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# Efficacy of Theobromine and Sodium Fluoride Solutions for Remineralization of Initial Enamel Caries Lesions

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**ABSTRACT**

**Objectives:** This study investigated the efficacy of theobromine in comparison with 0.05% sodium fluoride solution for remineralization of initial enamel caries lesions (IECLs).

**Materials and Methods:** Ninety non-carious extracted premolars were sectioned longitudinally into buccal and lingual segments. Caries-like lesions were induced in each segment using acidified gel. Forty-five buccal segments were used for surface microhardness (SMH) test, and 45 buccal segments were used for energy-dispersive X-ray spectroscopy (EDS). The lingual segments were used as the control group for EDS and SMH test. The baseline SMH was measured with a Vickers hardness tester, and the baseline calcium content was analyzed by EDS. Each test group was divided into three subgroups for treatment with (1) artificial saliva, (2) 1.1 mol/L theobromine, and (3) 0.05% sodium fluoride. Remineralization and demineralization were done by daily pH cycling to simulate the oral environment. Samples in each group were immersed in treatment solutions for 1 min before and after the remineralizing cycle. After a 7-day cycle, the SMH test and EDS analysis were performed again. Data were analyzed using one-way ANOVA, Tukey's post-hoc test, and paired sample t-test ($P<0.05$).

**Results:** All treatments effectively remineralized the IECLs ($P<0.05$), and theobromine caused the maximum increase in SMH, which was significantly higher than the value in sodium fluoride group (mean value of 36.56±4.95 versus 23.25±3.92; $P=0.000$). EDS showed the highest calcium deposition in theobromine group (3.82±1.83wt%).

**Conclusion:** Theobromine is an effective cariostatic agent, and can be considered as a safe alternative to fluoride in preventive dental care.

**Keywords:** Theobromine; Dental Caries; Tooth Remineralization; Sodium Fluoride; Mouthwashes

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## INTRODUCTION

Dental caries is one of the most common childhood diseases causing pain and discomfort [1]. Based on studies, 61% of children between 6-12 years have experienced dental caries [2,3]. Simultaneous interaction of pathological and protective factors determines the disease progression [1]. Nowadays, minimally invasive dentistry is an important approach [4]. Remineralizing agents are among the caries management strategies to preserve the tooth structure. Different remineralization agents such as fluoride, casein phosphopeptide, xylitol, calcium sodium phosphosilicate, and arginine have been introduced to the market [5].

Fluoride is the most important remineralizing agent for prevention of dental caries [1]. Different mechanisms have been proposed for the role of fluoride in remineralization but the most important mechanism is the formation of fluorohydroxyapatite crystals which are larger and more resistant to acid dissolution than normal hydroxyapatite crystals [6]. Fluoride has some adverse effects especially when consumed in excessive amounts. Fluorosis is one major concern regarding the use of fluoride [7,8]. Fluoride can pass through the cell membrane and affect the function of a wide range of cell types. Fluoride overdose can irritate the digestive system [9] and lower the IQ of children [10].

Theobromine was introduced as a new agent with remineralizing potential by Nakamato et al [11]. Theobromine is a bitter alkaloid that belongs to the methylxanthine class of chemical compounds (3, 7 dimethylxanthine), and only differs from the caffeine (1, 3, 7 dimethylxanthine) in one methyl group [12]. It is a white crystalline water-soluble powder which is naturally derived from the cocoa beans. It is also found in the tea plant leaves and cola. It is believed that theobromine forms large crystallites that are more resistant to acid dissolution in apatite-forming medium [5,13]. Some in vitro studies revealed that theobromine can improve enamel hardness [14,15]. Further results demonstrated that theobromine had greater antimicrobial effects against Streptococcus mutans than other commercially available fluoridated tooth-pastes [16]. Theobromine toothpaste has been released and is available in the United States but information regarding its properties is limited [17]. Moreover, some recent studies revealed that theobromine did not have any remineralizing potential or cariostatic effect [18,19]. According to such disparate findings, more research is needed to investigate the remineralizing potential of theobromine. Thus, the aim of this study was to evaluate and compare the remineralizing potential of theobromine and 0.05% sodium fluoride solutions on initial enamel caries lesions (IECLs).

