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Original Article

Dental Caries: The Reverse Gear – Comparative Synergistic Remineralizing Potential of Sodium Fluoride Mouthrinse with Xylitol and Essential Oils: An *In situ* Study

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Abstract

Background: With the universal use of topical fluorides, there has been a decrease in the prevalence of dental caries, but still the quest is on for a better topical agent which can provide a superior remineralizing effect. **Aims:** The present in situ study was to evaluate the remineraliizing efficacy of sodium fluoride mouth rinse and two experimental mouth rinses xylitol sodium fluoride, essential mouth rinses. **Materials and Methods:** Partially demineralized human enamel slabs were used in the study; five enamel slabs were embedded in each of the Hawley's appliances worn by nine participants enrolled in the study. The participants were divided equally into three groups. Each group used one of the experimental mouthrinses. Hence, in each mouthrinse group, there were 15 demineralized enamel slabs in each appliance used by three participants in each mouthrinse group. The study was conducted for a period of 14 days, after which the enamel slabs were retrieved and analyzed using quantitative wavelength-independent microradiography with the assistance of image processing. **Results:** Analysis of the samples showed a statically significant increase in the amount of remineralization with xylitol sodium fluoride mouthrinse. The order of increase in the remineralization was as follows: xylitol sodium fluoride > sodium fluoride > essential oil sodium fluoride > baseline demineralization (control). **Conclusion:** The xylitol sodium fluoride mouthrinse can prove to be a great weapon in reversing the initial dental caries. Essential oil mouthrinses are known for their antigingivitis and anti-inflammatory properties, and their efficacy in synergistically acting with sodium fluoride in remineralizing dental caries is questionable under *in vivo* conditions.

Keywords: Essential oil, microradiography, remineralization, sodium fluoride, xylitol

Introduction

Worldwide, the contribution of dental caries to the burden of oral diseases is about ten times higher than that of periodontal disease, the other common oral condition. Owing to its globally high prevalence, dental caries is a "pandemic" disease characterized by a high percentage of untreated carious cavities causing pain, discomfort, and functional limitations. Untreated carious cavities, furthermore, have a significant impact on the general health of children and on the social and economic well-being of communities. A surgical approach to the elimination of carious lesion was developed a century ago; this approach was necessary at that time because there was no valid alternative. The focus on caries has recently shifted to the development of methodologies for the detection of the early stages of caries lesions and the noninvasive treatment of these lesions. The noninvasive treatment of early lesions by remineralization has the potential to be a major advance in the clinical management of the disease. Remineralization of white spot lesions may be possible with a variety of currently available agents containing fluoride, bioavailable calcium and phosphate, and phosphate. This concept bridges the

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conventional gap between prevention and surgical procedures, which is just what dentistry needs today.

The present *in vivo* study was undertaken to compare the remineralizing potential of fluoride mouthrinse with two experimental combinations: essential oil (Listerine) with sodium fluoride and Xylitol with sodium fluoride. These agents were chosen because essential oil mouthrinse is the oldest over-the-counter rinse and most popularly used with proven antiplaque and anti-inflammatory properties. Coming to xylitol, this sugar subjective has been extensively studied for its anticariogenic properties. The remineralization was checked using wavelength-independent microradiography which gives the direct measure of de- and remineralization and is highly reliable in detecting mineral changes.[1]

Materials and Methods

The study was conducted in the Department of Pedodontics and Preventive Dentistry, Dental College and Hospital, Khammam. A total of 15 impacted third molars were embedded in enamel slabs. The samples were randomly divided into three groups, namely, control group, sodium fluoride group, and Xylitol with 0.005% NAF group with 15 samples in each group. The samples of each group were subjected to demineralization process for a period of 14 days. The samples were then mounted on the enamel slabs and subjected to remineralization and pH cycling for a period of 48 h. The samples were evaluated for remineralization using quantitative wavelength-independent microradiography and were statically analyzed.

The samples were selected based on the following inclusion and exclusion criteria.

Inclusion criteria

- Caries-free teeth
- Teeth extracted for orthodontic reasons only.

