamel lesions selectively absorb fluoride ions onto incomplete enamel defects with demineralized hydroxyapatite crystals or reoffer fluorohydroxyapatite. As a result, increasing fluoride can boost remineralization and build a low-solubility veneer on the remineralizing crystals, this mineral is acid-resistant [4].

Herbal components Sanguinary, Propolis, Miswak, Charcoal, Clove, and *Azadirachta indica* (neem), are the most frequent herbal compounds found in items for oral hygiene; dentrifice and mouthwash. Through animal research, *in vivo* and *in vitro* studies any leaves or herbalist have been marketed as having anti-inflammatory, anti-pyretic, analgesic, anti-bacterial, anti-viral, anti-carcinogenic, and anti-oxidant effects. As a result of these issues, several items for oral care makers and global corporations have begun to incorporate botanical ingredients into their goods [5].

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The Himalaya herbal toothpaste contains many ingredients, its key ingredients are Miswak, Babool, Neem and Pomegranate [6]. It contains also calcium fluoride. Miswak contains roughly 1.02 g/g fluoride, and also releases high amount of calcium in saliva which are essential for remineralization [7]. Babool contains fluoride and have the benefits in remineralization of the teeth [8]. Neem contains 2.8 µg/g fluoride which helps in preventing dental caries. The uses Calcium fluoride in toothpastes are an essential strategy for preventing tooth decay [7]. In the existence of accessible calcium (Ca<sup>2+</sup>) ions, the fluoride ion causes remineralization of caries-affected dental hard tissue, resulting in fluoro-hydroxyapatite. As a result, adding calcium to fluoride toothpaste increased remineralization of enamel underlying lesions much more than fluoride toothpaste only [9]. Pomegranate is known also to possess bactericidal, antioxidant, and anti-inflammatory traits so it controls the remineralization of Himalaya toothpaste [10].

So, this clinical research was carried out to compare the remineralization potential of herbal toothpaste preventive regimen versus a preventive regimen using sodium mono fluorophosphate toothpaste among high caries risk patients having initial caries lesions patients. The null hypothesis tested was that caries prevention regimen including herbal toothpaste would have remineralization potential similar to fluoride-based toothpaste.

## MATERIALS AND METHODS

### Sample size calculation

We were planning a study of a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. In a previous study by Güçlü et al in 2016 the response within each subject group was normally distributed with standard deviation 3.1. If the true difference in the experimental and control means is 3.58, we will need to study 13 experimental patients and 13 control patients to reach a total of 26 subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. This was increased by 20% to compensate for dropouts to be 32 patients (16 per group). The Type I error probability associated with this test of this null hypothesis is 0.05. Sample size was calculated using PS version 3.1.2 for windows using t test for independent variables.

### Protocol registration

This study was registered in clinical trials ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) with I.D: NCT04446390. The study was approved by Research Ethics Committee (CREC), Faculty of Dentistry, with approval number 22/7/20, and informed consent was obtained from all participants. It was a triple blinded study where the patients, data analyzer and outcome assessors were blinded where all toothpastes were sprayed with black paint.

### Study design

This two-armed, parallel-design, and randomized clinical trial study was conducted out over a period of three months with a total of three visits: first visit (baseline: T0), second visit (1 week: T1), and final visit (12 weeks: T2). The primary outcome was assessment of remineralization of initial carious lesions by diagnodent, and secondary outcome was visual assessment of initial lesions by International Caries Detection and Assessment System (ICDAS II).

### Eligibility criteria for patients

Guclu et al., Karabekiroglu, and Ünlü the patients in this study were selected following different exclusion and inclusion criteria,

### Inclusion criteria of the participants

- Healthy patients with free medical history.
- Age range 20-40 years.
- Patients with high caries risk assessment according to Cariogram.
- Patients with initial carious lesions at the facial surface of any teeth.
- High plaque index.
- Not under antibiotic therapy either at the time of the study or up to the last month before the start of the study.
- Patients having any appliances.

### Exclusion criteria of the participants

- Patients with a compromised medical history.
- Patients with dentin caries.
- Extreme plaque accumulation and periodontal problems.
- Participants with a history of allergy to any of the drugs or chemicals used in the study.
- Patients received any recent remineralizing agents or bleaching.
- Patients under antibiotic therapy.

