

Assessing the Impact of Silver Diamine Fluoride (SDF) on Periodontal Pathogens in an In Vitro Study

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INTRODUCTION

Early Childhood Caries (ECC), commonly known as baby bottle tooth decay, is a dental condition that primarily affects the teeth of infants and young children. ECC emerges when the teeth, particularly the primary ones, undergo rapid decay, leading to dental problems in the early years of life (Kim and Kim, 2023). This condition is often linked to prolonged exposure to sugary substances, inadequate oral hygiene practices, and a lack of routine dental care. Addressing ECC involves preventive measures, emphasizing the importance of early dental interventions and promoting proper oral hygiene habits in young children (Li et al., 2023).

Silver Diamine Fluoride (SDF) has recently emerged as a potential solution for addressing dental caries and, notably, periodontal diseases. SDF is a topical antimicrobial agent traditionally used to halt dental caries by leveraging its antibacterial properties and its capacity to facilitate tooth remineralization (Okuwaki et al., 2021). While its effectiveness against cariogenic bacteria is well-established, a growing interest exists in investigating its influence on periodontal pathogens.

This pilot in vitro study aims to explore the impact of SDF exposure on several dental caries causing bacteria that are frequently associated with dental caries. We want to demonstrate the antibacterial and cytotoxic efficacy of SDF against these pathogens through carefully controlled laboratory research and contribute to the expanding body of knowledge regarding its application in maintaining oral health (Okuwaki et al., 2021; Joshi et al., 2023).

The main objective of this study is to provide informative data regarding the potential use of SDF as an antibacterial agent in the management of dental caries. By examining its effect on periodontal bacteria in an in vitro context, we may be able to gain some understanding of the potential of this treatment strategy in managing and preventing caries, thereby enhancing patient well-being and dental health.

MATERIALS AND METHODS

Antimicrobial activity

The antimicrobial activity of SDF gel was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15-20 minutes. After sterilization, the medium was poured on to the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*Streptococcus mutans*, *Lactobacillus* sp, *E. faecalis*, *Candida albicans*) was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations (25 µg, 50 µg, 100 µg) of CuO NPs. An antibiotic (e.g., Bacteria-Amoxyrite, Fungi- Flucanazole) was used as a standard. The plates were incubated at 37°C for 24 hours and 48 hours for fungal cultures. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters (mm) and the zone of inhibition was calculated.

Time kill curve assay

A 1 mL aliquot of the bacterial and fungal suspension (*Streptococcus mutans*, *Lactobacillus* sp, *E. faecalis*, *Candida albicans*) was added to 9 mL of Mueller Hinton broth containing the SDF gel at a concentration of 25 µg, 50 µg, 100 µg. The final microbial concentration was approximately 106 CFU/mL. The mixture was then incubated at 37°C with shaking at 200 rpm for varied time intervals (0, 4, 6, 8, 10, 12, & 24hr). Then the percentage of dead cells is calculated at wavelength of 600nm at regular time intervals.

RESULTS

Antimicrobial activity of SDF gel

Organisms	25 µg/mL	50 µg/mL	100 µg/mL
<i>S. mutans</i>	28	31	34
<i>Lactobacillus sp</i>	24	26	28
<i>E. faecalis</i>	23	25	30
<i>C. albicans</i>	33	38	41

