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# The Effect of Silver Diamine Fluoride with or without Glutathione and Potassium Iodide on Discoloration, Fluoride Release, and Microhardness of Primary Teeth

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## ABSTRACT

**Background and Objective:** Silver diamine fluoride (SDF) is a minimally invasive alternative to conventional restorative methods. Despite proven benefits, it causes discoloration of teeth and gingiva. The present study was conducted to investigate the effect of silver diamine fluoride (SDF) with or without glutathione (GSH) and potassium iodide (KI) on discoloration, fluoride release, and microhardness of primary teeth.

**Methods:** This in vitro study was conducted on 51 human anterior primary teeth extracted due to trauma or becoming loose. After artificial induction of dentin demineralization, the teeth were randomly assigned to 3 groups of 17: SDF, SDF+KI, and SDF+20%GSH. The color change (ΔE) was measured using a spectrophotometer, dentin microhardness was measured using a Vickers hardness tester, and the concentration of fluoride released was measured using a potentiometer immediately after treatment and also after one and three months of immersion in artificial saliva.

**Findings:** The mean dentin microhardness in all three groups decreased significantly over time. In the SFD group, it decreased from 25.17±4.69 to 21.09±4.12, in the SFD+KI group from 22.35±3.41 to 18.62±2.19, and in the SFD+GLU group from 18.35±2.21 to 15.65±2.61 after three months ($p<0.001$). The mean dentin microhardness in the SDF group was significantly higher than in the other groups at all time points ($p<0.001$). Fluoride release in all three groups increased significantly over time ($p<0.001$). Fluoride release in the SDF+KI group was significantly higher than in the other two groups at all time points (2.41±0.47) ($p<0.001$). The color change (ΔE) in the SDF+KI group was significantly greater than that in the SDF group three months after treatment compared to immediately after treatment ($p<0.01$).

**Conclusion:** The results of the study showed that SDF alone produced the highest microhardness, while SDF+KI showed the highest fluoride release and the greatest color change three months after treatment.

**Keywords:** *Silver Diamine Fluoride, Hardness, Tooth Discoloration, Fluorides*.

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## Introduction

Silver was used in medicine even before its antimicrobial properties were fully recognized (1). After years of use in developing countries, silver diamine fluoride (SDF) received U.S. Food and Drug Administration (FDA) approval as an alternative to invasive dental procedures (1). SDF is a colorless, odorless liquid that was initially used as a dental desensitizing agent and for molar-incisor hypomineralization (2). SDF can be used as a minimally invasive alternative to conventional restorative procedures in the management of dental caries, especially in communities at high risk of dental caries (3, 4). Carious lesions treated with SDF show a reduction in size and an increase in mineral density, indicating their remineralization (5). Evidence suggests that SDF can repair early caries and inhibit the proliferation of Streptococcus mutans on demineralized dentin (6).

SDF can also inhibit the activity of matrix metalloproteinases and collagen degradation (6). SDF has been shown to be more effective than sodium fluoride and acidified fluoride phosphate in preventing dental caries (7). Due to the high concentration of silver (25.5%) and fluoride (44,800 ppm) in its composition, it has been reported that annual application of 78% SDF is more effective in restoring and preventing dental caries than 7 months of fluoride (7-10). Furthermore, a systematic review showed that application of SDF to carious lesions every 6 months for 2-7 years provides the best results in preventing dental caries (11). Despite the aforementioned benefits, SDF leads to dark brown or black discoloration of the teeth and gingiva, which may limit its use and raise aesthetic concerns (7). Several strategies have been proposed to prevent or reduce discoloration caused by SDF, such as replacing SDF with Zinc fluoride and Ammonium hexafluorosilicate (7, 12), using saturated potassium iodide (KI) immediately after SDF application (13), and using silver nanoparticles (14), and 20% glutathione (GSH) after SDF application (15). KI reacts with silver ions to form silver iodide, which has a beige color and masks the discoloration caused by SDF (15, 16).

GSH is one of the most common intracellular non-protein thiols that act as an antioxidant (15) and is a tripeptide thiol which is adsorbed by the metal surface and forms a coating around silver particles that prevents their aggregation and release, thereby minimizing discoloration caused by SDF over time (15). 38% SDF contains 44,800 ppm fluoride, which is used to stop dental caries (17). Examination of carious lesions after in vitro application of SDF or sodium fluoride varnish has shown that their hardness increases, indicating optimal efficacy of these products (17). Fluoride release assessment is a common method for evaluating the antimicrobial activity of restorative materials (18).

Since a study that investigates the effects of using GSH and KI together with SDF on tooth discoloration immediately and one and three months after treatment was not found, the aim of this study was to evaluate the effects of SDF with or without GSH and KI on discoloration, fluoride release, and microhardness of primary teeth.

## Methods

After approval by the Ethics Committee of the Faculty of Dentistry, Ahvaz Jundishapur University of Medical Sciences with the code IR.AJUMS.REC.1403.356, this in vitro study was conducted on 51 human anterior primary teeth that were extracted due to trauma or becoming loose. The teeth were randomly assigned to 3 groups of 17: SDF, SDF+KI, and SDF+20%GSH.