## MATERIALS AND METHODS

The study protocol was approved by the ethics committee of Babol University of Medical Sciences (MUBABOL.HRI.REC.1369.213). Non-carious premolar teeth freshly extracted for orthodontic reasons were collected. The teeth were examined by a trans-illuminator and 90 teeth without demineralization, enamel cracks and enamel malformations were selected. The teeth were stored in 0.1% thymol solution at room temperature until they were used.

*Specimen preparation:*

All the teeth were cleaned with pumice to remove debris and dental plaque. Each tooth was sectioned approximately 2 mm below the cementoenamel junction by a water-cooled diamond disc to cut the roots. The coronal part of each tooth was longitudinally sectioned in mesiodistal direction. Forty-five buccal segments were used for surface microhardness (SMH) test and 45 buccal segments were used for energy-dispersive X-ray spectroscopy (EDS). The lingual segments were used as the control group for EDS and SMH test. For the SMH test, the buccal segments of the tooth samples were mounted in self-cure acrylic resin, keeping the buccal surface exposed. Then, each tooth surface was polished with 800, 1200, and 2400-grit silicon carbide paper to have a flat and smooth enamel surface. Finally, an acid-resistant nail varnish (maral, Iran) was applied on the enamel surface to leave a 3×3 mm window in the middle part. The same process was done to prepare the lingual segment as the control group.

*Inducing IECLs:*

To induce IECLs, all teeth were immersed in an acidified gel for 7 days [20].

The demineralization gel was prepared by adding 100 mmol/L of sodium hydroxide to 100 mmol/L of lactic acid to obtain a final pH value of 4.5. Also, 6% w/v hydroxyethyl cellulose was added to this solution while vigorously stirring [20].

*Baseline SMH measurement:*

Forty-five prepared premolar tooth samples were used for the SMH test. The baseline SMH was measured using a Vickers hardness tester (model MH1; Koopa Pazhoohesh, Tehran, Iran) with 200 g load for 15 s [7]. For each enamel specimen, the mean of the three indentation scores was calculated and presented the specimen's hardness value. The specimens were randomly divided into three treatment groups (n=15) as below:

1) Artificial saliva
2) 0.05 % sodium fluoride solution made by dissolving sodium fluoride powder (Sigma Aldrich, Hamburg, Germany) in distilled water.
3) 200 mg/L theobromine solution made by dissolving theobromine powder in distilled water (Sigma Aldrich, Hamburg, Germany).

After measuring the SMH, the treatment procedures were performed on all teeth, as explained below, and then the SMH was measured again.

*Treatment procedure:*

To simulate the remineralization/demineralization process in the oral environment as closely as possible, a pH cycling model was used for 7 days. Artificial saliva was used as the remineralization medium in all treatment procedures [21]. An acidified gel with the following composition was used as the demineralization medium: 2.2mmol/L $KH_2PO_4$, 2.2 mmol/L $CaCl_2$, 50 mmol/L acetic acid, and 0.5 µg F/L, with a pH of 4.5 [22]. The one-day treatment cycle is shown in Figure 1.

*Energy-dispersive X-ray spectroscopy (EDS):*

The enamel surface of the teeth was polished with silicon carbide papers. After the induction of artificial carious lesions, the samples were divided into three treatment groups (n=15) including fluoride, theobromine, and artificial saliva. The treatment procedures were done for the buccal surface of the teeth. The lingual surface of each tooth was considered as the control for the buccal surface of the same tooth. After the treatment procedures, all tooth samples (both buccal and lingual surfaces) were rinsed with distilled water and left to air dry at room temperature for 24 h. Before examining the teeth with EDS, they were sputter-coated with gold-palladium (EMITECH K450X Sputter Coater, Emitech Ltd., Ashford, UK) and placed in a vacuum chamber. The EDS analysis was performed by a scanning electron microscope (Tescan VEGA-II, Czech Republic) equipped with an EDS analyzer (Rontec, Bruker Company, Berlin, Germany) to assess the mineral content. All analyses were performed at the center of each sample.

