ORIGINAL RESEARCH

Comparison of Cariostatic and Remineralizing Potential of Two Commercial Silver Diamine Fluoride Preparations Using Confocal Laser Microscopy and EDX-SEM Spectroscopy: An *In Vitro* study

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Abstract

Aim: To investigate the cariostatic and remineralizing effect of two commercial silver diamine fluoride (SDF) preparations on enamel and dentinal caries using a bacterial plaque model.

Materials and methods: A total of 32 extracted primary molars were divided into two groups (n = 16), group I (FAgamin), and group II (SDF). Plaque bacterial model was used to induce caries on enamel and dentin. Preoperative evaluation of samples was done using confocal laser microscopy (CLSM) and energy-dispersive X-ray spectroscopy-scanning electron microscope (EDX-SEM). All samples were treated with test materials and evaluated for postoperative remineralization quantification.

Results: Energy-dispersive X-ray spectroscopy (EDX) revealed that mean preoperative levels (in weight %) of silver (Ag) and fluoride (F_2) in carious enamel lesions were 0.0 and 0.0, which increased postoperatively to 11.40 and 31.05 for FAgamin and 13.61 and 31.87 for SDF, respectively. For dentinal caries, EDX revealed mean preoperative levels (in weight %) of Ag and F_2 were 0.0 and 0.0, which increased to 11.47 and 48.71 for FAgamin and 10.16 and 47.82 for SDF, respectively postoperatively. Both the groups showed evident demineralization with exposed collagen under SEM. The mean value of enamel lesion depth for the group I and II were 38.64 and 39.30 µm, that reduced to 28.02 and 28.70 µm while for dentinal caries, the mean depth from 38.05 and 38.29 µm that reduced significantly to 28.96 and 30.10 µm, respectively ($p < 0.001$). Caries depth declined significantly after the application of both FAgamin and SDF ($p < 0.001$).

Conclusion: FAgamin and SDF show similar cariostatic and remineralization potential for dental caries. The bacterial plaque model used in this study is an efficient method to induce artificial carious lesions in teeth.

Clinical significance: A comparative evaluation of these two cariostatic and remineralizing agents will aid in identifying the efficacy of both commercial products in treating initial caries lesions in an effective noninvasive and child-friendly manner.

Keywords: Confocal laser scanning microscopy, Dental caries, Fluoride, Silver diamine fluoride, X-ray emission spectroscopy.

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Introduction

Dental caries continues to remain a global health problem, despite various advancements in dental care. Many chemical agents have been added to stop the caries process.[1] Ag, which is bactericidal, is used as an antimicrobial agent to arrest caries, with one of these compounds being SDF, which was introduced in 1960 by Suzuki et al.[2] Though SDF is available in many concentrations, but 38% of SDF is most commonly used. Many studies reported that an economical, simple, and noninvasive method to arrest dental caries is the application of the SDF.[3,4] Antibacterial effect of SDF[5] also helps to inhibit matrix metalloproteinase.[6] An increase in elemental content of the carious enamel lesions[7] and the microhardness of the carious dentin lesions has been shown by the action of SDF.[8,9] This cariogenic microflora produces acid, which causes the demineralization of dentin. As a result, to prevent dentin demineralization and collagen degradation and to promote remineralization of dentin, the application of SDF is recommended.[10,11]

Silver diamine fluoride (SDF) acts on caries by forming Ag phosphate crystals by reacting with the enamel of the tooth surface. Whenever SDF is applied to an established carious lesion, it enters dentinal tubules by blocking the lumen of dentinal tubules. Compared to other Ag halides, AgF_2 is highly soluble in water. In addition to AgF_2, SDF also has ammonium in it. Ag diamine ion [Ag $(NH3)_2$] is formed by combining ammonium ions with Ag one. This Ag $(NH3)_2$ is reversible and highly stable compared to AgF_2, remaining

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at high intensity for an extended time. Regardless of its advantages, SDF is not preferred by many clinicians in routine practice.[12]

This study aims to define the outcome of two commercial solutions of SDF on the enamel and dentinal caries using a bacterial plaque model and compare it with CLSM and EDX.

Materials and Methods

We carried out this *in vitro* experimental study at the Department of Pediatric and Preventive Dentistry and the Department of Microbiology after gaining clearance from the Institutional Ethical Committee, letter no. MIDSR/STU/560/714/2019.

Inclusion Criteria

- Primary maxillary and mandibular molar, with an intact and healthy crown.
- Teeth extracted close to exfoliation (age 9–12 years male/female), showing physiological root resorption.

Exclusion Criteria

- Teeth are showing evidence of caries, fractures, or other pathological defects.

Collection of Tooth Samples

The external surface of selected teeth was cleaned with curettes to remove soft tissue and debris. All specimens were stored in 5% sodium hypochlorite for 10 minutes to achieve removal of debris and soft tissues, disinfection, and finally were rinsed with double-deionized water. All the samples were kept in normal saline in air and light protected jar at room temperature until the beginning of the experiment.

