

Original Article

The Effect of Silver Diamine Fluoride, CPP-ACP/NaF, and Sodium Fluoride Varnish on Deciduous Enamel Erosion by Daily Snack (Drink) in Children/ An In Vitro Study

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Abstract

Objective: Most children consume carbonated soft drinks and fruit juices daily, inducing detrimental low pH and contributing to dental erosion. The current study aimed to assess and compare the efficacy of a single application of 38% Silver diamine fluoride (38% SDF), CPP-ACP/NaF varnish, and Sodium fluoride varnish (NaF) in the prevention of enamel demineralization in primary teeth against daily snack drink in children.

Methods: Forty primary anterior teeth (n=40) were divided into four groups and exposed to the following treatments (n=10): G1= 38%SDF (e- SDF TM); G2= CPP-ACP/NaF (MI VarnishTM); G3= NaF varnish (FluoroDose®); G4= Distilled water. The specimens were submitted for four days, with six pH cycles per day (10 minutes in orange juice) (SUNQUICK, IRAQ). Artificial saliva was used to remineralize the specimens after erosion challenges and as storage media between cycles. After four days of pH cycling, all the specimens were subjected to an atomic absorption spectrometer (AAS) to measure the amount of Calcium and phosphorus loss in each acidic solution. Scanning Electron Microscope evaluated the enamel topographic characteristics.

Results: All varnishes promoted better results for protecting enamel than the distilled water, with a difference. However, 38% of SDF demonstrated less Calcium and phosphorus loss than CPP-ACP/NaF, NaF varnish, and distilled water ($p < 0.001$).

Conclusions: Considering calcium and phosphorus loss values, a single application of each 38% SDF, MI varnish, and Naf effectively inhibited enamel erosion after four days of an erosive challenge, although the best protective effect was in favor of SDF.

Keywords: *Tooth erosion, Tooth demineralization, CPP-ACP, Primary teeth, Sodium fluorides, Silver diamine fluoride, Fluoride varnish.*

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Introduction

Dental erosion is an irreversible loss of tooth structure by chemical processes of acid dissolution without bacterial involvement. Dental erosion is a prevalent issue in modern society, owing to the increased intake of acidic beverages such as soft drinks, sports drinks, and fruit juices⁽¹⁾.

Topical applications of highly concentrated fluorides, such as oral rinses, gels, or varnishes, have been considered to prevent enamel deterioration and enhance its resistance against erosive attacks^(2,3).

Sodium fluoride varnishes (NaF) have been used due to their ability to attach to the tooth surface and their high fluoride concentration, which increases the formation of calcium fluoride (CaF₂) deposits that act as a mineral reservoir and which can partially behave as a physical barrier avoiding contact between the acid and the underlying enamel⁽⁴⁾.

CPP-ACP is a technology based on amorphous calcium phosphate (ACP) stabilized by casein phosphopeptides (CPP). The benefits of CPP-ACP nanocomplexes are the high concentration of calcium and phosphate ions that promote enamel remineralization. In addition, the production of an ACP phase (CPP-ACP addicted to fluoride ions) occurs in the presence of fluoride ions. Therefore, when the fluoride ions are disassociated, this phase may help with remineralization⁽⁵⁾.

Both have proved their preventive and remineralization abilities in vitro and in vivo studies⁽⁶⁻⁸⁾.

Silver diamine fluoride (SDF) has been used as a preventive measure in pediatric populations at high risk of developing early childhood caries due to SDF's bactericidal effect and its ability to remineralize the affected and softened tooth structures through the deposition and incorporation of fluoride ions. Fluoride ions present in 38% SDF solution are retained by the demineralized dental hard tissue to form fluoridated hydroxyapatite, which is harder and has a greater resistance to bacterial acidic challenges. In addition, SDF solution has a bactericidal effect because it includes free silver ions that bond to the surface of bacteria and kill their cells as a result⁽⁹⁾.

It is considered that both the prevalence and severity of dental erosion have been increasing over the years among children and adolescents in different age groups in many countries due to modern diet habits^(10,11). Additionally, due to the subsequent demineralization of

dental hard tissue, dental enamel might be subjected to scathing attack and loss of its inorganic component. Accordingly, the application of remineralization agents to protect and prevent primary teeth from dental erosion might have beneficial outcomes. So, the current study aimed to assess and compare by Atomic Absorption Spectrometer (AAS) and Scanning Electron Microscope (SEM) the efficacy of a single application of (38%) Silver diamine fluoride; CPP-ACP/NaF varnish, and Sodium fluoride varnish in the prevention of enamel demineralization in primary teeth against daily snack drink in children.