### Eligibility criteria for teeth: Inclusion criteria of the teeth

- The WSLs with laser fluorescence measurements inside the range 14-20 were involved in this study,
- The lesions with scoring either 2 or 3 according to International Caries Detection and Assessment System (ICDAS) scoring were included in the study.

### Exclusion criteria of the teeth

- The white spot lesion scoring is (<14) or (>20) according to diagnodent,
- The white spot lesion scoring is other than 2, 3 according to International Caries Detection And Assessment System (ICDAS),
- White spot lesions due to hypomineralization or fluorosis. After 3 months all participants 32 (16 males, 16 females) completed the follow-up with 100% retention rate.

### Before intervention

Nothing done to the patient except examination no scalling and polishing. A clinical examination was undertaken to document the DMFT index, where D indicates decaying teeth, M represents missing teeth, and F reflects filled teeth, according to WHO criteria (Karabekiroğlu, and Ünlü). Without applying any pressure, a clinical testing was done on dry teeth using a flat mouth mirror and ball ended dental probe with the aid of a dental seat light. The DMFT index score was calculated and placed at the cariogram program applying the given codes in the cariogram handbook according to age. 32 patients with a high caries risk were chosen based on a cariogram and with 32 initial lesions on their maxillary and mandibular teeth based on diagnodent

measurement. Based on the set regimen, participants were randomly assigned to one of two groups, with (group 1) denoting participants that were exposed to herbal-based toothpaste regimen (Himalaya complete care) and (group 2) denoting participants that used a fluoride-based toothpaste preventive regimen (Colgate cavity protection).

Oral hygiene advice was given to each patient in the same way. A small mirror was used to show them the patient's teeth to determine if there was any plaque, and the importance of cleaning was highlighted. Standard oral hygiene instructions for at-home care were given to all patients. Each participant was receiving the same dietary guidance, such as reducing the amount and frequency of sugar consumption, as well as avoiding sugary foods and drinks before night. Patients also informed about potentially cariogenic foods and beverages such as cakes and biscuits, sugared soft drinks, honey, and chocolate. The participants followed a chemotherapeutic and caries prevention regimen that is split into two parts: at home and in the office;

**Home-based chemotherapeutic and preventive regimen:** Patients were given three home-use packages containing soft toothbrush, toothpaste according to the tested regimen, and xylitol chewing gum in order to change the toothbrush every month. Toothpastes was sprayed black so the patient was blinded to the toothpaste type. They were instructed to brush their teeth twice a day (in the morning after breakfast and at night before bedtime) with a pea-sized quantity of assigned toothpaste using the Bass technique. Chew sugar-free trident xylitol gum 2 pieces for 5 minutes four times a day, after meals.

**In-office based chemotherapeutic and preventive regimen:** The dentist was performing a professional tooth cleaning at the start of treatment to remove any existing calculus. All carious lesions on the teeth were filled with resin composite. Volunteers were told not to brush their teeth or do any other oral hygienic practices for 24 hours before their dental appointment, and not to eat or drink anything except water for an hour before the appointment.

**For intervention group:** Himalaya complete care was used by the participants.

**For comparator group:** Colgate cavity protection was used by the participants.

### Assessment criteria

**Remineralization of initial carious lesions:** The examiner was blinded, at each timeline (T0, T1 and T2), DIAGNOdent (KaVo Dental GmbH, Germany) was used to quantify Laser Fluorescence (LF) for each obviously opaque lesion on air-dried teeth, which was adjusted before each patient. Organic substances in the demineralized lesion glow when exposed to light of wavelength 655 nm on the tooth surface, providing an indirect indicator of the extent of demineralization of non-cavitated caries. The gadget converts the fluorescence intensity into an arbitrary numerical scale. Only white spot lesions with initial LF readings between 14 and 20 were included. After air drying the probe tip was placed on the lesion and a sound spot was recorded. The assessments were repeated for three times to reduce measurement errors, and the mean values of three consecutive assessments were reported for each area as shown in Table 1.

**Table 1:** Scoring of diagnodent for assessment of lesions.

Score	LF assessment criteria (measurements)
0-14	Healthy tooth
14-20	Initial carious lesions

>20 Dentinal caries

**Visual examination assessment:** Plaque was eliminated and the teeth were cleaned on the first day (T0). The enamel surfaces were visually inspected with a mouth mirror, ball ended dental probe and light. Two examiners were in charge of the visual evaluation, which followed the grading criteria in the table. Only lesions with a score of 2 or 3 were considered for the investigation. The same visual assessment was used to assess the efficacy of the various treatment regimens at each timeline (T0, T1, and T2) as shown in Table 2.