Assuming $\alpha=0.05$, $\beta=0.2$, 80% power, and mean and standard deviation of 0.96 based on a previous study (19), the sample size for each group was calculated as 17 teeth. After extraction, the teeth were immersed in thymol solution (0.1 wt% ADRICH, USA) for disinfection. The occlusal enamel was then

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removed with a low-speed diamond saw (NTI Superflex, Kerr Crop) to expose the mid-coronal dentin. The teeth were then polished using 600 grit silicon carbide abrasive paper under tap water for 10 seconds. The teeth were then immersed in a demineralizing solution containing 50 mM acetate, 2.2 mM potassium dihydrogen phosphate, and 2.2 mM calcium chloride (with a pH of 4.4) for 7 days at 37°C to simulate dentin demineralization. The teeth were then randomly assigned to 3 groups of 17 to receive SDF, SDF+KI, and SDF+20%GSH.

In the SDF group, one drop of 30% SDF (Cariestop 30%, Biodinamica, Brazil) was applied to the tooth surface using a microbrush and rinsed with saline solution for 30 seconds after 1 minute. In the SDF+KI group, after applying SDF, KI was applied to the tooth surface and rinsed with saline solution for 30 seconds after 1 minute.

In the SDF+GSH group, a mixture of SDF and 20% GSH was applied to the tooth surface for 1 minute and then rinsed with saline solution for 30 seconds. Then, the teeth were immersed in artificial saliva and the following tests were performed after one and three months of storage.

The color change ($\Delta E$) of the teeth was assessed using a spectrophotometer. The color parameters were measured immediately after treatment and after one and three months of immersion in artificial saliva and $\Delta E$ was calculated. A potentiometer (PH/ion meter, Metrohm, Switzerland) with an ion-sensitive electrode (Metrohm, Switzerland) was used to measure the dissolved fluoride concentration after one and three months of immersion. The potentiometer was first calibrated with standard fluoride solutions. Then, the electrode was placed in the solution, the container was shaken thoroughly to ensure uniform distribution of fluoride ions, and then the fluoride concentration was measured and reported as ppm. After each measurement, the electrode was rinsed with distilled water and dried (18).

The dentin microhardness was measured immediately after the teeth were removed from the artificial saliva. The dentin surface was divided into three hypothetical medial and distal sections. The first hypothetical section from the left was divided into three parts: upper, middle and lower, and the microhardness of each region was measured using a digital hardness tester (Nexus 4000 Innova test, Holland). The average of the measured values was reported as the microhardness of the corresponding tooth. After applying the load, the diamond indenter created a trapezoidal indentation with diameters d1 and d2. By dividing the load by the surface area of the indentation, the Vickers hardness number was calculated in $kg/mm^2$ and the average of the three values was recorded as the final hardness of the corresponding tooth. In this study, a load of 300 g was applied for 15 s according to a previous study (20). The normal distribution of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Accordingly, comparisons between the three groups were made using One-Way ANOVA followed by pairwise comparisons using Tukey's post-hoc test. Intra-group changes over time were examined using Repeated Measures ANOVA. All statistical analyses were performed using SPSS version 22 (SPSS Inc., IL, USA) and $p<0.05$ was considered significant.

## Results

The results showed that the mean dentin microhardness in all three groups decreased significantly over time. Nevertheless, this decrease was greater in the SDF+GSH group compared to other two groups. Moreover, the difference in microhardness between the three groups was significant at all time points ($p<0.001$ for all comparisons), such that the mean dentin microhardness in the SDF group was significantly greater than that the SDF+KI and SDF+GSH groups ($p<0.001$) (Table 1).

**Table 1. Dentin microhardness in the three groups at different time points**

| Group | Time<br>Immediately after treatment<br>Mean±SD | After one month<br>Mean±SD | After three months<br>Mean±SD | p-value$_{time}$# |
|---|---|---|---|---|
| SDF | 25.17±4.69 | 22.33±4.49 | 21.09±4.12 | <0.001 |
| SDF+KI | 22.35±3.41 | 20.24±3.47 | 18.62±2.19 | <0.001 |
| SDF+GLU | 18.35±2.21 | 17.75±2.63 | 15.65±2.61 | <0.001 |
| p-value$_{group}$* | <0.001 | <0.001 | <0.001 | – |

***ANOVA, #Repeated measures ANOVA**

Intra-group comparisons showed that fluoride release increased significantly over time in all three groups (p<0.001 for all groups). Inter-group comparisons showed significant differences in fluoride release at all three time points (p<0.001 for all), such that fluoride release in the SDF+KI group was significantly greater than the other two groups at all time points (p<0.001 for both groups) (Table 2).