*Statistical analysis:*

Statistical analysis of the data was conducted using SPSS 23.0 (SPSS Inc., Chicago, IL, USA) with 0.05 level of significance. The Kolmogorov-Smirnov test assessed the normality of the data. The pretest and posttest parameters of SMH and calcium level in each group were compared using paired sample t-test. The inter-group comparisons were performed with one-way ANOVA and Tukey's post-hoc test ($P<0.05$).

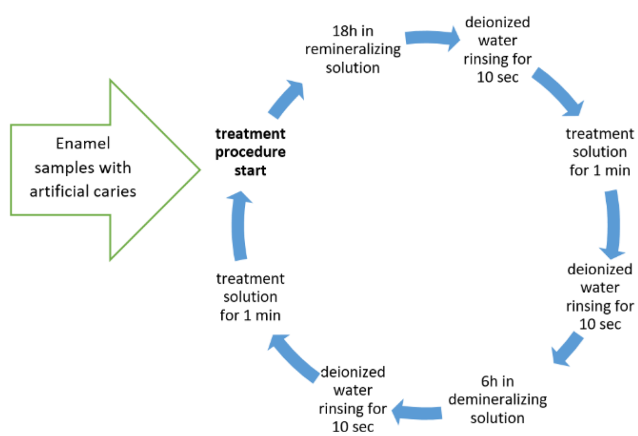


**Fig. 1.** Treatment procedure

## RESULTS

*SMH:* The mean and standard deviation values of enamel SMH in each group are shown in Table 1. All groups showed statistically significant increase in SMH after treatment ($P<0.05$). Comparison of the groups showed a significant difference in SMH after treatment ($P=0.001$).

Theobromine showed the maximum increase in SMH with a mean Vickers hardness number of 36.56±4.95 followed by sodium fluoride

**Table 1.** Results of statistical analysis regarding the microhardness values and calcium deposition pre- and post-treatment

| Groups | Vickers hardness number | | | | | Calcium Deposition | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-treatment | | Post-treatment | | P | Pre-treatment | | Post-treatment | | P |
| | Mean | SD | Mean | SD | | Mean | SD | Mean | SD | |
| **Artificial Saliva** | 273.05 | 28.07 | 275.98 | 27.41 | 0.002* | 41.30 | 1.95 | 41.95 | 1.99 | 0.001* |
| **Sodium Fluoride** | 242.90 | 37.71 | 266.15 | 36.85 | 0.001* | 40.03 | 2.34 | 42.21 | 2.28 | 0.001* |
| **Theobromine** | 245.43 | 41.81 | 282.00 | 42.94 | 0.001* | 40.47 | 2.00 | 44.29 | 2.05 | 0.001* |

SD: Standard deviation
*Indicates statistically significant difference at $P<0.05$

with a mean Vickers hardness number of 23.25±3.92.

*EDS:* Calcium deposition in all three treatment groups was significantly higher than that in the control surfaces ($P<0.05$, Table 1). The highest calcium deposition value was seen in the theobromine group (3.82±1.83wt% increase, $P<0.05$), and sodium fluoride played a more important role in remineralization compared with artificial saliva ($P=0.003$).

## DISCUSSION

The present study was designed to compare the remineralization of IECLs by theobromine and sodium fluoride solutions using an in-vitro pH cycling model, which is widely utilized by the researchers to simulate the oral environment in vitro. Fluoride is the gold standard for remineralization [23]. It is available in different forms such as toothpastes, solutions, gels, and varnishes [1]. Using other types of topical fluoride such as mouth rinses in addition to toothpastes has shown a significant reduction in the rate of caries especially in patients at high risk of caries [24]. The concentration of fluoride used in this study was 0.05%, which is preferred in young children [25]. Although 0.05% sodium fluoride concentration is optimal especially for daily use of mouthwash, it has not been examined in comparison with theobromine.

The most effective dose of theobromine for cariostatic activity is not clearly known; thus, various doses have been examined in previous studies [14,26,27]. As 200 mg/L has been considered as the minimum concentration to achieve the desired crystal enlargement [20], we chose this concentration of theobromine in the present study.