Materials and methods

An in vitro study was carried out from the beginning of December 2021 till the end of March 2022. Approval from the scientific committee and ethical committee of the College of Dentistry/ University of Sulaimani was obtained to conduct the study on 9/11/2021 with a research number of 71/21.

Three different fluoride varnishes were tested:

CPP-ACP/ NaF varnish (MI Varnish, GC Corporation, Tokyo, Japan), NaF varnish (FluoroDose, Australia), 38% Silver diamine fluoride (e-SDF, Globus Medisys, India).

Sample selection

A total of forty extracted sound human primary anterior teeth were selected for this study. The buccal surfaces of the teeth were evaluated under a stereomicroscope (ERMA optical works, Tokyo) to ensure the absence of structural defects, cracks, and caries. The teeth have been cleaned with tap water to wash away their blood, followed by storage in distilled water at room temperature until required^(12,13).

Sample preparation

The enamel surface of all extracted primary anterior teeth was cleaned and polished using non-fluoridated pumice and a slow handpiece. The remaining roots had been cut at the cemento-enamel junction level using a straight diamond bur with copious water irrigation to avoid harming the crowns. All the teeth were stored in a normal saline solution at room temperature⁽¹⁴⁾.

After that, the samples were cleaned in an ultrasonic device (FLOUREON VGT-900, CHINA) with distilled water for five minutes⁽¹⁵⁾. Next, the teeth were disinfected in 0.5% chloramine solution, prepared by dissolving 0.5 grams of chloramine T in 100 liters of distilled water one week before undertaking the study^(14,16).

To standardize the exposed enamel surface, a 3×3 mm window was created in the center of the buccal surface of the teeth by sticking adhesive tape on it. To maintain a sound reference surface, all teeth were painted with a thin coat of acid-resistant nail polish (ASMA & BEAUTY 3D, China) on all surfaces, excluding the window area^(17,18).

Design of the study

The total forty numbers of specimens were divided into four main groups at random (n=10) as follows:

G1= 38% Silver diamine solution (e- SDF™).

G2= CPP-ACP/NaF (MI Varnish™).

G3= 5% NaF Varnish (FluoroDose®).

G4= Distilled water.

For G1, the specimens were exposed to a drop of 38% SDF solution for two minutes. Following the treatment, the specimens were immersed in artificial saliva for six hours to simulate the clinical situation; then lightly dried with absorbent papers and introduced to the pH cycling.

For G2 and G3, after single applications of the varnishes in a thin layer using a micro brush, the specimens were immersed in artificial saliva for six hours. Moreover, the layer of varnishes was removed with the plastic scaler, and cotton was soaked with acetone. Finally, specimens were cleaned using distilled water, lightly dried with absorbent papers, and then introduced to the pH cycle.

For G4, the specimens did not receive treatment. Instead, they were immersed in artificial saliva for six hours and then introduced to the pH cycle^(5,15,19).

pH cycling model

To mimic the oral environment in vitro and carry out the remineralization process in artificial saliva. All the teeth were immersed in six cycles of orange juice per day for 10 minutes for four days, which means 60 minutes/day using freshly opened bottles of orange juice; the pH of orange juice was measured with a pH meter at room

temperature. Furthermore, the samples of all groups were submerged in artificial saliva between each erosive cycle for 60 minutes. After each erosive period, samples were rinsed with distilled water for 10 seconds. The specimens were also stored in artificial saliva overnight in an incubator at 37 °C after the final day of the erosive challenge^(20,21).

Demineralization cycle

After the varnishes were applied as described before, the specimens were immersed for six cycles per day for 10 minutes using freshly opened bottles of orange fruit drink (SUNQUICK, Iraq), with a pH of 2.5, 6 ml/sample, at room temperature 25 °C using separate containers for each specimen. The orange juice solution was composed of (3.4 mg/ml of Calcium and 6.5 mg/ml of Phosphorus). During demineralization cycles, the specimens were kept in hermetically sealed containers because removing gas from the drink may increase its pH and decrease its potential to dissolve hydroxyapatite⁽²²⁾. The orange juice was refreshed daily during the experimental period⁽²³⁾.

Remineralization cycle (Artificial saliva)

Between each demineralization cycle, the specimens in each group were rinsed with distilled water and immersed in 15 ml of artificial saliva for 60 minutes in separate containers, which were prepared by mixing 1.5 mM CaCl₂× 2H₂O, 0.9 mM KH₂PO₄, 150 mM KCl, 20 mM HEPES(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) in one liter of distilled water. The pH of the artificial saliva was adjusted to 7.0 using KOH. And the artificial saliva solution was changed every day⁽²⁴⁾.