**Table 2. Concentration of fluoride released in the three groups at different time points**

| Group | TIME<br>Immediately after treatment<br>Mean±SD | After one month<br>Mean±SD | After three months<br>Mean±SD | p-value$_{time}$* |
|---|---|---|---|---|
| SDF | 1.41±1.39 | 1.61±0.43 | 1.78±0.43 | <0.001 |
| SDF+KI | 1.96±0.55 | 2.13±0.47 | 2.41±0.47 | <0.001 |
| SDF+GLU | 0.91±0.13 | 1.29±0.34 | 1.54±0.47 | <0.001 |
| p-value$_{group}$# | <0.001 | <0.001 | <0.001 | – |

***ANOVA, #Repeated measures ANOVA**

ΔE was highest in the SDF group after one month compared to immediately after treatment, and highest in the SDF+KI group after three months compared to immediately after treatment. When comparing three months with one month, ΔE was highest in the SDF+KI group and lowest in the SDF group. Pairwise comparisons using Tukey's test showed that ΔE1-3 in the SDF+KI group was significantly higher than that in the SDF group (p<0.01) (Table 3). No other significant differences were observed at other times (p<0.05).

**Table 3. ΔE values in the three groups at different time points**

| Group | ΔE0-1<br>Mean±SD | ΔE0-3<br>Mean±SD | ΔE1-3<br>Mean±SD |
|---|---|---|---|
| SDF | 3.47±4.86 | 4.38±4.84 | 2.14±0.92 |
| KI+SDF | 2.98±1.19 | 5.24±1.59 | 3.55±1.48 |
| GLU+SDF | 2.30±0.67 | 3.42±1.63 | 3±1.48 |
| p-value* | >0.05 | >0.05 | <0.01 |

***ANOVA, ΔE0-1: color change after one month compared to immediately after treatment, ΔE0-3: color change after three months compared to immediately after treatment, ΔE1-3: color change after three months compared to one month.**

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## Discussion

The present results on microhardness showed that the microhardness of demineralized dentin increased significantly in all three groups. However, this increase was greater in the SDF group and less in the SDF+GSH group. SDF has a strong and direct effect on the organic phase of dentin. It changes the crystal structure of dentin hydroxyapatite and causes shrinkage of dentin, which affects its microhardness (21). Similarly, Cömert et al. reported that the increase in microhardness of demineralized dentin after application of SDF and SDF+KI was significantly greater than that of SDF+GSH (22). Rogalnikovaitė et al. evaluated the effect of SDF application on carious dentin and showed that it increases dentin microhardness (23). The present results showed a decrease in microhardness over time in all three groups, which emphasizes the need for its reapplication.

Another study reported that SDF activity decreases over time and requires frequent renewal. Horst et al. recommended renewal of SDF every 6 months for 2 years after initial application (24). However, if the patient does not return for regular follow-up, restorations with glass ionomer cement or the SMART technique are recommended (25). In the present study, fluoride release was observed in all three groups and this release increased over time in all groups. Fluoride release was highest in the SDF+KI group. The results demonstrated that the addition of KI and GSH had no negative effect on fluoride release. The 30% SDF used in this study contained 70,499 ppm fluoride and an alkaline pH of approximately 8 (26). In this study, the amount of fluoride released from the surface of the samples after application of the materials was measured, but the amount of fluoride absorbed was not measured; therefore, the fluoride concentration in the solution was lower than predicted. The reaction of fluoride with enamel and dentin depends on its concentration and the pH of the environment. In other words, fluoride is more reactive in acidic environments.

Ariffin et al. confirmed the effect of lowering pH on fluoride release (27). They showed that applying a silver fluoride (AgF) layer to glass ionomer and resin-modified glass ionomer resulted in increased fluoride release and also showed that decreasing the environmental pH increased fluoride release. It should be noted that commercial fluoride products should have an alkaline pH to ensure stability and optimal caries control (26). In this study, all teeth were immersed in artificial saliva with a neutral pH. However, GSH is acidic in nature and can reduce the environmental pH and increase fluoride release (11, 28). On the other hand, it has been shown that silver in the SDF composition may interfere with the formation of fluoride deposits and the formation of silver phosphate may compete with fluoride products. When SDF is mixed with GSH or KI, some of the silver ions bind to iodide ions and GSH, and thus, fluoride can be released more effectively. This explanation could be one of the reasons for the increased fluoride release when SDF was used together with GSH or KI (29).

The present study also showed that SDF caused a black discoloration that remained constant over the three-month study period. GSH partially reduced the black discoloration that also remained constant over the three-month study period. However, application of KI after SDF application resulted in the formation of a white precipitate that reduced the SDF-induced discoloration, but this discoloration did not persist and the dark discoloration caused by SDF returned, probably due to washing out of the white precipitate. Kamble et al. showed that application of KI and GSH reduced the SDF-induced discoloration; however, the discoloration was still present at day one, and one week, and four weeks after treatment (19). Furthermore, Roberts et al. showed that KI reduced the SDF-induced discoloration (30).

The present study had an in vivo design and could not fully simulate the clinical oral environment. Therefore, generalization of the results to the clinical environment should be done with caution. Clinical trials are needed to confirm the present results and achieve more general conclusions.

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Based on the results of this study, it was determined that SDF alone produced the highest microhardness, while the combination of SDF+KI showed the highest fluoride release and the most color change three months after treatment.

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