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Original Research Article

Enhancement of silver diamine fluoride antimicrobial efficacy through zinc incorporation

Ahsan Abdullah*, Anurag Mishra, Bhawana Tiwari, Haya Shadab Usmani

Department of Pediatric and Preventive Dentistry, Career Dental College, Lucknow, Uttar Pradesh, India

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***Correspondence:**
Dr. Ahsan Abdullah,
E-mail: klahsan@gmail.com

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ABSTRACT

Background: Silver diamine fluoride (SDF) is widely used for arresting dental caries because of its potent antimicrobial properties. Zinc possesses antimicrobial and biofilm-inhibitory effects that may further enhance the efficacy of SDF. This study evaluated the antimicrobial activity of zinc-incorporated SDF against *Streptococcus mutans* and *Enterococcus faecalis*. Objective of the study was to compare the antimicrobial efficacy of conventional 38% SDF with zinc-modified SDF at different zinc concentrations.

Methods: An in vitro experimental study was conducted using four groups: group 1 (n=10), 38% SDF; group 2 (n=10), 5 M zinc + SDF; group 3 (n=10), 10 M zinc + SDF; and group 4 (n=10), 20 M zinc + SDF. Bacterial isolates of S. mutans and E. faecalis were standardized to 0.5 McFarland (1.5×10^8 CFU/ml). Antimicrobial activity was evaluated using the agar disc diffusion method on Mueller–Hinton agar, and zones of inhibition were measured using a Vernier caliper. Statistical analysis was performed with the level of significance set at $p<0.05$.

Results: A concentration-dependent increase in antimicrobial activity was observed against both microorganisms. For *S. mutans*, the mean zone of inhibition was 12.36±0.58 mm with 38% SDF, 11.28±0.55 mm with 5 M zinc + SDF, 17.82±0.65 mm with 10 M zinc + SDF, and 20.75±0.92 mm with 20 M zinc + SDF. Similarly, for *E. faecalis*, the mean zone of inhibition was 9.80±0.72 mm, 8.95±0.64 mm, 15.99±0.70 mm, and 18.96±1.08 mm, respectively. Intergroup differences were statistically significant ($p<0.05$).

Conclusions: Higher concentrations of zinc significantly enhanced the antimicrobial efficacy of SDF against both *S. mutans* and *E. faecalis*. Zinc-modified SDF may represent a promising strategy for improving caries management; however, further *in vivo* studies are warranted to confirm its clinical applicability.

Keywords: Silver diamine fluoride, Zinc, Antimicrobial activity, Dental caries, *Streptococcus mutans*, *Enterococcus faecalis*

INTRODUCTION

Dental caries remains a major oral health concern, particularly in children, and is driven by acidogenic microorganisms within the oral biofilm.[1,2] Among these, *Streptococcus mutans* plays a pivotal role in the initiation and progression of dental caries through its ability to produce extracellular polysaccharides, generate organic acids, and enhance bacterial virulence.[3] In contrast, Enterococcus faecalis is predominantly associated with persistent endodontic infections, where its ability to survive under adverse environmental conditions and resist antimicrobial agents contributes to treatment challenges.[4]

In recent years, silver diamine fluoride (SDF) has gained widespread acceptance as an effective non-restorative agent for caries management because of its potent antimicrobial activity and ability to arrest carious lesions.[5-7] Its sustained antibacterial effect is primarily attributed to the penetration and retention of silver ions within dental hard tissues, while fluoride ions promote remineralization and inhibit further demineralization.[7] Despite these

advantages, there remains a need to further enhance its antimicrobial efficacy. Zinc, an essential trace element, has demonstrated significant antimicrobial and anti-cariogenic properties, including inhibition of bacterial metabolism, acid production, and biofilm formation.[8,9] Emerging evidence suggests that incorporating zinc into silver-based systems may produce synergistic effects, thereby improving antimicrobial performance.[10]

Therefore, the present study aimed to evaluate and compare the antimicrobial efficacy of conventional 38% silver diamine fluoride and zinc-enhanced silver diamine fluoride at different zinc concentrations against *Streptococcus mutans* and *Enterococcus faecalis* using the agar disc diffusion method.