Exclusion criteria

- Teeth with fracture of either crown or root detected using FOTI
- Teeth with caries lesions including white spot lesions detected using quantitative light induced fluorescence, and fiberoptic trans illumination (QLF and FOTI)
- Teeth with QLF score >1
- Teeth with hypoplastic lesions detected visually as well as on QLF
- Teeth with intrinsic stains
- Teeth with any wasting diseases such as attrition, abrasion, and erosion
- Teeth with developmental anomalies
- Teeth with any restoration.

To detect a clinically relevant difference of 20% at 5% level of significance with 80% power, the required sample size was arrived at 15 samples in each group.

The teeth were cleaned with slurry of pumice and then stored in 1% thymol solution. The tooth samples were cut in the cementoenamel junction. Each selected tooth had two windows on the middle 1/3rd of the buccal and lingual surfaces of size 5 mm × 3 mm. Leaving the window, the rest of the tooth was painted with acid-resistant nail varnish and immersed in demineralizing solution (0.01 M acetate buffer) and incubated at 50°C for 48 h. At the end, the teeth were retrieved and washed with deionized water which served as control. Of the rest three sections, one section was kept in deionized water which served as the control. The rest three sections were embedded in the palatal appliances of the participants using the three rinses. This was done with each of the tooth involved in the study. Maxillary Hawley's appliances were fabricated which carried the enamel slabs. The enamel slabs were flush with three rinses.

Nine healthy postgraduate students of 25–30 years were enrolled in the study. The participants were not on any medications and had no active carious lesions, restorations, or periodontal disease. They used a nonfluoridated toothpaste during the study. Participants were divided into three groups equally depending on the mouthrinse used: [Figure 1]

- Group N – used 0.005% of sodium fluoride
- Group X – 20% Xylitol with 0.005% sodium fluoride
- Group E – essential oils with 0.005% sodium fluoride.

Each of the palatal appliances has five enamel slabs. Hence, enamel slabs were present in each group. The participants wore the appliances from 9.15 am daily for a period of 14 days. They were asked to rinse with their respective 20 ml mouthrinse for 30 s and to refrain from eating or drinking for 30 min after rinse. After 14 days of the *in situ* experiment, the appliances were retrieved and washed with deionized water. The enamel slabs were removed with the help of a dental bur

Table 1: The Balteau X-Ray equipment used for quantitative wavelength in depended micrographic evaluation

Equipment features	
X-ray machine	**Baltaeu-NDT Baltograph – LS1**
Voltage range	20–160 KV
Current range	1–30 m A max
Focal spot size	0.4 and 1.5 mm (dual focus)

NDT: Baltean NDT is known for its unique and unequalled engineering and manufacture of portable equipment

Table 2: Optimized radiographic features

Measurement	Value
Voltage used	40 KV
Exposure (current)	3 mA
SFD	1000 nm
Type owf film	D2 Agfa
Processing	Manual standard

SFD: Source to film distances

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and then washed again with deionized water and stored in in Zip lock covers duly numbered in depended microradiography [Tables 1 and 2]. The enamel slabs were grouped as C, N, X, and E, and each set was arranged in a row. For example, C, N1, X1, and E1 of the first set, similarly, the rest of the 14 sets were arranged [Figure 2]. The arranged sets were then placed on X-ray (40 kV). This was done by using the film digitizer with the resolution of 500 DPI (dots per inch). The scanned images were 8 Bit (256 grey shades i.e. 0-255). The radiograph was digitized using a film digitizer with a resolution of 500 DPI. The scanned images were 8 bit (256 gray shades, i.e., 0–255). The image processing of the scanned images was done using the (Java based image processing program developed at the National institute of Mental and laboratory for optical and computational instrumentation; 2010; Bethesda, Maryland, USA). Each sample was profiled with gray values as the Y-axis and the know the levels of mineral content [Figure 3]. The profiles of the C, N, X, and E were compared to know the levels of mineral content [Figure 3].