**Table 2:** Visual examination criteria used in the selection and assessment of samples.

Score	Visual assessment criterion
1	No, or slight, change in enamel translucency after air-drying for 5 seconds
2	Opacity or discoloration hardly visible on the wet surface but visible after air-drying
3	Visible opacity or discoloration without air-drying
4	Localized enamel breakdown with opacity or grayish discoloration from the underlying dentin
5	Cavitation in opaque or discolored enamel exposing the dentin

### Statistical analysis

Data was analyzed using Medcalc software, version 19 for windows (MedCalc Software Ltd, Ostend, Belgium). Data was explored for normality using Kolmogorov Smirnov test and Shapiro Wilk test. Continuous data showed normal distribution and were described using mean and standard deviation. Intergroup comparison between continuous data was performed using independent t test, while intragroup comparison was performed using repeated measures ANOVA and two-way ANOVA was used to test interaction of variables followed by tukey post-hoc test. Categorical data was described as frequency and percentage; comparisons between categorical variables were performed using the chi square test. A value less than or equal to 0.05 was considered statistically significant and all tests were two tailed.

## RESULTS

### DIAGNOdent score

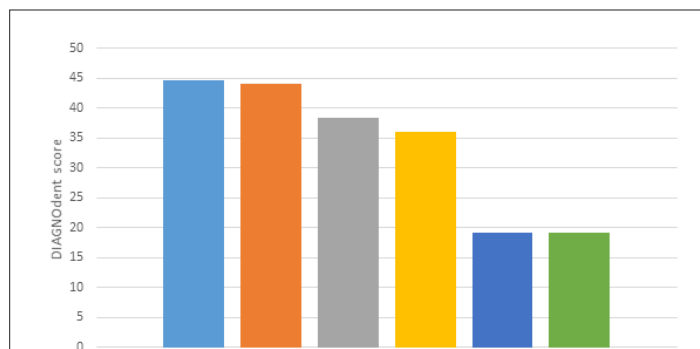
**Effect of material and follow-up on DIAGNOdent score:** Intergroup comparison between both materials have shown no statistically significant difference within follow up periods; baseline and 1 week (P=0.6669), while at 3 months' intergroup comparison revealed statistically significant difference with herbal toothpaste higher than fluoride toothpaste (P=0.0155). Intragroup comparison within Himalaya or Colgate have shown statistically significant difference between different follow-up periods (P<0.0001) (Table 3) and (Figures 1 and 2).

**Table 3:** Mean and standard deviation of DIAGNOdent score of both materials at each follow-up.

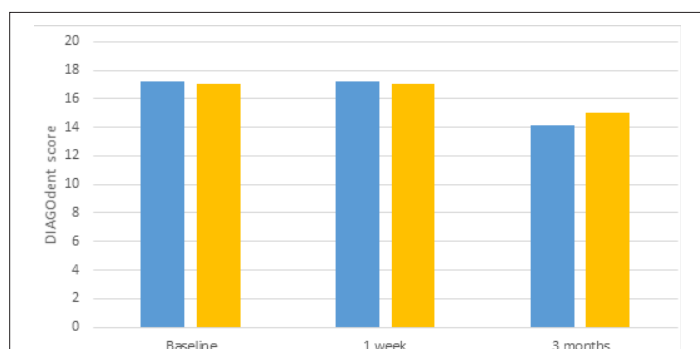
Intervention	Himalaya		Colgate		P value
	Mean	SD	Mean	SD	
Follow-up					
Baseline	17.200a	1.082	17.000a	1.414	P=0.6669
1 week	17.200a	1.082	17.000a	1.414	P=0.6669

3 months	14.133b	0.743	15.000b	1.069	P=0.0155*
P value	<0.0001*		<0.0001*		

**Note:** Means that do not share a letter are significantly different, \*: Corresponds to statistically significant difference, a: mean there is no statistically significant difference between them, b: mean there is statistically significant difference between them.