METHODS

Study design

An in vitro experimental study was conducted at Career Post Graduate Institute of Dental Sciences and Hospital, Lucknow, Uttar Pradesh, in collaboration with the Department of Microbiology, Career Medical College and Hospital, Lucknow, Uttar Pradesh, from November 2025 to January 2026. The study aimed to evaluate and compare the antimicrobial efficacy of conventional 38% silver diamine fluoride (SDF) and zinc-enhanced SDF containing different zinc concentrations (5 M, 10 M, and 20 M) against Streptococcus mutans and Enterococcus faecalis.

Grouping

The samples were divided into four groups as follows: group 1: 38% SDF (control), group 2: 5 M zinc solution + 38% SDF, group 3: 10 M zinc solution + 38% SDF, and group 4: 20 M zinc solution + 38% SDF.

Preparation of zinc solutions

Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$; molecular weight: 297.49 g/mol; Molychem, Mumbai, India) was accurately weighed and dissolved in phosphate-buffered saline (PBS; buffer solution pH 7.0, Molychem, Mumbai, India) (Figure 1) to prepare zinc solutions of 5 M, 10 M, and 20 M concentrations (Figure 2). The prepared solutions were thoroughly homogenized to ensure uniform distribution of zinc ions before use.

Preparation of test discs and application of agents

Sterile 6-mm Whatman No. 1 filter paper discs were used for antimicrobial testing. For the experimental groups, each disc was pre-treated with 25 µl of the respective zinc solution (5 M, 10 M, or 20 M), followed by application of 38% silver diamine fluoride (e-SDF®, Kids-e-Dental LLP, India).

The zinc solution and SDF were allowed to interact on the filter paper disc for 1 minute before placement on the inoculated agar plates. Discs in the control group received 38% SDF alone.



Figure 1: Silver diamine fluoride (SDF) and materials used for preparation of zinc nitrate solutions.



Figure 2: Prepared stock solutions of zinc nitrate hexahydrate in phosphate-buffered saline (PBS).

Microbiological procedure

Pure cultures of *Streptococcus mutans* and *Enterococcus faecalis* were obtained from the Department of Microbiology and subcultured on Mitis Salivarius agar and brain heart infusion agar, respectively. The cultures were incubated aerobically at 37°C for 24–48 hours for *S. mutans* and 24 hours for *E. faecalis*. Identification of both microorganisms was confirmed by Gram staining and standard biochemical tests.

Preparation of bacterial suspension

A single well-isolated colony of each confirmed microorganism was inoculated into sterile normal saline. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard (approximately 1.5×10^8 CFU/ml) to obtain a standardized bacterial concentration for antimicrobial testing.

Antimicrobial assay

Antimicrobial activity was evaluated using the agar disc diffusion method. A standardized bacterial suspension was

uniformly spread over Mueller–Hinton agar plates using sterile cotton swabs to obtain a confluent bacterial lawn. The inoculated plates were allowed to stand for 10 minutes to facilitate absorption of the inoculum. Sterile filter paper discs containing 38% SDF alone or zinc-enhanced SDF (5 M, 10 M, and 20 M) were then placed on the agar surface using sterile forceps. Ten discs were tested for each group against each microorganism. The plates were incubated aerobically at 37°C for 24 hours.

Measurement of zone of inhibition

Following incubation, the diameter of the zone of inhibition surrounding each disc was measured in millimetres using a Vernier caliper. Measurements were recorded from one edge of the clear inhibition zone to the opposite edge, including the diameter of the filter paper disc. The mean zone of inhibition for each group was calculated and recorded for statistical analysis.

Statistical analysis

The data were expressed as mean ± standard deviation (SD) of the zone of inhibition (mm). Intergroup comparisons were performed using one-way analysis of variance (ANOVA). A p value of <0.05 was considered statistically significant.

RESULTS

The antimicrobial efficacy of conventional 38% SDF and zinc-enhanced SDF at varying concentrations (5 M, 10 M, and 20 M) was evaluated against *Streptococcus mutans* and *Enterococcus faecalis* using the agar disc diffusion method.

Antimicrobial activity against Streptococcus mutans

The mean zone of inhibition against *S. mutans* is presented in Table 1. Group 2 (5 M zinc + SDF) exhibited the lowest mean zone of inhibition (11.28±0.55 mm), followed by group 1 (38% SDF) (12.36±0.58 mm). A marked increase in antimicrobial activity was observed in group 3 (10 M zinc + SDF) (17.82±0.65 mm), while group 4 (20 M zinc + SDF) demonstrated the highest mean zone of inhibition (20.75±0.92 mm). The results indicate a concentration-dependent increase in antimicrobial activity with increasing zinc concentration (Table 1 and Figure 3).