Table 3: Master chart showing demineralization and remineralization measurements in gray values of the 15 samples

Samples number	Control (C)	Sodium fluoride	Xylitol sodium fluoride	Essential sodium fluoride
1	123.4	148	160	141.7
2	21	38	42	28.
3	100.9	118	176.1	106
4	71	84	94	73
5	72	90	111	84
6	64	69	95	65
7	67	76	101	74
8	77	81	148	80.8
9	89	142	226	134
10	107	115.9	236	111
11	117.3	139	141	129
12	137	145	154	141.9
13	119	156	226	150
14	64	69	95	65
15	137	145	154	141.9

Table 4: The mean scores of the gray values and their standard deviation and range

	C	N	X	E
Mean	91.1	107.7	143.9	101.7
SD	32.7	37.3	56.1	37.3
Range	21.0–137.0	38.0–156.0	42.0–236.0	28–150

SD: Standard deviation, Group N- used 0.005% sodium fluoride. Group X- 20% Xylitol with 0.005% sodium fluoride. Group E- Essential oils with 0.005% sodium fluoride, E: Enamel slabs

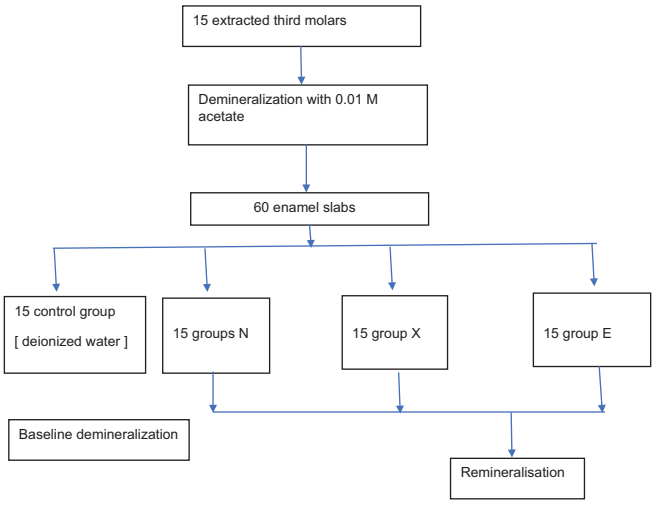

Figure 1: Study design

Statistical analysis

All data were entered into MS Excell sheet and analyzed by Microsoft Excel software (SPSS-17.0, SPSS Inc, Chicago, Illinois, USA) to assess the remineriling potential of three materials. The differences between three materials were statistically analyzed by ANOVA and Post hoc Newman Keuls Range test.A P value <0.005 was considered significant. All the results and statistical analyses were tabulated.

Results

The results of the microradiograph analysis were in the form of graphs plotted which show the mineral content of each of the enamel samples [Figure 3].

Table 1 shows the features of the Balteau X-ray equipment used for quantitative wavelength-independent microradiographic evaluation.

Table 2 shows optimized radiographic features.

Table 3 shows demineralization and remineralization measurements in gray values of the 15 samples.

Table 4 shows the demineralization and remineralization agents and the mean scores of the gray values and their standard deviation and range.

Table 5 shows the comparison of remineralization of the three mouthrinses with that of baseline demineralization study by applying ANOVA test. $P < 0.01$ was considered statistically significant.

Table 6 shows the comparison of three different groups' mouthrinses with that of baseline demineralization study by applying *post hoc* Newman–Keuls test. $P < 0.01$ was considered statistically significant.

Figure 2: Example of a microradiograph

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Discussion

The teeth are subjected to alternating periods of demineralization and remineralization. If there is a disruption in this balance, then the tooth results in cavity. The concept of remineralization was described at the beginning of the 21st century. Remineralization is analogous to wound healing in the soft tissues of the body which results in the arrest or reversal of a carious lesion through reduced cariogenic activity or increased resistance of the tooth surface or combination of both these process. These changes are associated with the deposition of mineral in the microspaces created in dental caries by the mineral dissolution that resulted from the early cryogenic activity.

Table 5: Comparison of remineralization of the mouthrinses with that of baseline demineralization

Groups compared	Mean differences	*P*
N-C	16.6	*P*>0.05 (NS)
X-C	52.8	*P*<0.01 (S)
E-C	10.6	*P*<0.05 (NS)

Post hoc Newman–Keuls range test. MSR, κ=40.5 *P*>0.05, NS. =50.0 *P*<0.01, S. NS: Not significant, S: Significant, MSR: Mean, standard deviation, range

Table 6a and 6b: Remineralization among the groups

Groups	Mean ± SD	*P*
N-C (Nd)	16.6 ± 13.5	0.001 (S)
X-C (Xd)	52.8 ± 41.2	<0.01 (S)
E-C (Ed)	10.6 ± 12.3	<0.01 (S)
Groups compared	**Mean differences**	***P***
Xd-ND	36.2	<0.01 (S)
XD-ED	42.2	<0.01 (S)
ND-ED	6	<0.05 (NS)