In the present study, theobromine showed significant remineralization potential which was in agreement with other studies introducing the cariostatic effects of theobromine [28,29]. The enamel remineralized by theobromine is more resistant against acid attacks [30]. This improved resistance can be due to larger crystal formation after treatment with theobromine. Nakamoto et al. [11] reported the size enlargement up to 4 times larger (2μm) than normal crystals (0.5μm). Larger crystals provide less surface for reaction with acid in comparison with smaller crystals [31].

In this study, theobromine caused maximum calcium deposition among the treatment groups. When enamel is exposed to acid, hydroxyapatite crystals start to dissolve. The theobromine molecules are smaller than the micro-channels formed after acid dissolution of enamel; thus, they can pass through the enamel micro-channels and penetrate into the hydroxyapatite crystalline structure. The higher electronegativity of oxygen and nitrogen in theobromine molecule ($C_7H_8N_4O_2$) attracts calcium (Ca) and phosphate ions which have low electronegativity and create new crystals of theobromine apatite [Ca10(PO4)6 (OHC7H8N4O2)] [32]. This can explain higher calcium deposition in the theobromine group in our study.

EDS is a specific method to quantify the mineral elements on substance surfaces [33] and it was used in the present study to quantify the calcium level of enamel surface.

The mineral content of enamel surface is directly related to its hardness [34]. Similar to a study by Taneja et al, [35] EDS showed a significant increase in calcium level in both treatment groups. They found no significant difference in remineralization potential between fluoride and theobromine. However, in our study, theobromine had the highest remineralization potential. This difference can be explained by different study protocols since Taneja et al. [35] used both theobromine and fluoride in the form of toothpaste and applied them by a cotton roll for 2 min on the tooth surface with a 24-h interval without any demineralization cycle. In a similar study, Gündoğar et al. [36] compared the effect of theobromine (200 mg/L and 500 mg/L) and fluoride (500 ppm and 1450 ppm) on IECLs on 20 bovine enamel samples. All treatment groups showed significant increase in SMH ($P<0.05$), and 1450 ppm fluoride caused the highest SMH value and calcium level compared with other groups ($P<0.05$). Although our study showed the remineralization effect of both theobromine and fluoride, but in contrast to a previous study [36], the remineralization potential of theobromine was significantly higher than that of fluoride in our study. Difference in the results of studies can be due to the type of employed pH cycling model, specimen preparation, and study protocol.

A number of recent studies did not consider any remineralization potential for theobromine or gave the lowest score to theobromine in comparison with fluoride [18,19,37]. This was in contrast to our results. This discrepancy can be due to a number of reasons. First is the inherent difference in the composition, time duration, and pH range of artificial caries induction medium, which lead to carious lesions with different severities at baseline [19]. Second is the difference between the study methodologies, for example diverse pH cycling models versus continuous exposure to treatment medium [18]. Third is the different forms of using the experimental agents, for example as part of a plaque-fluid phase [18] or in the form of a toothpaste (Theodent) that has other components such as calcium and phosphate as well [37]. Some studies reported that theobromine has dose-dependent effects [14,29]. In this study, we compared 0.0011 mol/L (200 mg/L) theobromine solution with 0.01191 mol/L of 0.05% sodium fluoride solution. Although the theobromine concentration was about 11 times lower than that of fluoride, it showed even better effects on enamel remineralization. This result approved that of Amaechi et al [20].

Theobromine is easily absorbed and metabolized in the human body; thus, it does not have the adverse effects of fluoride [38]. Starvic [39] stated that a 65-kg individual should eat about 18 g of theobromine/day (171 milk chocolate bars each weighing 1.05 oz) to reach the toxic dose, which is almost impossible to occur. Accordingly, theobromine can be a safe alternative to fluoride especially in children.In vitro studies such as the present study are important tools for evaluation of new agents and their characteristics; but they have limitations such as inability to completely simulate the oral environment. Moreover, caries development is a multi-factorial and complicated process, which cannot be exactly simulated in vitro; thus, clinical trials are recommended to confirm the results of in vitro studies.

## CONCLUSION

This study was designed to compare the remineralization potential of theobromine and sodium fluoride solution for IECLs. The results showed that although both agents had remineralizing effects, theobromine was more effective at a lower concentration and may serve as a safe and effective alternative to fluoride in mouthwashes, toothpastes, and other cariostatic products.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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