Table 1: Mean±SD of zone of inhibition against *S. mutans* (n=10).

Groups	Mean±SD (mm)
5M zinc + SDF	11.28±0.55
38% SDF	12.36±0.58
10M zinc + SDF	17.82±0.65
20M zinc + SDF	20.75±0.92

Antimicrobial activity against Enterococcus faecalis

The antimicrobial activity against *E. faecalis* followed a similar trend (Table 2). Group 2 (5 M zinc + SDF) showed the lowest mean zone of inhibition (8.95±0.64 mm), followed by group 1 (38% SDF) (9.80±0.72 mm). Increased antimicrobial activity was observed in group 3 (10 M zinc + SDF) (15.99±0.70 mm), whereas group 4 (20 M zinc + SDF) exhibited the highest mean zone of inhibition (18.96±1.08 mm). These findings also demonstrated a concentration-dependent increase in antimicrobial activity (Table 2 and Figure 4).

Table 2: Mean±SD of zone of inhibition against *E. faecalis* (n=10).

Groups	Mean±SD (mm)
5M zinc + SDF	8.95±0.64
38% SDF	9.80±0.72
10M zinc + SDF	15.99±0.70
20M zinc + SDF	18.96±1.08

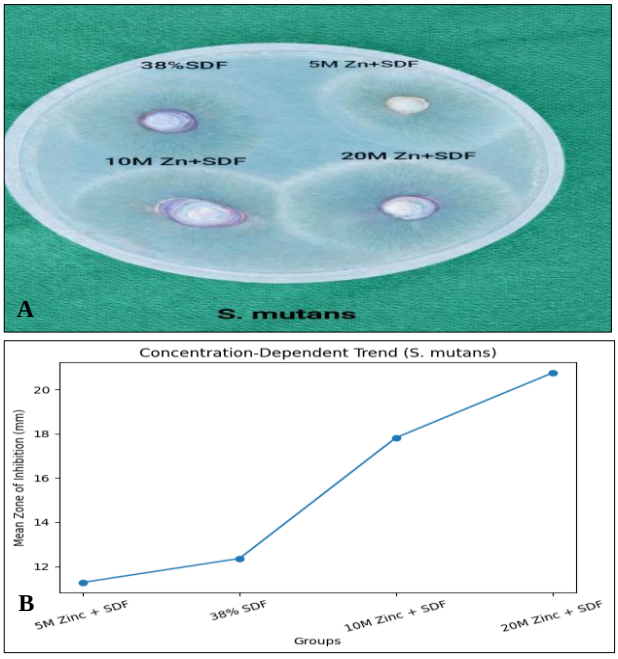


Figure 3: Antimicrobial efficacy of 38% SDF and zinc-enhanced SDF against *Streptococcus mutans*; (A) Representative agar disc diffusion plate showing zones of inhibition; and (B) graphical representation of the mean zone of inhibition.

Table 3: One-way ANOVA comparing antimicrobial activity among groups (n=10).

Microorganism	Statistical test	P value	Interpretation
S. mutans	One way ANOVA	0.001	Significant
E. faecalis	One way ANOVA	0.002	Significant

Statistical analysis

One-way analysis of variance (ANOVA) demonstrated statistically significant differences among the four groups for both microorganisms. The differences in the mean zone of inhibition were significant for *S. mutans* (p = 0.001) and *E. faecalis* (p=0.002), indicating that zinc concentration significantly influenced the antimicrobial efficacy of SDF (Table 3).