**Figure 1:** Bar chart of DIAGNOdent scores for both materials after different follow-up period. **Note:** (■) Colgate/3 months, (■) Himalaya/3 months, (■) Colgate/1 month, (■) Himalaya/3 months, (■) Himalaya/1 week, (■) Himalaya/baseline



**Figure 2:** Bar chart showing effect of material on DIAGNOdent score at each follow-up. **Note:** (■) Himalaya, (■) Colgate

### ICDAS

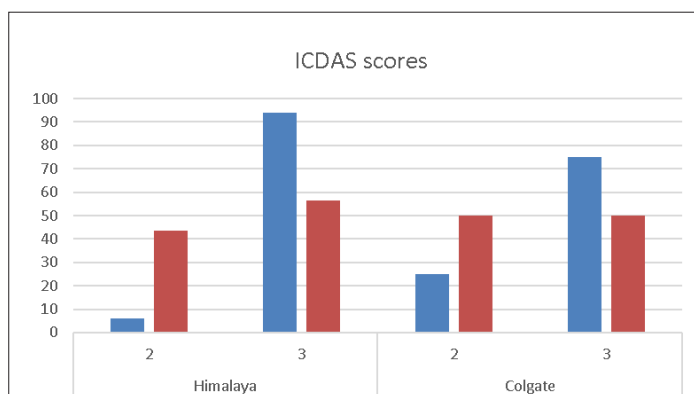
Intergroup comparison between both materials have shown no statistically significant difference within different follow up periods; baseline and 3 months respectively (P=0.1506 and P=0.7273). Intragroup comparison within Himalaya have shown statistically significant difference between different follow-up periods (P=0.0159\*). Intragroup comparison within Colgate have shown no statistically significant difference between different follow-up periods (P 0.1506). (Table 4) and (Figures 3 and 4).

**Table 4:** Frequency, percentage and P value for ICDAS scores for the intergroup comparison between materials within each follow-up and intragroup comparison within each material between different follow-up periods.

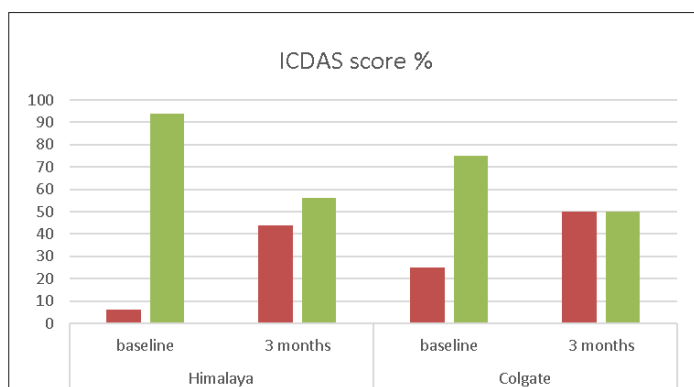
Material	Himalaya		Colgate		P value
	2	3	2	3	
Baseline	1(6.2%)	15 (93.8%)	4 (25%)	12 (75%)	P=0.1506 NS
3 months	7(43.7%)	9(56.3%)	8(50%)	8(50%)	P=0.7273NS

P value	P=0.0159*	P=0.1506
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**Note:** \*:Corresponds to statistically significant difference.



**Figure 3:** Bar chart showing percentage of each ICDAS score within each material at different follow-up periods. **Note:** (■) Baseline, (■) 3 months.



**Figure 4:** Bar chart showing percentage of each ICDAS score within each follow-up period for both materials. **Note:** (■) 2, (■) 3.

### DISCUSSION

The main objective of conservative dentistry is maintaining the health of the dentition free from dental caries. A paradigm shift in caries management has occurred in recent periods, moving away from restorative procedures toward non-restorative caries risk management including early preventive methods to fulfill the goal of new dentistry (minimum intervention). The dentist can use regimens (fluoride toothpastes, liquids, patient health education, Etc) to control initial caries lesions. This is based on understanding of the pathophysiology of dental caries as it is a multifactorial infectious dynamic disease resulting from an interaction between the host (tooth structure and saliva), microbial biofilm, and diet. Dental caries is associated with loss of tooth structure caused by organic acids produced by cariogenic bacteria in the dental biofilm. Tooth enamel is made up of 90% substituted hydroxyapatite, which goes through a series of dissolution (demineralization) and re-crystallization cycles (remineralization). Non-cavitated White Spot Lesions (WSLs) are just the first sign that the vastly complicated biophysical systems that keep enamel healthy have shifted in the direction of demineralization. When the surface of the lesion is undamaged and the body of the lesion is net dissolving calcium and phosphate ions, it is possible to reverse the early stages of enamel caries. A combination of good oral hygiene, food restriction, and fluoride administration is commonly recommended for the



prevention and reversal of early caries [11]. Recommending reduced daily sugar consumption through a dietary assessment and guidance on eating fewer cariogenic alternatives is the most common way for reducing fermentable carbohydrates and arresting the caries process [12]. The goal of this study was to see how herbal or fluoride-based toothpastes, combined with sugar-free gum including xylitol affected the remineralization of early carious lesions in high-caries-risk patients over a period of 12 weeks follow up.