Grouping of Samples

A total of 32 extracted primary molars were divided into two experimental groups ($n = 16$) as follows:

- Group I**:** (Treated with FAgamin) eight teeth for enamel caries and eight teeth for dentinal caries.
- Group II**:** (Treated with SDF) eight teeth for enamel caries and eight teeth for dentinal caries.

Preparation of Tooth Surface for Caries Induction

Induction of enamel and dentinal caries was done using the plaque bacteria model as follows: a small rounded window of 5 × 5 mm was prepared till the dentin-enamel junction on every specimen exposed the dentin of the tooth. Similarly, a rim of 5 mm intact enamel was left around the dentin well to induce enamel caries. All the remaining surfaces of the tooth except the window were coated with two layers of nail varnish, rendering them resistant to caries induction medium.

Preparation of Biological Caries Induction Media

Plaque from buccal surfaces of the maxillary and mandibular posterior and facial surface of anterior teeth, as well as debris from the carious lesion on teeth, were collected from a patient with having high caries index. The plaque and carious dentin were mixed in 20 mL of brain heart infusion (BHI) broth, followed by vortexing to gain homogeneous bacterial suspension to get the biological caries induction model. The BHI broth was incubated in the microaerophilic environment using a candle jar for 24 hours at 37°C in the incubator to enhance the growth of cariogenic bacteria. Around 1 mL of biological caries, induction media was added to 16 test tubes containing 5 mL nutrient and BHI broth, respectively, into which tooth samples were immersed completely. All the test tubes were screw-capped tightly to prevent contamination and incubated at 37°C in aerobic as well as the microaerophilic environment. After every 5 days, broth from test tubes was replenished with freshly prepared broth to maintain the viability and cariogenic potential of the bacteria. This process was repeated for 30 days for enamel carries induction and 90 days for dentinal caries induction.[13]

Grouping of Samples

After 30 days, all the specimens were evaluated under a stereomicroscope for determination of the depth of demineralization and the establishment of enamel and dentinal caries lesion. All the samples were equally separated into two groups, that is, group I (treatment with FAgamin) and group II (treatment with SDF) ($n = 16$). Eight teeth from each group were evaluated for enamel caries, and another eight teeth for dentinal caries.

Application of Test Materials

All the specimens from group I were treated with FAgamin, while group II will be treated with SDF as recommended by the manufacturer, respectively.

Application of FAgamin

All specimens were removed from the broth and rinsed with double-deionized distilled water. The caries lesion was dried using tissue paper, and excess debris was removed using a micro brush. The applicator tip was immersed in a drop of FAgamin and applied on the lesion gently for 2 minutes. After 2 minutes of application, the teeth were washed with water spray and these specimens were kept in neutral broth for 1 hour.

Application of SDF

All specimens were removed from the broth and rinsed with double-deionized distilled water. The caries lesion was dried using tissue paper and excess debris was removed using a micro brush. The applicator tip was dipped in a drop of SDF and applied on the lesion gently for 2 minutes. After 2 minutes of application, the teeth were washed with water spray, and these specimens were kept in neutral broth for 1 hour.

Thermocycling of Samples

All the specimens were subjected to thermocycling at a temperature of 5–55°C for 500 cycles with a dwell time of 30 seconds.[14,15] All the samples were checked for loss of acid-resistant varnish from their surface during this procedure.

Microscopic Evaluation of Samples

Surface Morphology

All samples from both groups were washed with distilled water ultrasonically three times, dehydrated in a desiccator, and finally sputter-coated with gold. The surface morphologies of the samples were then observed using SEM.

Elemental Analysis

Elemental analysis was carried out on the surface of all the specimens from each group. The levels of F_2, calcium (Ca), phosphorus (P), and Ag ions were estimated by EDX under SEM. The elemental analysis was done on the enamel and dentinal surface of each sample, and the mean weight percentage of F_2, Ca, P, and Ag was calculated for the quantification of demineralization and remineralization.

Lesion Depth

All samples from both groups were scanned by a confocal laser microscope using Z-resolution sectioning. The three-dimensional (3D) photos were observed and managed using FlouView data analyzing software (FV10-ASW 4.2 viewer). From the reconstructed 3D model of each sample, Z-sectional photos were traced, and the lesion depth was measured.

Statistical Analysis

The data obtained will be subjected to statistical analysis with Statistical Package for the Social Sciences (SPSS) version 22.0 statistical package for M3 Window (SPSS Inc. Chicago, Illinois, United States of America.). Intragroup comparison in FAgamin (group I) and SDF (group II) in cariostatic and remineralization potential in enamel caries was made. Intragroup mean and standard deviation were analyzed using the paired "*t*-test."