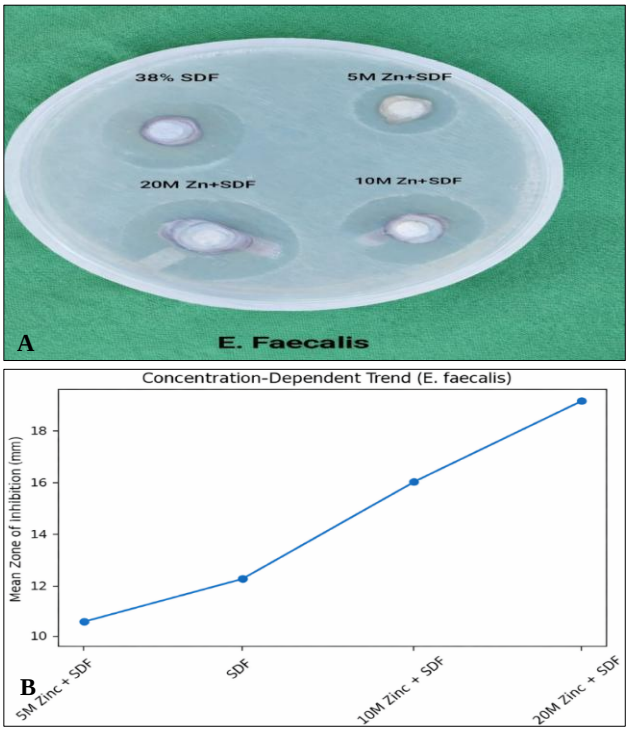


Figure 4: Antimicrobial efficacy of 38% SDF and zinc-enhanced SDF against *Enterococcus faecalis*; (A) representative agar disc diffusion plate showing zones of inhibition; and (B) graphical representation of the mean zone of inhibition.

DISCUSSION

The present *in vitro* study evaluated the antimicrobial efficacy of zinc-enhanced SDF against *Streptococcus mutans* and *Enterococcus faecalis*. The findings demonstrated a concentration-dependent increase in antimicrobial activity following zinc incorporation, with 20 M zinc-enhanced SDF demonstrating the largest zone of inhibition against both microorganisms. These findings suggest that zinc incorporation enhances the antimicrobial performance of conventional SDF. Previous studies have established SDF as an effective caries-arresting agent because of its broad-spectrum antibacterial activity and remineralization potential.[5-7] The antibacterial effect of SDF is primarily attributed to silver ions, which disrupt bacterial cell walls, denature proteins, and interfere with DNA replication, while fluoride ions promote remineralization and inhibit further demineralization.[6] The penetration and retention of silver ions within dentinal tubules further contribute to its sustained antibacterial activity.[7]

The enhanced antimicrobial activity observed after zinc incorporation may be explained by the complementary antibacterial properties of zinc. Zinc has been reported to inhibit bacterial metabolism by reducing acid production, impair bacterial enzyme activity, disrupt membrane integrity, and inhibit biofilm formation.[8,9] Several studies have also demonstrated the antibacterial activity of zinc-based compounds against oral pathogens, supporting their role in enhancing antimicrobial efficacy.[15,16] When combined with silver ions, these mechanisms may produce a synergistic antimicrobial effect, thereby enhancing the antibacterial efficacy of SDF.[10] The improved inhibition against *Streptococcus mutans*, the primary cariogenic microorganism responsible for biofilm formation and caries initiation, suggests that zinc-enhanced SDF may improve caries prevention and arrest.[3] Similarly, *Enterococcus faecalis* is recognized as a resistant microorganism frequently associated with persistent endodontic infections.[4] The increased inhibition observed against *E. faecalis* in the present study indicates that zinc-enhanced SDF may have potential applications against resistant oral pathogens. In addition to improving antimicrobial efficacy, zinc incorporation may also help overcome one of the major limitations of SDF, namely tooth discoloration. Previous studies have reported that SDF-induced staining adversely affects patient acceptance, whereas recent evidence suggests that zinc incorporation may reduce discoloration while maintaining antimicrobial efficacy.[12,13,17] Furthermore, zinc deposition within dentinal tissues may contribute to enhanced remineralization and prolonged antibacterial effects.[14,18]

Limitations

Despite these promising findings, the present study has certain limitations. As an *in vitro* investigation, it does not completely replicate the complex oral environment, including the influence of saliva, host immune responses, and multispecies biofilms. Furthermore, only antimicrobial activity was evaluated using the agar disc diffusion method, without assessing remineralization potential, mechanical properties, or long-term ion release. Therefore, further in vivo studies and randomized clinical trials are required to validate these findings and establish the long-term clinical effectiveness and safety of zinc-enhanced SDF.

CONCLUSION

Within the limitations of this *in vitro* study, zinc incorporation into 38% SDF significantly enhanced its antimicrobial efficacy against *Streptococcus mutans* and *Enterococcus faecalis*. A concentration-dependent increase in antimicrobial activity was observed, with higher zinc concentrations demonstrating greater inhibitory effects than conventional SDF. These findings suggest that zinc-enhanced SDF may represent a

promising modification for improving the antibacterial performance of conventional SDF in caries management. However, further *in vivo* studies and randomized clinical trials are required to confirm its long-term clinical effectiveness and safety.

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