Measurement of Calcium and Phosphorus (Quantitative evaluation)

3 mL from each specimen was obtained to analyze Calcium and phosphorus loss. The concentration of both minerals was obtained automatically using an atomic absorption spectrometer with induced argon as the source of agitation (Varian Spectra AA 220 FS, USA). The calcium and phosphorus loss from the enamel specimens were determined by subtracting the calcium and phosphorus content of the demineralization solution blanco solutions were analyzed before the enamel exposure from the total Calcium and phosphorus content of the solution. Calcium and phosphorus loss amounts were then calculated in mg/ml⁽¹⁷⁾.

Scanning electron microscopy analysis (Qualitative evaluation)

An enamel specimen from each group was randomly selected for evaluation of structural changes using scanning electron microscopy (EDAX, CAM, SCAN, 3200LV, UK).

Enamel specimens were coated with a thin layer of gold atoms using a gold sputter machine (EDAX, EMITECH, K550X, UK). Initially, the samples were analyzed with panoramic vision (500x) to notice the relation between the windows (sound and experimental); photomicrographs of the most representative areas of each group were obtained with a magnification of (1000 x) to observe any changes in detail^(15,25).

Statistical analysis

Data were analyzed using the statistical package for social sciences (SPSS, version 25). The normality of data was checked using the Shapiro-Wilk test. Accordingly, parametric tests were used. Repeated measures ANOVA test was used to compare the calcium and phosphorus readings throughout the four days of the study in each of the materials. One-way analysis of variance (ANOVA) was used to compare the means of Calcium and Phosphorus between the materials on each study day. A p-value of ≤ 0.05 was considered statistically significant.

Results

Table 1 summarizes the amount of Calcium (Ca^{+2}) released into an acidic solution, showing the mean concentration values and standard deviations. All tested varnishes showed a preventive effect against erosion after four days because they statistically differed from the G4 for calcium loss concentration. The highest mean of Ca^{+2} of the four days (42.83 mg/ml) was found in the G4, and the lowest (14.2 mg/ml) was in the G1. The means of Ca^{+2} of the G2 and G3 were 26.21 and 26.46 mg/ml, respectively ($p < 0.001$).

Though, considering phosphorus loss concentration, by one-way ANOVA. Note that the post-hoc test (LSD test) showed that all the differences in Phosphorus mean between every two materials were significant ($p < 0.001$). The highest mean of Phosphorus (P) of the four days was 172.49 mg/ml in the G4, and the lowest (21.57 mg/ml) was in the G1. The means of P of the four groups were 107.09 and 114.36 mg/ml in the G2 and G3, respectively ($p < 0.001$), as presented in Table 2.

Table 1: Mean of calcium loss during four days in study groups.

Groups	N	Mean (mg/ ml)	(SD)	p-value
G1= (e- SDF™)	10	14.20	(0.10)	
G2=(MI Varnish™)	10	26.21	(0.11)	
G3=(FluoroDose®)	10	26.46	(0.09)	< 0.001
G4=(Distilled water)	10	42.83	(0.22)	

N: number; mg/ml: milligram/ milliliter; SD: standard deviation.

Table 2: Mean of phosphorus loss during four days in study groups.

Groups	N	Mean (mg/ ml)	(SD)	p-value
G1= (e- SDF™)	10	21.57	(0.17)	
G2=(MI Varnish™)	10	107.09	(0.46)	
G3=(FluoroDose®)	10	114.36	(0.84)	< 0.001
G4=(Distilled water)	10	172.49	(1.08)	

N: number; mg/ml: milligram/ milliliter; SD: standard deviation.

All groups significantly increased surface roughness after four days of the experiment; these results were evident when examining the SEM photomicrographs. The biggest variances between exposed and unexposed areas were observed for G4, which was more expressive than in other groups, and very slight variations were seen for G1. When comparing the exposed areas on

SEM photomicrographs, the enamel surface of specimens treated with 38% SDF showed slight changes in enamel topography. In contrast, G2 and G3 showed some surface irregularities and minor tiny porosities than in G1. Still, the more significant variations between exposed and unexposed areas were detected for G4, demonstrating a destructive character with clear irregularities and porosities. Figure 1.