In toothpastes and mouth rinses, a variety of chemical agents have been used to minimize the production of dental plaque. As a result of increased recognition of folk medicinal traditions in many areas of the world, the use of "herbal" therapy has inspired interest and supported the emergence of complementary therapies in health care development. Herbal health products have got a lot of attention on the market. Because of the presence of many active ingredients, several toothpastes on the market like Himalaya complete care claim to be anti-cariogenic [5,13] miswak is one of the key ingredients of Himalaya complete care toothpaste which promote the remineralization of incipient lesions. *Salvadora persica* (S.P.) (Miswak) is a plant that grows in the desert and can be found from west India to Africa. In these places, S.P. roots and sticks are commonly utilized to clean teeth. Arak tree, Natural toothbrush, chewing stick, and Miswak are some of the various names for this plant. It contains more than 10 naturally occurring elements essential for maintaining good oral health, it contains calcium carbonate, produced significant increases in calcium (22-fold) saliva calcium absorption prevents dental enamel demineralization and encourages remineralization. It is also a natural source of topical fluoride, it contains nearly 1.02 µg/g fluoride which prevents caries and has anti-decay effect. [7,14,15]. Babool is another ingredient of Himalaya toothpaste [8] mentioned that sticks from babool contain fluoride and have the benefits in remineralization of the teeth. Other component is Neem (*Azadirachta indica*), [7] mentioned that Neem contained 2.8 µg/g fluoride which helps in preventing dental caries and have the benefits in remineralization of the teeth [8]. Pomegranate which has astringent and antibacterial assets, which helps to combat dental plaque, it controls the remineralization of Himalaya toothpaste [10]. Himalaya herbal toothpaste also contains calcium fluoride 500 mg. The result of [9] showed that adding calcium to Fluoride toothpastes meaningfully improved remineralization of a subsurface lesion in the enamel when compared to Fluoride-only toothpaste *in situ*, the level of accessible calcium in the Fluoride dentifrice was found to be substantially linked with improved remineralization.

Fluoride's capacity to remineralize teeth is regarded as the gold standard against which all remineralization treatments compared. Fluoride is now well recognized as the primary active ingredient in a variety of fluoridated products aimed at remineralization and the prevention of caries development [16]. Fluoride works in four ways. It helps to avoid demineralization by producing fluoro-apatite crystals from enamel apatite particles, which are more acid tolerant than HAP crystals. Second, fluoride accelerates the production of new fluoro-apatite crystals by combining calcium and phosphate ions together. Third, it restricts the action of acid-producing carious germs by interfering with the formation of Phosphoenol Pyruvate (PEP), a critical intermediate in the glycolytic cycle in bacteria. The F also clings to tooth hard tissue, oral mucosa, and dental biofilm to stimulate remineralization and prevent demineralization [17]. By blocking bacterial metabolism, fluoride is thought to reduce acid generation and bacterial development. Fluoride toothpaste activity is determined by fluoride content, frequency of use, and the volume of paste used [18]. Fluoride can be found in toothpastes in a variety of chemical