Results

Using EDX spectroscopy, it was found that there was an increase in Ca, F_2, and Ag levels (in weight %) after the application of FAgamin (group I) and SDF (group II). In contrast, the decline in P levels (in weight %) was seen in active carious lesions (Table 1 and Fig. 1).

Elemental Analysis

The EDX results were constant with the characteristic mineral concentration outline. In enamel caries, an increase in F_2, Ag levels (in weight %) postapplication was higher in both the groups, and the difference was found to be statistically significant. In both groups increase in Ca levels and a decrease in P levels (in weight %) was seen, but the difference was not found to be statistically significant Table 1.

Whereas in dentinal caries, EDX analysis showed that an increase in F_2 and Ag levels (in weight %) postapplication was higher in both the groups and the difference was found to be statistically significant. There was an increase in the level of P in both groups, but the increased level of P in group II was found to be statistically significant as compared to group I (Table 2 and Figs 2 and 3).

Evaluation of Surface Morphology

The surface morphology of the samples was evaluated using SEM. In the arrested dentinal lesion, a smooth surface with exposure to dentine collagen fibers was seen (Figs 4A to D). When viewed under high magnification, a disorganized and sparse distribution of collagens, and a dense structure of spherical granules in the intertubular area was found (Figs 4E to H).

Table 1: Intragroup comparison in FAgamin (group I) and SDF (group II) in cariostatic and remineralization potential in enamel caries, that is, before and after application levels (in weight %) of minerals using EDX spectroscopy

Enamel caries				
FAgamin (Group I)	*Before application Mean (SD)*	*After application Mean (SD)*	*Paired t-test*	*p-value, significance*
Calcium	16.66 (8.63)	31.60 (8.13)	$t = -3.561$	$p = 0.003$
Phosphorus	12.55 (3.83)	7.91 (2.06)	$t = 3.01$	$p = 0.009$
Fluoride	0.0 (0.0)	11.40 (3.17)	$t = -10.14$	$p < 0.001$**
Silver	0.0 (0.0)	31.05 (8.36)	$t = -10.50$	$p < 0.001$**
FAgamin (Group I)	*Before application Mean (SD)*	*After application Mean (SD)*	*Paired t-test*	*p-value, significance*
Calcium	22.08 (8.31)	28.09 (5.87)	$t = -1.670$	$p = 0.117$
Phosphorus	12.09 (6.10)	10.83 (2.11)	$t = 0.553$	$p = 0.589$
Fluoride	0.0 (0.0)	13.61 (3.77)	$t = -10.205$	$p < 0.001$**
Silver	0.0 (0.0)	31.87 (5.99)	$t = -15.07$	$p < 0.001$**

*$p < 0.05$, significant difference; **$p < 0.001$, highly significant difference; SD, standard deviation

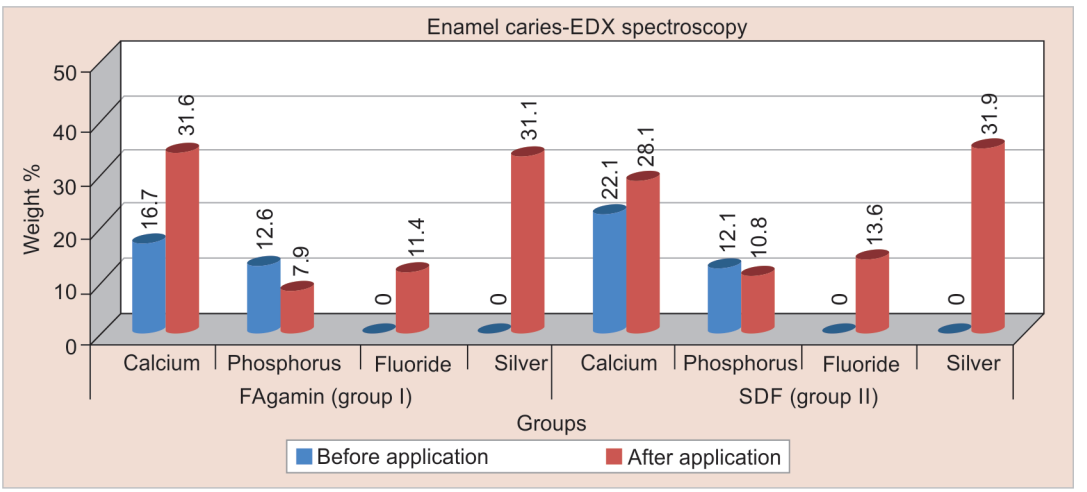


Fig. 1: Graph showing an intragroup comparison in FAgamin (group I) and SDF (group II) in cariostatic and remineralization potential in enamel caries

Lesion Depth

Confocal imaging showed that there was a decline or decrease in depth (in µm) of enamel and a dentinal lesion, that is, increases in cariostatic and remineralization properties was found using both interventions (Table 3 and Fig. 5). The decline in the depth of enamel and the dentinal lesion was seen in both groups FAgamin (group I) and SDF (group II), and the difference was found to be statistically significant ($p < 0.05$) (Fig. 6).

