



Research Article

New Modulation of Fluoride Remineralization Against Dental Caries: an in Vitro Study

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ABSTRACT: This study aims to assess the local application of a novel fluoride supplement modification, achieved by dissolving fluoride tablets in deionized water, to achieve concentrations of 2500 ppm on demineralized tooth surfaces, thereby demonstrating its effectiveness as a remineralizing agent. **Materials and Methods:** 10 permanent molars were employed. Each tooth is enrolled in Teflon tape, and only the enamel of the occlusal surface is exposed to a demineralizing solution (acid etch) for 45 seconds in vitro. The teeth are then randomly assigned to two groups: Group 1 is treated with distilled water, and Group 2 is treated with a sodium fluoride solution. All groups except the control baseline group were treated with their respective remineralizing solutions for 10 days. Vickers' Microhardness Number (VHN) and Densometric X-Ray Analysis were completed at baseline, following demineralization, and subsequently 10 days after remineralization. **Results:** Densometric X-ray analysis and Microhardness Number (VHN) showed a remineralizing effect of sodium fluoride solution on enamel density and surface hardness after remineralization, with a significant difference observed after 10 days ($p \leq 0.05$). The mean value in the group treated with sodium fluoride solution was higher than the recorded mean value for the group treated with distilled water. **Conclusion:** Sodium fluoride solution is considered an effective remineralizing agent, significantly increasing enamel density and surface hardness.

Keywords: Densometric analysis; Fluoride solution; Microhardness; Remineralization.

INTRODUCTION

Dental caries is a significant public health issue that affects a substantial number of people worldwide. A dynamic balance of demineralization and remineralization determines the occurrence of dental caries. ⁽¹⁾

The early phase of a caries lesion is known as a white spot lesion (WSL), and it represents subsurface enamel demineralization.⁽²⁾ Numerous substances, such as fluoride, phosphate, or calcium ions, can be applied to the tooth surface to prevent or postpone this. ⁽³⁾

By inhibiting demineralization, promoting remineralization, and leveraging its antimicrobial properties, fluoride has been demonstrated to halt and reverse the carious process.⁽⁴⁾ Nonetheless, high doses might result in toxicity and fluorosis. As a result, efforts to provide efficient remineralization and anti-caries with little side effects have increased. ⁽⁵⁾

Fluoride can be provided in various methods, including topically (through toothpastes, mouthwashes, varnishes, and gels) and systemically (via fluoridated water, fluoridated salt, and dietary supplements). ⁽⁶⁾

It is utilized to produce fluorapatite crystals, which are more resistant to acids because they contain fluoride rather than the hydroxyl ions present in enamel. ⁽⁷⁾

The fluoride ion prevents demineralization by altering the metabolism of the bacteria in tooth plaque, lowering demineralization, and promoting early remineralization of carious lesions, especially at low concentrations.⁽⁸⁾

The objective of the current study is to assess the in vitro effects of the local application of a novel fluoride supplement modification, achieved by dissolving fluoride tablets in deionized water, on demineralized tooth surfaces to demonstrate its effectiveness as a remineralizing agent.

MATERIALS AND METHODS

In the current in vitro study, 10 sound permanent molar teeth extracted with age grouping (18-25) years and collected from Alhur specialized dental center in Karbala and different private dental clinics, the study was approved by the Research Ethics Committee and Scientific Committee Department of Dental Basic Science/ College of Dentistry/ University of Mosul, under approval number (UoM. Dent/ A.H.L.83/ 22).

The teeth were clinically sound, and cleaned with a scalpel to remove the periodontal ligament and intercrestal bone remnants, and stored in 37% Formalin solution and the teeth were to be used for no more than 1 month after extraction.

To remove any remnant of formalin saturation, all the teeth were rinsed under a running tap waterfall for 24 hours. After that, each tooth was mounted in a wax-hardened block.

For standardization, the area of treatment and evaluation was the cusps of the occlusal surface on the mesial side, and each tooth was enrolled in Teflon tape with only the occlusal surface exposed. The induction of demineralization on the enamel occlusal surface was done by dryness with air for 10 seconds. After that, acid etch (37% phosphoric acid) was applied on the enamel for 45 seconds to induce a demineralizing effect, then washed with triple syringe water and dryness for 5 seconds, which showed a chalky white appearance of the occlusal surface that represented the white spot lesion.