Post hoc Newman–Keuls range test. MSR, κ=21.0, *P*>0.05 NS, 29.3, *P*<0.01 S. NS: Not significant, S: Significant, SD: Standard deviation, MSR: Mean, standard deviation, range

Today, extensive laboratory and clinical research have established the remineralization concept as an indisputable fact, which can enhance therapeutically.[2-5] In the past two decades, there has been reduction in the caries prevalence. The reasons could be attributed to the near-universal use of fluoride-containing products such as dentifrices, mouthrinses, and topical gels which are applied in the dental office by twined personnel. Weak fluoride solutions as in the mouthrinses were provided to be an alternative to water fluoridation in community level of caries prevention.[6,7] Dental caries of the enamel, typically, is first observed as the so-called white spot lesion. This is a small area of substrate demineralization beneath the dental plaque. Complete delete the substances.. The body of the subsurface lesions may have lost as much as 50% of its original mineral content by an apparently intact surface layer,[8] this surface is remineralized as follows: as the saliva flows over the plaque, its components neutralize the acid, raising the PH and thereby stopping demineralization. The saliva which is supersaturated with calcium and phosphate drives back into the tooth.[9,10] The partially demineralized crystal surface within the lesion acts as nucleators, and new surfaces grow on these crystals.

Neutral sodium fluoride has been the most frequently tested fluoride compound because of its ease in preparation, acceptability, nature of preventing caries by 50%, and is also economical. Sodium fluoride mouthrinses are usually formulated at the concentrations of their 0.2% (900 ppm) for weekly use of 0.05% (226 ppm) for daily use.[11,12] Fluoride has three principal topical mechanisms of action in combating the caries process. It inhibits the bacterial metabolism and demineralization and enhances the remineralization. Various agents have been added to fluoride such as chlorhexidine, Xylitol, essential oil (Listerine), and glycyrrhizin to increase the remineralization capacity of fluoride. Xylitol is a sugar substitute found naturally in the same food, but in mass

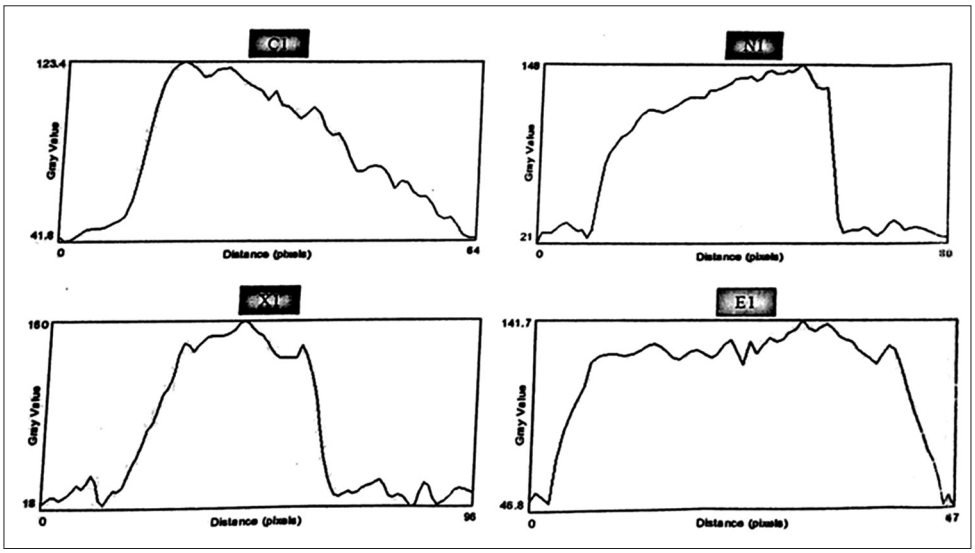

Figure 3: Graphs plotted with gray values after profiling of the microradiograph.