Discussion

This was the first research to examine the cariostatic and remineralization potential of two commercial preparation of SDF

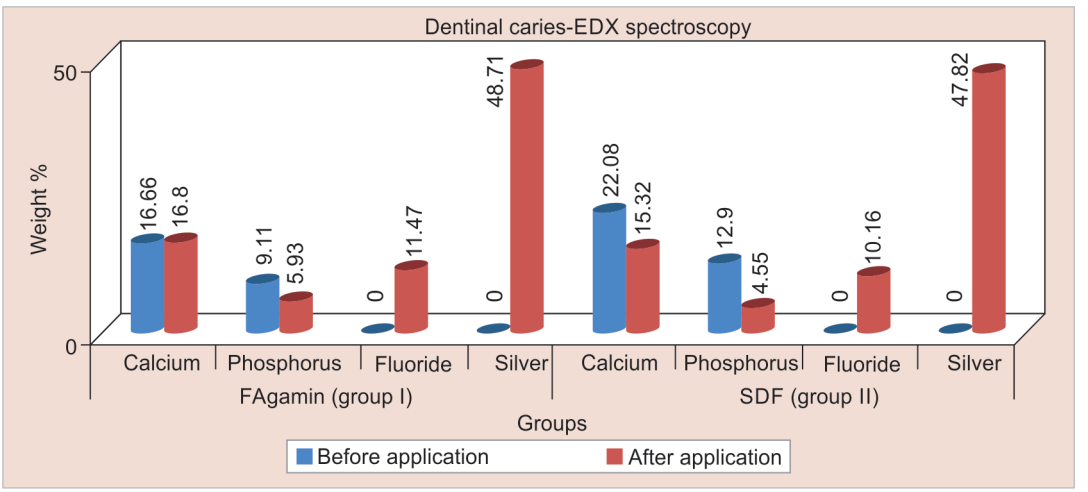


Fig. 2: Graph showing an intragroup comparison in FAgamin (group I) and SDF (group II) in cariostatic and remineralization potential in dentinal caries

Table 2: Intragroup comparison in FAgamin (group I) and SDF (group II) in cariostatic and remineralization potential in dentinal caries, that is, before and after application levels (in weight %) of minerals using EDX spectroscopy