Solutions Preparation: Sodium fluoride solution was prepared by dissolving 2.5 mg of sodium fluoride tablet in 1 ml of de-ionized water to get 2500 ppm of fluoride, which shows effect as a remineralizing agent according to the equation below: -

$$1\text{ppm} = 1\text{ mg/liter}$$

$$2500\text{ ppm} = 2500\text{mg/liter}$$

$$1\text{ tablet} = 0.25\text{mg}$$

So

$$0.25\text{mg} \longrightarrow 1\text{ tablet}$$

$$2500 \longrightarrow X$$

$$X = \frac{2500}{0.25} = 10000\text{ tablets}$$

For that, get on 2500 ppm, which is equal to 2500mg/liter

We need to dissolve 10000 tablets in 1 liter of de-ionized water

$$\text{So, } X = \frac{10000\text{ tablet}}{1000\text{ ml}} = 10\text{ tablets} / 1\text{ ml.}$$

Study design

Teeth samples were randomly divided into two study groups: one for the sodium fluoride solution and the other for distilled water. Each group consisted of five teeth that were measured with a microhardness test and Densometric analysis.

The enamel microhardness and densometric analysis were measured initially for sound enamel (control) and after induction of demineralization and remineralization effects.

After preparation of the enamel specimen and induction of demineralization, each tooth emerged separately in 20 ml of the selected agent's solution for three minutes at 10 AM and 8 PM with manual shaking, then the teeth were restored in de-ionized water for the next day at a temperature of 37 °C. The procedure was repeated daily for 10 days. It is worth mentioning that each solution agent was replaced in each day

during the time of procedures, the samples were reexamined for the microhardness and densometric analysis, and compared between groups. ⁽⁹⁾

Statistical Analysis

The statistical data analysis was performed using the Statistical Package for the Social Sciences (SPSS for Windows, Version 25.0, Chicago, Illinois, United States). The mean and standard deviation of each group were subsequently calculated.

Kolmogorov-Smirnov's tests were used to verify the normal distribution of quantitative variables. Student's test was performed to compare sodium fluoride solution and distal water. An ANOVA test was also performed for inter-group comparisons. A p-value less than 0.05 was considered statistically significant, and $p < 0.01$ was highly significant.

RESULTS

Vickers hardness test.

Distal water

The mean values and standard deviation of the microhardness of enamel specimens at baseline (control) before demineralization were (324.2 ± 29.10) and became (234.54 ± 9.83) post-demineralization, and (233.66 ± 7.46) after 10 days of remineralization for the distal water group Figure (1) displays the mean values of both sodium fluoride solution and distal water. There was a statistically significant difference between the baseline, post-demineralization, and after 10 days' groups of distal water ($p \leq 0.05$). As shown in (Table 1).

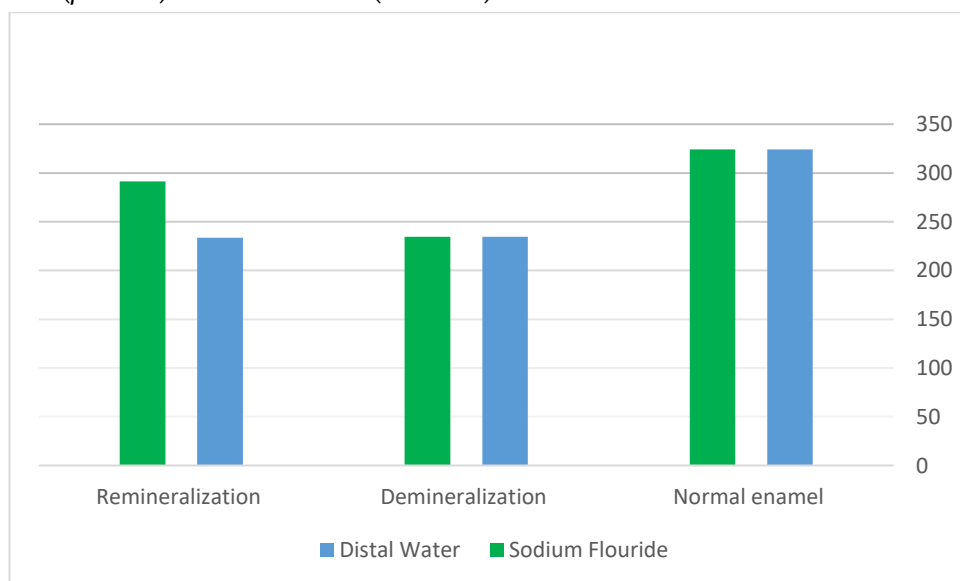


Figure (1): The mean value of microhardness of the enamel surface before and after remineralization between sodium fluoride solution and distal water.