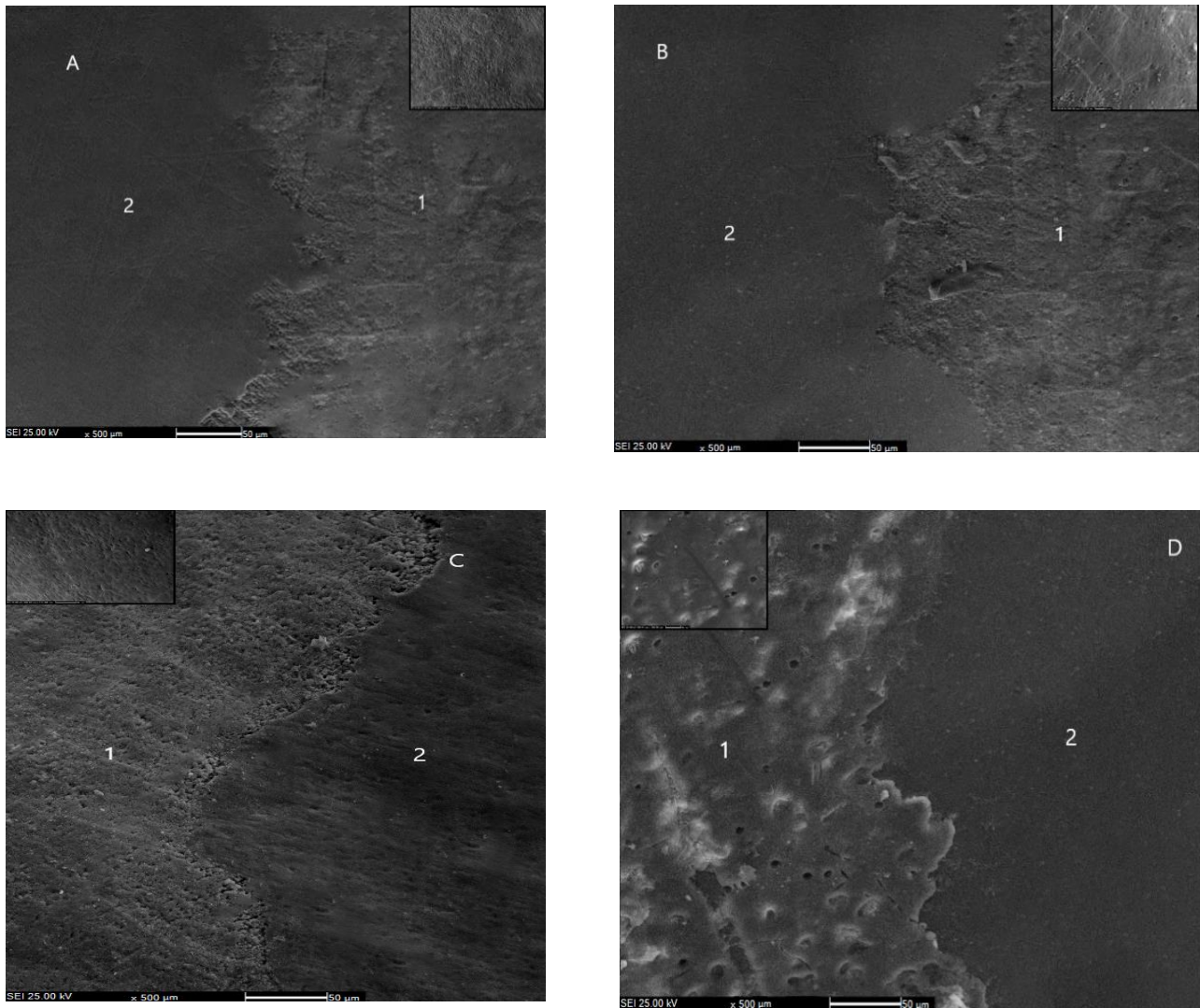


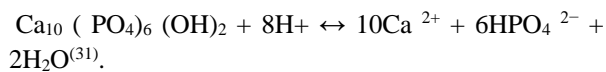
Figure 1: Surface scanning electron microscope (SEM) images of enamel samples after treatment and erosion challenge at 500 \times (Photomicrographs of the interface: 1= exposed area (after erosion), 2= unexposed area (sound enamel)) and 1000 \times (Photomicrographs of exposed area in the upper corner of each photo). (A) G1 = e- SDF TM (B) G2 = MI VarnishTM (C) G3 = FluoroDose[®] and (D) G4 = Distilled water.