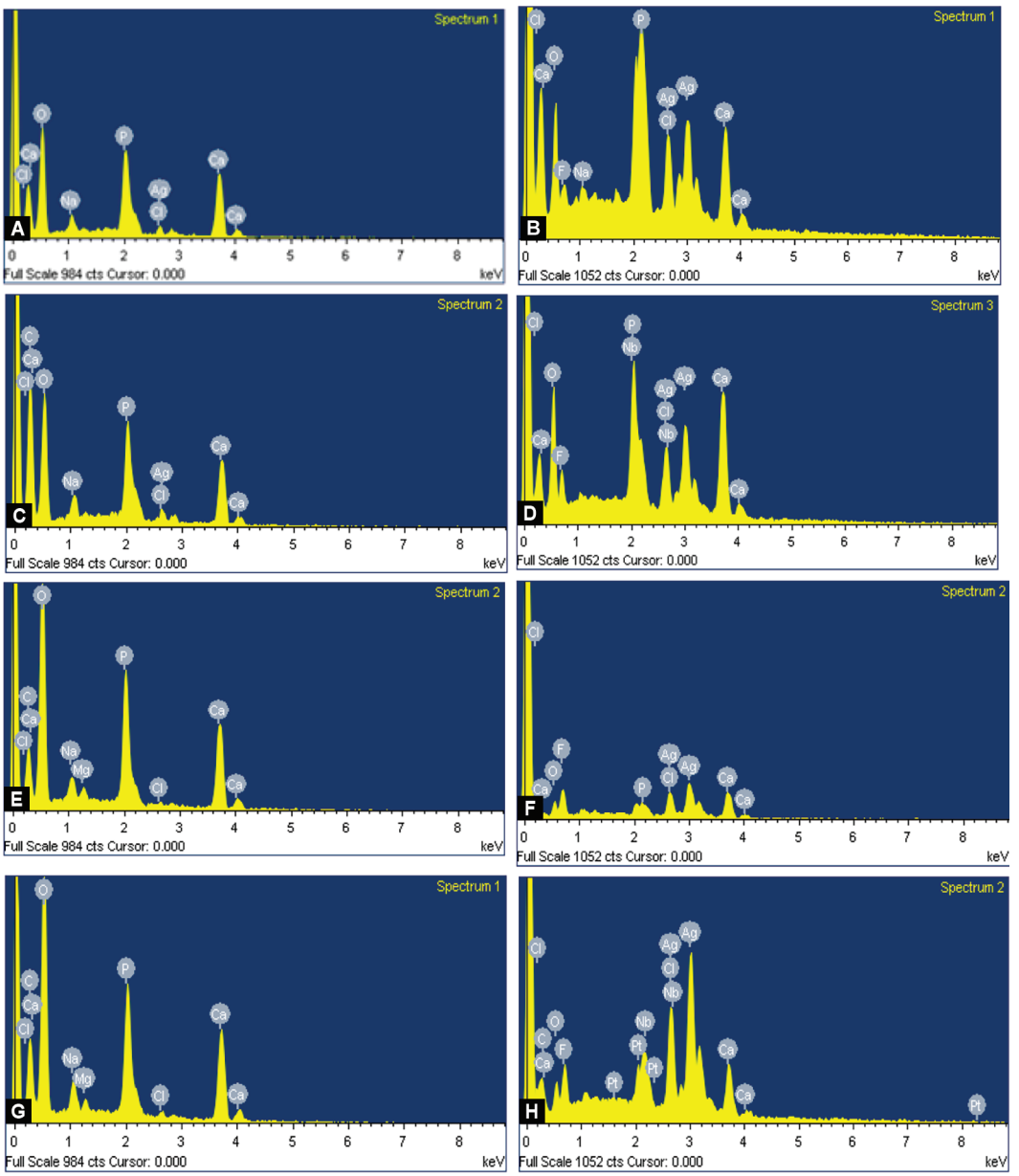
Dentinal caries				
FAgamin (Group I)	*Before application Mean (SD)*	*After application Mean (SD)*	*Paired t-test*	*p-value, significance*
Calcium	16.66 (8.63)	16.80 (4.93)	$t = -0.042$	$p = 0.967$
Phosphorus	9.11 (5.29)	5.93 (3.21)	$t = 1.452$	$p = 0.169$
Fluoride	0.0 (0.0)	11.47 (2.73)	$t = -11.86$	$p < 0.001$**
Silver	0.0 (0.0)	48.71 (9.49)	$t = -14.51$	$p < 0.001$**
FAgamin (Group I)	*Before application Mean (SD)*	*After application Mean (SD)*	*Paired t-test*	*p-value, significance*
Calcium	22.08 (8.31)	15.32 (4.15)	$t = 2.059$	$p = 0.059$
Phosphorus	12.90 (3.70)	4.55 (3.04)	$t = 4.918$	$p < 0.001$**
Fluoride	0.0 (0.0)	10.16 (2.02)	$t = -14.17$	$p < 0.001$**
Silver	0.0 (0.0)	47.82 (4.19)	$t = -32.24$	$p < 0.001$**

*$p < 0.05$, significant difference; **$p < 0.001$, highly significant difference; SD, standard deviation

Table 3: Intragroup comparison of FAgamin (group I) and SDF (group II, respectively) in cariostatic and remineralization potential, that is, change in depth (in µm) of enamel lesion and a dentinal lesion, that is, before and after application levels of minerals respectively by confocal imaging

Enamel caries				
FAgamin (Group I)	*Before application Mean (SD)*	*After application Mean (SD)*	*Paired t-test*	*p-value, significance*
FAgamin (Group I)	38.64 (1.61)	28.02 (1.63)	$t = 13.089$	$p < 0.001$**
SDF (Group II)	39.30 (1.53)	28.70 (1.75)	$t = 12.872$	$p < 0.001$**
Dentinal caries				
SDF (Group I)	*Before application Mean (SD)*	*After application Mean (SD)*	*Paired t-test*	*p-value, significance*
FAgamin (Group I)	38.05 (1.98)	28.96 (1.77)	$t = 9.676$	$p < 0.001$**
SDF (Group II)	38.29 (2.29)	30.10 (0.88)	$t = 9.409$	$p < 0.001$**