Table (1): Enamel Microhardness Between Groups of the Material.

ANOVA for Groups of Materials		
	df	Sig.
Between Groups of Distal Water	2	0.00**
Between Groups of Sodium fluoride solution	2	0.00**

** highly Significant differences between groups. $P \leq 0.01$

Sodium fluoride solution

The mean values and standard deviation of the microhardness of enamel specimens at baseline (control) before demineralization were (324.2 ± 29.10) , then became (234.54 ± 9.83) post demineralization, and were raised to (291.47 ± 5.63) after 10 days of remineralization with sodium fluoride solution. The comparison between the mean value of the sodium fluoride solution and the distal water is shown in (Figure 1).

There was a statistically significant difference between the baseline, post-demineralization, and after 10 days' groups of sodium fluoride solution, where ($p \leq 0.05$). As shown in (Table 1). After remineralization, the microhardness of the group that was treated with distal water and the group that was treated with sodium fluoride solution shows a highly significant difference, as shown in (Table 2).

Table (2): Comparison between control and sodium fluoride solution by t-test values.

	T value	P value
Distal water Sodium fluoride	-13.871	0.00 **

**highly Significant differences, $P \leq 0.05$

Densometric analysis

The density of the enamel surface was studied using this examination.

Distal water

The mean values and standard deviation of the enamel density specimens at baseline (control) before demineralization were (193.8 ± 12.25) and became (141.2 ± 11.43) post-demineralization, and stayed at (140 ± 13.37) after 10 days of remineralization with distal water. The relationship between the mean value of distal water and sodium fluoride solution is shown in (Figure 2).

There was a statistically significant difference between the baseline, post-demineralization, and after 10 days groups of distal water ($p \leq 0.05$). As shown in (Table 3).

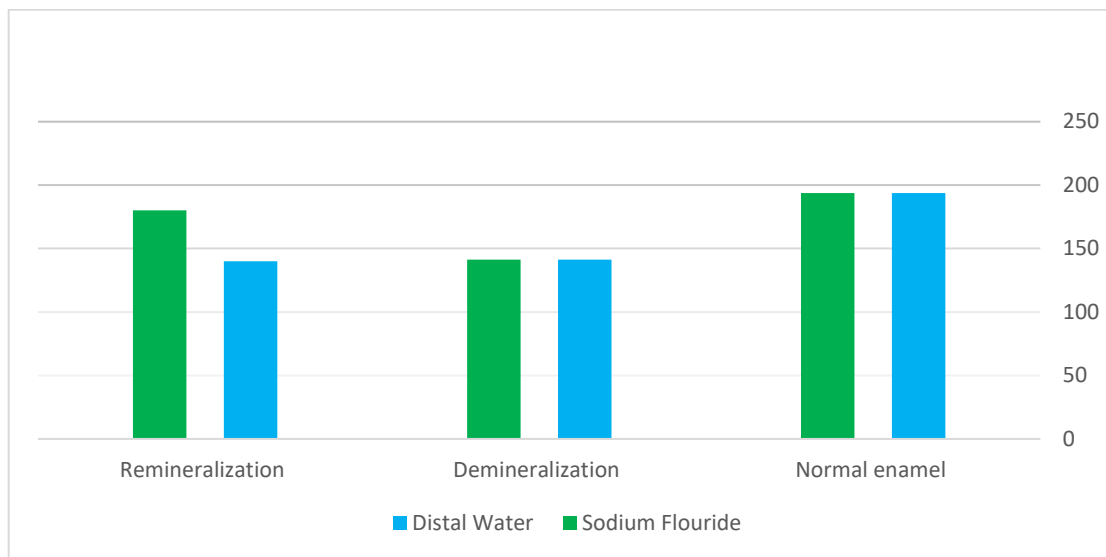


Figure (2): The mean value of enamel density before and after remineralization between sodium fluoride solution and distilled water.