Discussion

Progressive softening of the surface layer of enamel can occur as a result of the dental erosion process⁽¹⁷⁾. This process is probably a consequence of the irreversible loss of dental tissue by exogenous or endogenous acids. Due to the increased intake of acidic beverages, dental erosion has become prevalent in modern societies⁽²⁶⁾. Additionally, most children consume carbonated soft drinks and fruit juices daily, inducing detrimental low pH and contributing to dental erosion. Accordingly, evaluating the efficacy of a single application of 38% SDF, CPP-ACP/ NaF varnish, and 5% Sodium fluoride varnish to prevent enamel demineralization in primary teeth against daily snack drinks in children is crucial.

Treatments with fluorides have been the point of controversy. However, some authors regard topical fluoride application in various forms (e.g., fluoride varnish, stannous fluoride, titanium tetrafluoride, dentifrices, and acidulated gel) as an effective measure for dental erosion^(18,27). While this fact is not backed up by others^(11,28). It's important to emphasize that the methodological diversity of the literature makes it difficult to compare data directly, including the results of the current study (e.g., different fluoride concentrations, varying acidic challenges, and heterogeneous samples).

Phosphorus and calcium loss determination using atomic absorption spectrophotometer has been considered a reliable and sensitive method to quantify erosive wear based on enamel composition⁽²⁹⁾. Inorganic minerals, predominantly Calcium-phosphate crystals, are responsible for about 95% of the enamel weight⁽³⁰⁾. Furthermore, hydroxyapatite dissolution happens according to the chemical reaction:



Based on the calcium and phosphorus loss analysis alterations, our result showed that all tested materials were better than the distilled water, showing the different capacities for protection against Calcium and phosphorus loss. As a result, these varnishes might be considered prospective clinical products.

Concerning the concentrations of Calcium and Phosphorus lost, after four days of an erosive challenge, CPP-ACP/NaF shows less mineral loss than NaF and distilled water, owing to the calcium and phosphate ions present in the CPP-ACP/NaF varnish penetrating the enamel and causing ion oversaturation. ACP from CPP-

ACP interacts with F from NaF to produce the ACPF phase, which is unstable and quickly changes into fluorhydroxyapatite. Additionally, this varnish's casein content may alter the mechanical characteristics of enamel, making it less vulnerable to mineral loss and slowing the erosive process⁽²⁰⁾.

Based on calcium and phosphorus loss concentrations that were significantly lower in the NaF group after four days of an erosive challenge than in the distilled water group, NaF treatment was capable of inhibiting dental erosion, as previously reported by other authors^(32,33).

In addition, a better effect in reducing erosion was observed for 38% SDF compared to the other tested varnishes and distilled water. Thirty-eight percent of SDF contains a high fluoride ion concentration; of 44,800 ppm. When the concentration of fluoride ions is relatively high in preventive materials, they combine with calcium ions to form calcium fluoride, promoting remineralization. Furthermore, it is well known that fluoride enhances enamel resistance to acid due mainly to the formation of fluorapatite⁽³⁴⁾.

In regard to our knowledge, no previous studies have evaluated and compared the ability of a single application of 38% Silver diamine fluoride, CPP-ACP/ NaF varnish, and 5% NaF varnish in inhibiting erosion, which makes it unfeasible to establish any comparison to these findings and draw definitive conclusions.

However, a study with a similar method focused on 10% SDF and 2%NaF inhibiting enamel erosion of primary teeth. After trials with 1% and 10% citric acid, 10% SDF seems to help avoid enamel erosion, while 2% NaF seems to work well after a 1% citric acid challenge. However, after challenges with 1 percent and 10 percent citric acid, neither fluoride treatment prevented enamel degradation in terms of calcium loss values⁽¹⁷⁾.

Also, according to Ainoosah et al., result, SDF was effective in reducing dental erosion on the surface of the enamel and dentin, which was obtained from bovine incisors using erosion and erosion-abrasion cycling models when compared to deionized water (DIW, negative control), potassium fluoride (KF, fluoride control), silver nitrate (AgNO₃, silver control), and fluoride varnish (FV, clinical reference) but dental erosion-abrasion only on dentin measuring the surface loss by non-contact profilometry they use⁽⁴⁾.

When deciding between Coca-Cola and juices in the beverage section, juices have long benefited from a health halo, making them a popular alternative to Coca-

Cola. However, unlike sugary Coca-Cola, fruit juice contains some vitamins and minerals. After all, some schools got rid of most of the colas used to offer kids at school, but they still serve them lots of juice⁽³⁵⁾.

Conclusions

In conclusion, considering calcium and phosphorus loss values, a single application of (38%) Silver diamine fluoride, CPP-ACP/NaF varnish, and NaF varnish effectively inhibited enamel erosion after four days of an erosive challenge. Among these, 38% of SDF had the most effective against erosion.

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