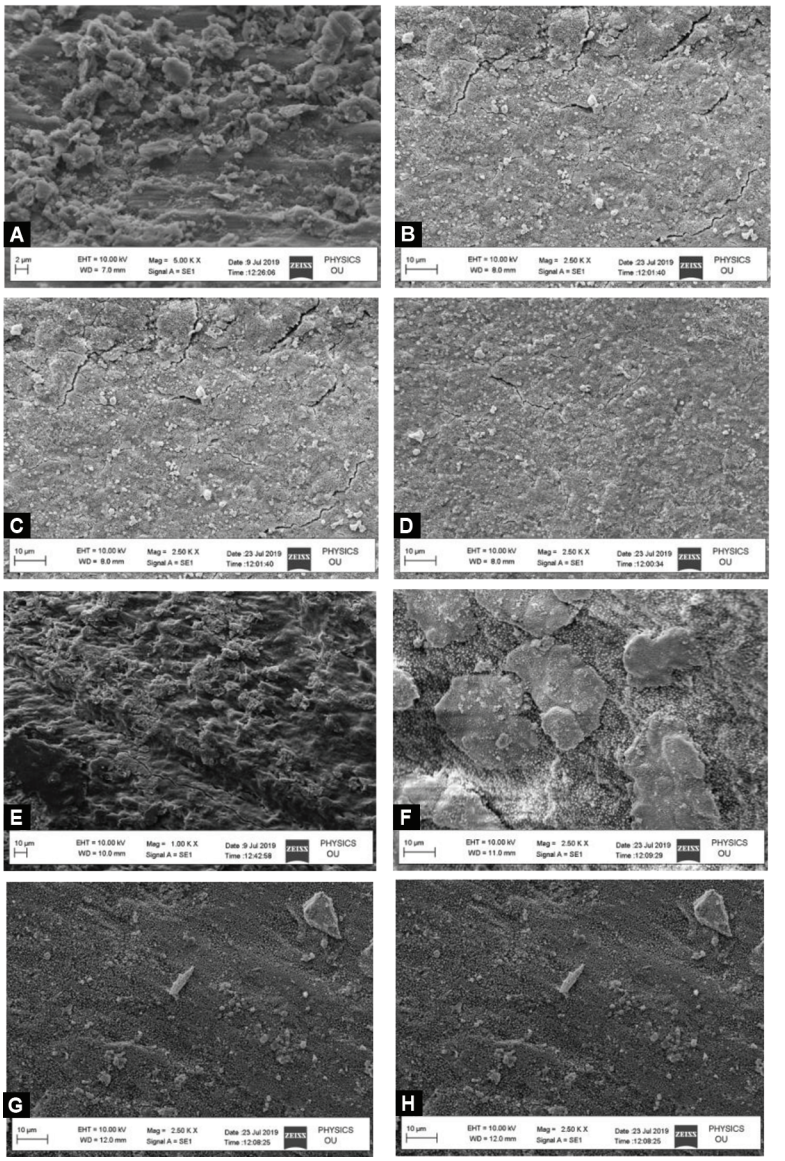
*$p < 0.05$, significant difference; **$p < 0.001$, highly significant difference; SD, standard deviation



Figs 3A to H: Shows elemental analysis (in weight %) using EDX: (A) Enamel specimen before application of FAgamin; (B) Enamel specimen after application of FAgamin; (C) Enamel specimen before application of SDF; (D) Enamel specimen after application of SDF; (E) Dentin specimen before application of FAgamin; (F) Dentin specimen after application of FAgamin; (G) Dentin specimen before application of SDF; (H) Dentin specimen after application of SDF

solutions, that is, FAgamin (Tadequim) and SDF (Kids-e-Dental). It showed that the application of both the commercial SDF preparations had a comparable cariostatic and remineralization potential on enamel and dentinal caries. Many types of research have been carried out to examine the demineralization and remineralization processes *in vitro*. In this research, we used a bacterial plaque model followed by pH cycling to induce artificial enamel and dentinal caries to mimic early caries formation.

The development of caries is an active natural procedure. It comprises demineralization and remineralization of the surface of the tooth,[16] which is induced by the acids produced by bacteria that settle on the surface of the tooth. The surface of the enamel contains these oral bacteria, which are part of the dental plaque. The demineralization and remineralization process is affected by the constituents of the dental plaque, which leads to the dissemination of carbohydrates, acids, and ions to and from the surface of the tooth.[17] Acid attack leads to the dissolution of enamel crystallites and caries formation begins.[16] It has been debated that successive demineralization of the surface and precipitation of mineral content of enamel surface build up the early carious lesions.[18] The 3D models of the investigational caries-like lesions permit an inspection of the extension of the lesion into the enamel and to define morphological characteristics of the lesion. Carious lesion induced by this bacterial plaque model was uniform in size and development when the SDF was applied topically. The elemental content and the depth of the lesion were uniforms among all the samples, the analysis was qualitative, and the result of the research could be widespread. Moreover, it is likely to define



Figs 4A to H: Representative SEM images of the enamel and dentin surface morphology before and after application of FAgamin and SDF: (A) Enamel specimen before application of FAgamin; (B) Enamel specimen after application of FAgamin; (C) Enamel specimen before application of SDF; (D) Enamel specimen after application of SDF; (E) Dentin specimen before application of FAgamin; (F) Dentin specimen after application of FAgamin; (G) Dentin specimen before application of SDF; (H) Dentin specimen after application of SDF

the volume of the lesion quantitatively with 3D reconstruction models.[19]