Table (3): ANOVA of enamel density between Groups of the same material

	df	Sig.
Between Groups of distilled water	2	0.00**
Between Groups of Sodium fluoride solution	2	0.00**

** highly Significant differences between groups

Sodium fluoride solution

The mean values and standard deviation of the enamel density specimens at baseline (control) before demineralization was (193.8 ± 12.25) and became (141.2 ± 11.43) post demineralization and was raised to (180 ± 12.083) after 10 days of remineralization with sodium fluoride solution.

The relationship between the mean value of distilled water and sodium fluoride solution is shown in (Figure 2).

There was a statistically significant difference between the baseline, post-demineralization, and after 10 days groups of sodium fluoride solution ($p \leq 0.05$). As shown in (Table 3).

After remineralization, the enamel density for the group that was treated with distilled water and the group that treated with sodium fluoride solution shows a highly significant difference, as shown in (Table 4).

Table (4): Comparison between distal water and sodium fluoride solution by t-test values.

	T value	P value
Distal water	-4.9614	0.00 **
Sodium fluoride		

**highly Significant Differences, $P \leq 0.05$.

DISCUSSION

Today, a variety of remineralizing substances are effective in fostering remineralization and avoiding dental caries, which has grown to be a significant public health issue. ⁽¹⁰⁾ Fluoride is regarded as the gold standard in the fight against tooth decay. ⁽¹¹⁾ it is an excellent remineralizing agent; however is associated with potential toxic side-effects. ⁽¹²⁾ Therefore, in recent times, there is an urgent need to restrict the use of fluoride as a treatment for dental caries due to dental fluorosis. ⁽¹³⁾

Featherstone, J.D., in 1983, first described the in vitro models that are the most conventional techniques in caries research and the production of artificial caries-like lesions. ⁽¹⁴⁾ The assessment of the remineralization period was chosen in accordance with the previous study, at 10 days, two weeks, and four weeks. ^(9, 15)

Surface microhardness is a physical characteristic that evaluates the impact of chemical and physical agents on the hard tissues of teeth. This is a practical approach to assessing the fluoride-treated enamel's resilience. ⁽¹⁶⁾ Because enamel has a tiny microstructure, is non-homogeneous, and is brittle, it is a good option for this examination. In investigations on demineralization and remineralization, microhardness indentation offers a comparatively easy, quick, and non-destructive approach. ⁽¹⁷⁾

The results of the following study showed an increase in the mean value of enamel hardness and density for the group treated with a sodium fluoride solution, indicating an effective remineralizing potential, whereas no remineralization effect was observed in the distal water.

The comparison between groups of the same solution showed that the distal water gives a statistically significant difference is attributed to the demineralization effect of acid etch compared with normal enamel, while the sodium fluoride solution shows a highly significant difference is attributed to remineralizing potential.

The remineralizing effect of sodium fluoride solution and distilled water was also confirmed by microhardness and densometric analysis, indicating an effective remineralization potential of sodium fluoride solution with a highly statistically significant difference.

Fluoride accelerates the development of fluorapatite crystals on the partly demineralized subsurface crystals in the carious lesion, enhancing tooth remineralization. This surface absorbs fluoride, which draws calcium ions to it. As a result, this new surface preferentially absorbs additional fluoride, creating a surface that looks like fluorapatite. ⁽¹⁸⁾

The inactivation of early carious lesions with fluoride solution has been utilized therapeutically. Fluoride works best when given topically, and its effects are amplified by good oral hygiene. ⁽¹⁸⁾ Supplements containing fluoride can be taken orally or topically as nutritional pills, lozenges, or drops. Fluoride concentrations in tablets and lozenges range from 1.0, 0.5, or 0.25 mg, and sodium fluoride is often the active component. Fluoride toothpaste containing 1000 ppm of fluoride has been shown to prevent dental caries in the permanent and primary dentition. ⁽¹⁹⁾

Comparing the prevention of caries to a placebo, the fluoride concentration increased from 1000/1055/1250 ppm to 240/2500/2800 ppm. This resulted in a 23% to 36% improvement. ⁽²⁰⁾

Most nations have toothpaste with a maximum fluoride content of 1,500 ppm available over the counter. On prescription, higher doses (2,800 ppm and 5,000 ppm) are offered. To lessen the danger of dental fluorosis, greater dosages should be recommended, but only used temporarily. ⁽²¹⁾

This study, parallel to the study by Caries Res (2016), concluded that toothpastes containing >1,500 ppm fluoride (2,500-2,800 and 5,000 ppm F) provide an additional caries preventive effect on root caries lesions in elderly patients compared to traditional dentifrices (1,000-1,450 ppm F). ⁽²²⁾