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produced from sustainable xylan from rich hardwood sources such as birch and beech wood. It has been used as a mouth rinse for more than 130 yrs.The active ingredients are thymol, menthol, eucalptol, and methlyol salicyclate. Microorganisms do not develop resistance to these oils. In long-term clinical trials, Listerine has shown to reduce plaque accumulations and severity of gingivitis up to 34%.[13,14]

Although there have been many studies regarding the efficacy of two agents as anticaries and antiplaque, respectively, less has been studied about their synergistic action of remineralization when combined with fluoride under *in vivo* condition. This was an intention of the present study. The results of the present study showed that there was a statistically significant increase in the remineralization with the Xylitol sodium fluoride mouthrinse when compared to the essential oil sodium fluoride and 0.5 ppm sodium fluoride *in vitro* study. The remineralization was greater in the combination of 20% Xylitol alone.[15]

Another study which investigated the remineralization effect of Xylitol chewing gum and fluoride *in vitro* condition using backscattered electron images showed that the gum extract consisting of Xylitol and sodium fluoride amplified the remineralization of the artificially demineralized enamel blocks to 96.5% when compared to the gum extract of Xylitol alone which was 78.6%.[16] Our study showed similar results even when Xylitol and sodium fluoride combination was tested *in vivo* conditions as a mouthrinse. Previous studies used mainly gum extracts of xylitol, whereas in our study, we have incorporated Xylitol and sodium fluoride in a mouthrinse. Xylitol has been added to mouthrinses as a sweetening agent, but we have incorporated Xylitol as a main ingredient[16] for therapeutic purposes. Xylitol with sodium fluoride delivered in the form of mouthrinse form has also shown to inhibit *Streptococcus mutans* counts.[17]

Coming to the next combination, the essential oil sodium fluoride mouthrinse, earlier studies showed that the combination was at least as good as the sodium fluoride rinse. In a study conducted to evaluate the remineralizing potential of a few formulations which contained essential oil (Listerine) with 0.022% sodium fluoride in the bovine enamel sample, a cyclic de- and remineralization model was used and the remineralization was checked using the surface microhardness. The results showed that there was an increase in the microhardness regained of about four times with essential oil sodium fluoride mouthrinse, whereas with sodium fluoride, there were three increases when compared to the nonfluoride group.[18] Another *in situ* study was conducted to assess the remineralizing effect of an essential oil fluoride mouthrinse in an intraoral caries test. The essential oil fluoride mouthrinse was compared with that sodium fluoride and essential mouthrinse alone.

The remineralization was analyzed with surface microhardness. The results of the study showed that the percentage of the surface microhardness recovery was 42% with essential oil mouthrinse. Whereas with sodium fluoride, it was 36%, and with essential oil mouthrinse alone, it was 16%. Hence, it was concluded that the combination of essential oil and sodium fluoride was at least as good as the sodium fluoride rinse alone.[19] Contradictory to the above studies, the essential oil sodium fluoride mouthrinse in our study showed a decrease in the gray values of about four to six units in all samples. The above studies used surface microhardness to assess remineralization, whereas we have employed wavelength-independent microradiography to assess remineralization. This method happens to be one of the direct methods to assess remineralization. Hence, there could be a variation in the results.

It is well established that the sodium fluoride whether in mouthrinse or dentifrices has known to remineralize the carious lesions.[11,20,21] The present in situ study was conducted to compare the remineralizing potential of sodium fluoride mouth rinse and two experimental mouth rinses ,xylitol Sodium fluoride and Essential moth rinses. Based on the results of our study, it can be deducted that the remineralization seen with Xylitol sodium fluoride mouthrinse was far more than sodium fluoride or the essential oil mouthrinse.

The essential oil sodium fluoride mouthrinse being at least as good as the sodium fluoride or the essential oil mouthrinse. The essential oil sodium mouthrinse being at least as good as the sodium fluoride under *in vivo* condition is questionable. An *in vitro* study which used the combination of listerine, xylitol, and sodium fluoride as a single formulation showed that it was superior in promoting enamel fluoride uptake over the conventionally available fluoridated products.

It is also demonstrated agent (xylitol) provided multifaceted and enhanced anticaries efficacy by promoting remineralization and reducing acidogenic bacteria.[20]

Limitations

However, further research is necessary to compare remineralizing ability in terms of net gain of calcium and phosphate gain by the enamel.

Conclusion

This study was intended in knowing which formulation was effective sodium fluoride remineralizing property; xylitol in therapeutic levels in rinse formulations was very effective in augmenting remineralizing property. Xylitol in therapeutic levels in a rinse formulation was very effective in augmenting remineralization is questionable.

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Conflicts of interest

There are no conflicts of interest.

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