There is a low level of F_2 on the surface layer of the normal, carious lesion, as described by earlier studies.[20] Food, saliva, or drinking water are some of the sources for this lower concentration of F_2 in the mouth. As soon as the pH of the oral cavity becomes less than the critical pH of saliva, this F_2 reacts with the hydroxyapatite, and low-solvable fluorapatite crystals are formed, which helps in decreasing the value of critical pH and also reduces the enamel dissolution.[21] This action of F_2 helps in the inhibition of demineralization. All the clinical situations of the early carious lesion were replicated in the model used for caries induction. The observations of the study showed significant changes in the absorption of F_2 before and after the application of both SDF solutions *via* EDX assessment. The results obtained from the present study were similar to the research led in the past by Mei et al.,[22] which showed that the application of SDF on the arrested caries of primary teeth has a highly mineralized area. There is induction of unbalanced F_2, which is present at the tooth-saliva boundary with the application of topical F_2. This unbalanced F_2 occurs in the form of CaF_2 deposits, and its suspension is reliant on the pH of the oral cavity. As soon as the rate of pH descends below critical pH, there is the release of labile F_2, which is bound firmly. Hence, the crystals of fluoride hydroxyapatite are the end result.[23] Another reason for the increase in F_2 intake may be due to the liquidity of the SDF preparations. The fluidity of SDF helps in the full contact of the solution with the carious surface within a very short period of time

The exposure of collagens in the active carious lesion in evasion of hydroxyapatite structure suggests that there was demineralization of the dentine. This collagen is the strength of the dentine, which helps in holding the apatite crystallites together.[24] The cross-section

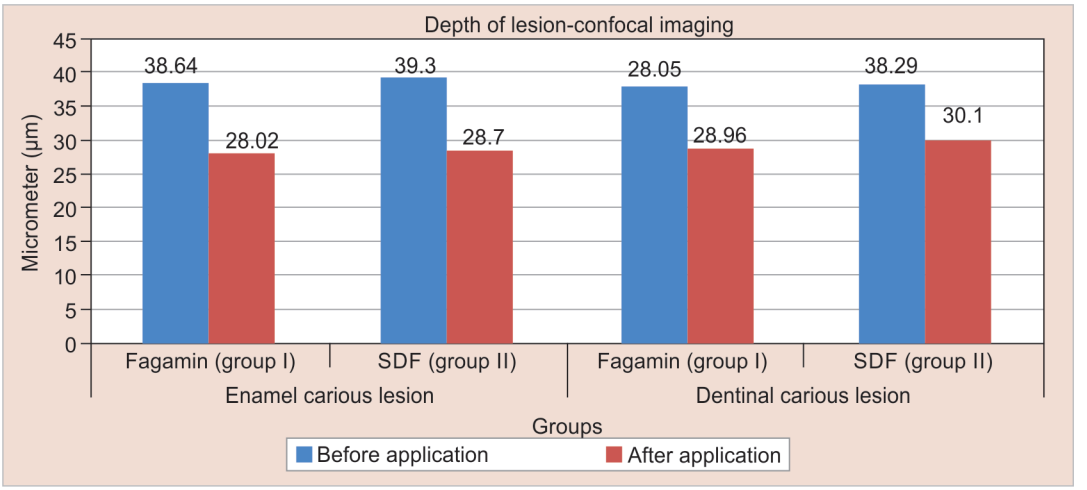
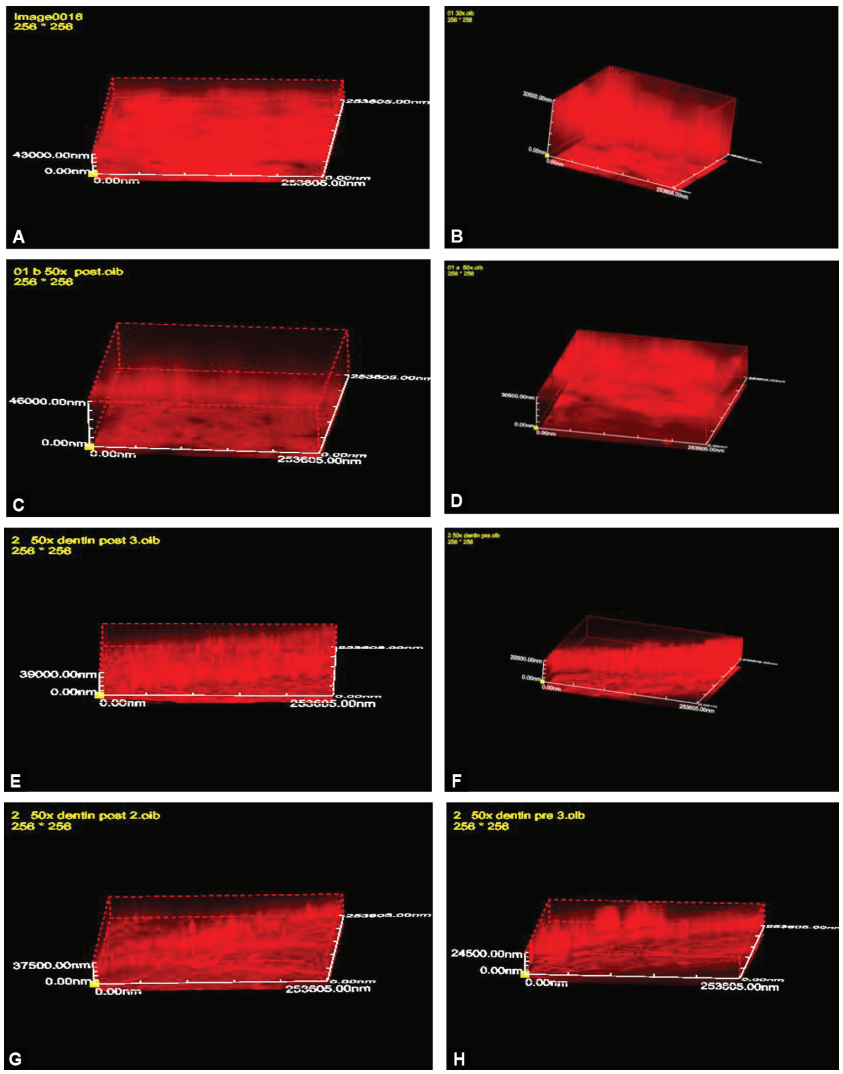