forms, the most common of which being Sodium Fluoride (NaF) and Sodium Monofluorophosphate (Na<sub>2</sub>FPO<sub>3</sub>). Free fluoride is produced directly by sodium fluoride. Sodium monofluorophosphate is the fluoride of preference when using nutrient abrasives. The fluoride released is adsorbed as free or bound CaF<sub>2</sub> or CaF<sub>2</sub>-like layers on the mineral surface. When compared to a placebo toothpaste with a fluoride concentration of 0 ppm. *In vitro*, toothpaste compositions with 1426 ppm F sodium fluoride showed considerable enamel protection against erosive acid exposures. When paired with twice-daily fluoride toothpaste use, 0.05 NaF mouth rinse successfully enhances the remineralization of initial caries, according to [17]. Xylitol chewing gum has been recommended as a follow-up measure in some remineralization instances. In addition to blocking *S. mutans*, xylitol can't be digested by plaque bacteria and is thought to act as a calcium ion channel. It's a carbohydrate-free sweetener that's widely used. To sustain salivary flow, bicarbonate concentration, and pH in the oral cavity, the chewing gum form of xylitol was used. Xylitol has been proven in several trials to reduce tooth plaque when the amount of *S. mutans* decreases (*in vivo* and *in vitro*). It has a long-term impact on lowering the occurrence of caries. The daily amount of xylitol employed in this study has been found to be a dose that has been found to be beneficial in past remineralization studies, and xylitol has been shown to reduce *S. mutans* in saliva in other studies [19], [11], and [20]. As in [11], Xylitol (chewing gum) was added to the routine oral hygiene treatment for all patients because of its antimicrobial activities against the extremely cariogenic *Streptococcus mutans* group of oral infections.

Physicians can use the International Caries Detection and Assessment System (ICDAS) II to visually examine the severity, size, and progression of WSLs. The DIAGNOdent (KaVo Dental) device is one of the fluorescent imaging techniques that can examine WSLs quantitatively to determine the aesthetic impact of therapy. Until white spot lesions have developed 200-300 µm beyond the enamel, physically, they are not evident [21]. The DIAGNOdent® measures early demineralization using laser fluorescence and is one of the noninvasive diagnostic procedures accessible. A lot of studies have established the utility of DIAGNOdent as a viable non-invasive caries-detecting tool. On the tooth surface, organic and inorganic substances absorb laser light emitted from DIAGNOdent and release fluorescence in the infrared range. An elevation in fluorescence indicates the presence of a demineralized region, which is signalled by an aural sound. Higher-pitched sounds and more sounds indicate greater levels of demineralization [22,23].

About the remineralization of initial lesions; The present trial showed that by a preventive regimen containing 1450 ppm fluoride-based toothpaste the remineralization potential of initial lesions as measured by Diagnodent device has been improved. The studies conducted confirmed the findings of the current study [11]. Miswak in Himalaya toothpaste enhance the remineralization and these findings support past *in vivo* researches [7], [14], and [15]. In this study although the usual oral hygiene regimen had no effect on the WSLs' aesthetic look in both groups, laser fluorescence assessments revealed considerable remineralization of the lesions during the 12-week program, and this result confirmed the result of [11] and also [21] which stated that an increase in the degree of mineralization that is significant statistically may not always reflect into a clinical enhancement in aesthetics. The remineralization of the lesions occurred, but although there was no cosmetic improvement. He also discovered a substantial difference in DIAGNOdent scores between baseline and 12 months, however this difference was not represented clinically. According to [18] and [11] many investigations have shown that a 12-week time span is sufficient for

identifying lesion regression in a semi-quantifiable way. Other research, on the other hand, indicated that a six-month interval is required to discover any negative or positive impacts of caries prevention measures. This study trial was limited to twelve weeks due to worries that patients with first lesions who were not acclimated to frequent dental visits might not participate with relatively long follow-ups for personal and/or socioeconomic reasons. In fact, all participants in this study stayed until the end of the follow-up period. Due to no adverse effects, none of the subjects withdrew (e.g., accelerated plaque accumulation, allergies, enamel-staining, and gingival inflammation). The findings of this clinical investigation highlight the critical impact of a caries prevention program using herbal- or fluoride-based toothpastes.

The null hypothesis was rejected in our research as there was a significant difference in diagnodont scores between the two materials. Limitation of the study: This study has certain limitations. To begin with, this study was mostly based on patient-centered treatment, which could lead to discrepancies in adherence to preventive regimen guidelines because brushing is dependent on patient participation, and there could have been a lack of cooperation. Second, the complexities of dental caries, as well as participant behavioral changes, could influence the final results. Finally, lengthier follow-up trials are needed to further examine the clinical effectiveness of various caries prevention regimens.

## CONCLUSION

Preventive regimens containing different toothpastes are positively influencing remineralization potential of initial carious lesions in high caries risk patients. Herbal based toothpaste was superior to fluoride based toothpaste in remineralization of initial carious lesions along three months follow up. Different preventive regimens didn't produce significant effect in altering visual appearance of initial carious lesions.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper

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