The study's disagreement results from Hasson et al.'s 2008 review of the research on fluoride supplements' ability to prevent dental fluorosis and caries. "There is weak and conflicting evidence that the use of fluoride supplements prevents dental caries in primary teeth," they declared in their conclusion. There is proof that these supplements guard against tooth decay in permanent teeth. The adverse effect of mild to severe dental fluorosis is considerable. ⁽²³⁾

Since there have only been a few randomized clinical trials, it cannot be said with absolute certainty that high fluoride toothpaste prevents root caries better than standard fluoridated toothpaste. However, using toothpaste with 5,000 ppm F has been shown to prevent or stop root caries lesions in the population of older adults, according to data from the few randomized clinical trials conducted in this field. ^(24, 25), including the elderly frail ⁽²⁶⁾ and vulnerable. ⁽²⁷⁾

Further research is needed to evaluate the potential risk of high concentrations of fluoride before it is used *in vivo* conditions.

CONCLUSIONS

According to the study's results, sodium fluoride solutions greatly enhanced enamel density and surface hardness and are thus regarded as efficient remineralizing agents. Additionally, topical fluoride supplementation looks to be a practical and affordable method of reducing tooth cavities.

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Authors' Contribution

AL-Shaibani MH, Al-Saffar MT, and Mahmood AS contributed to conceptualization, validation, and writing the original draft. AL-Shaibani MH, Al-Saffar MT, and Mahmood AS are responsible for formal analysis, methodology, and project administration. Al-Saffar MT and Mahmood AS reviewed and edited the manuscript. AL-Shaibani MH, Al-Saffar MT, and Mahmood AS contributed to the investigation, software development, validation, and visualization. AL-Shaibani MH, Al-Saffar MT, and Mahmood AS. are involved in data curation, resources, and review & editing. All authors have read and approved the final manuscript.

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Ethical statement: the study was approved by the Research Ethics Committee and Scientific Committee Department of Dental Basic Science/ College of Dentistry/ University of Mosul, under approval number (UoM. Dent/ A.H.L.83/ 22)

Conflict of interest

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

Availability of data and materials: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Generative AI and AI-assisted technologies

No artificial intelligence tools were used. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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تعديل جديد لإعادة تمعدن الفلورايد ضد تسوس الأسنان (دراسة مختبرية)

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الملخص

الأهداف: تهدف الدراسة الى تقييم تأثير إعادة التمعدن لمحلول فلوريد الصوديوم مقارنة مع الماء المقطر، بواسطة اختبار Vickers microhardness وتحليل الأشعة السينية. **المواد وطرائق العمل:** في هذه الدراسة المختبرية، تم استخدام 10 أضرار دائمة. تم احاطة كل سن بشرط عازل، ويتم تعريض جزء مينا لسطح الإطباق فقط لمحلول إزالة المعادن (حفر حمض) لمدة 45 ثانية في المختبر ويتم توزيعه عشوائيًا على مجموعتين: المجموعة 1 عولجت بالماء المعقم، المجموعة 2 عولجت بمحلول محلول فلوريد الصوديوم. تم علاج جميع المجموعات باستثناء مجموعة الأولية التي لم تتعرض الى شيء مع حلول إعادة التمعدن الخاصة بهم لمدة 10 أيام. تم الفحص في جهاز فيكرز الصلادة الدقيقة (VHN) وتحليل الأشعة السينية قبل البدء، بعد سحب المعادن، وبعد 10 أيام بعد إعادة التمعدن. **النتائج:** أظهر تحليل الأشعة وجهاز الصلادة الدقيقة (VHN) تأثير إعادة تمعدن محلول فلوريد الصوديوم في كثافة المينا وصلابة السطح بعد إعادة التمعدن، مع وجود فرق معنوي بعد 10 أيام (قيمة $p \leq 0.05$) وكانت القيمة المتوسطة في المجموعة المعالجة بمحلول فلوريد الصوديوم أعلى من القيمة المتوسطة المسجلة للمجموعة المعالجة بالماء المقطر. **الاستنتاجات:** يعتبر محلول فلوريد الصوديوم عامل فعال لإعادة التمعدن ويزيد بشكل كبير من كثافة المينا وصلابة السطح.

الكلمات المفتاحية: محلول الفلورايد، إعادة التمعدن، صلابة المعادن الدقيقة، التحليل القياسي.