Fig. 5: Graph showing an intragroup comparison of FAgamin (group I) and SDF (group II, respectively) in cariostatic and remineralization potential, that is, change in depth (in µm) of enamel lesion and dentinal lesion



Figs 6A to H: Representative CLSM images of the enamel and dentin showing the depth of the lesion (in nm) before and after application of FAgamin and SDF: (A) Enamel specimen depth before application of FAgamin; (B) Enamel specimen depth after application of FAgamin; (C) Enamel specimen depth before application of SDF; (D) Enamel specimen depth after application of SDF; (E) Dentin specimen depth before application of FAgamin; (F) Dentin specimen depth after application of FAgamin; (G) Dentin specimen depth before application of SDF; (H) Dentin specimen depth after application of SDF

of the arrested carious lesion showed compact granules of spherical grains under SEM. This compact granule suggests the formation of extrafibrillar minerals, which inhibited the collagens from being exposed. Also, the action of SDF helps in the inhibition of the action of matrix metalloproteinases,[6] cysteine cathepsins,[25] and bacterial collagenase.[10] This action of SDF might be responsible for the protection of the collagens from being destructed by the endopeptidases and collagenase. Also besides, the increased resistance of collagen and enhanced chemical and physical property of collagen might be due to the higher levels of Ag ion in the SDF solution. Similar results were found in the study conducted by Mei et al.,[10] which showed inhibition of demineralization and preservation of collagens from degradation in demineralized dentine after application of 38% SDF.

There was also a significant difference in the depth of the lesion before and after applying both products, measured by confocal laser microscopy. Lesion depth reveals the degree of mineralization and cariostatic effect of both solutions. The result shows that there was a similar degree of remineralization in both groups, that is, FAgamin and SDF. The concentration of F_2 in both 38% SDF preparation is 44,800 ppm. There is no commercial product in dentistry having this high level of F_2. Stable results were obtained with the data from the EDX test, which showed that there was comparable F_2 uptake in both groups, which decreased the depth of lesion measured by confocal microscopy.

Both products showed a more substantial remineralization effect and decreased the depth of the lesion, that is, the cariostatic effect. However, the exact action of SDF on carious lesions was not implicit. The presence of higher levels of F_2, Ag, and alkaline properties of SDF might be responsible for the cariostatic effect on the carious lesion.[26] The remineralization of hydroxyapatite is stimulated by F_2. The alkaline nature of SDF helps to encourage the remineralization and inactivation of dentinal collagenases.[27] The earlier studies clarified that the insolubility of the formed Ag composite and the antibacterial effect of Ag is responsible for the action of Ag in SDF on the carious enamel lesion.[26,27] In the current research, an increase in Ag was confirmed by EDX before and after the application of both SDF products. The presence of Ag nanoparticles on the hydroxyapatite crystals of both groups, which were treated with SDF, was confirmed using EDX. Hence, there is suppression of the bacterial adhesion by the Ag-containing hydroxyapatite, which helps in the arrest of the caries process.

Conclusion

Based on the results of the study, it can be concluded that FAgamin and SDF show similar cariostatic and remineralization potential for dental caries. The bacterial model used in this study is an efficient method to induce artificial carious lesions in teeth.

Clinical Significance

To the best of our knowledge, this is the first study to compare the cariostatic and remineralizing potential of two commercial SDF preparations. A comparative evaluation of these two cariostatic and remineralizing agents will aid in identifying the efficacy of both commercial products in treating initial caries lesions in an effective noninvasive and child-friendly manner. As both products have similar efficacy, these products can be used in daily practice.

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