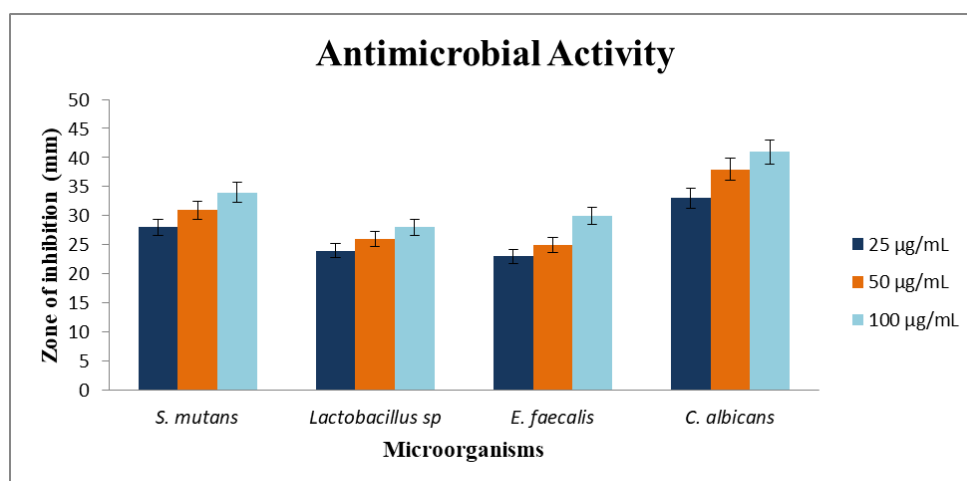


Figure 1: The antimicrobial activity of Silver Diamine Fluoride (SDF) gel against selected oral microorganisms is presented in terms of zone of inhibition at concentrations of 25, 50, and 100 µg/mL. A clear concentration-dependent increase in antimicrobial efficacy was observed across all tested organisms. Among them, *Candida albicans* demonstrated the highest susceptibility, exhibiting the largest zones of inhibition at all concentrations (33–41 mm). *Streptococcus mutans* showed moderate sensitivity, while *Lactobacillus* species and *Enterococcus faecalis* exhibited comparatively smaller zones, indicating relatively lower susceptibility. These findings suggest that SDF gel possesses broad-spectrum antimicrobial activity, with greater effectiveness against fungal organisms.

Time kill curve assay

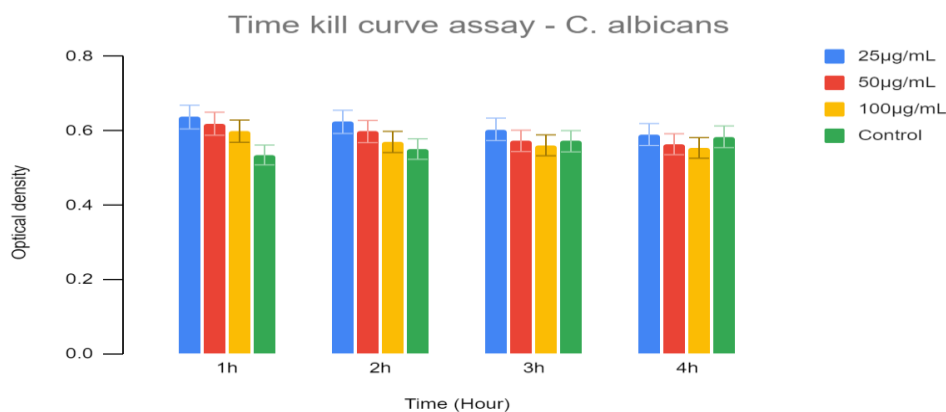


Figure 2: The time-kill curve assay for *Candida albicans* demonstrated a progressive reduction in optical density with increasing concentrations of SDF gel over a 4-hour period. The highest concentration (100 µg/mL) exhibited the most significant inhibitory effect, indicating rapid antifungal activity. In contrast, the control group showed a steady increase in optical density, reflecting uninhibited growth. These findings confirm the time- and dose-dependent antifungal efficacy of SDF gel.

Time kill curve assay

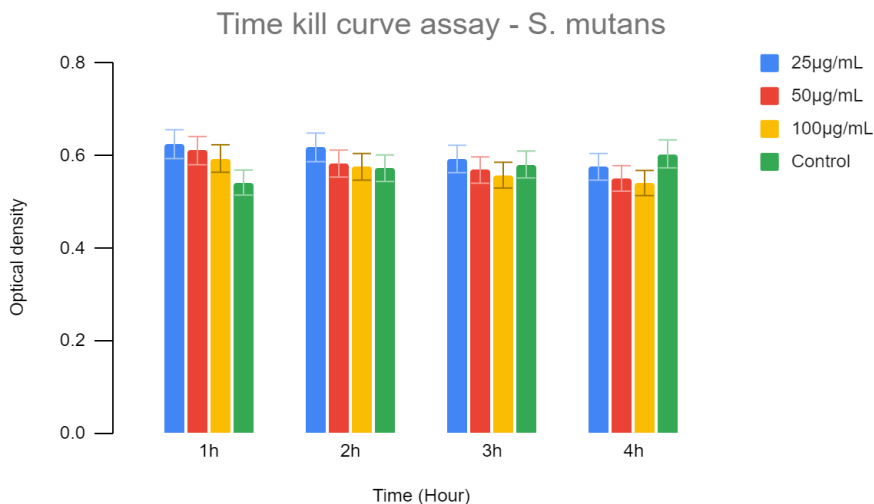


Figure 3: The time-kill assay for *Streptococcus mutans* revealed a concentration-dependent decline in optical density over time, indicating effective bacterial inhibition by SDF gel. The 100 µg/mL concentration demonstrated the most pronounced reduction in bacterial growth across all time intervals. In contrast, the control group maintained higher optical density values, signifying continuous growth. These results highlight the potent antibacterial activity of SDF gel against cariogenic bacteria.

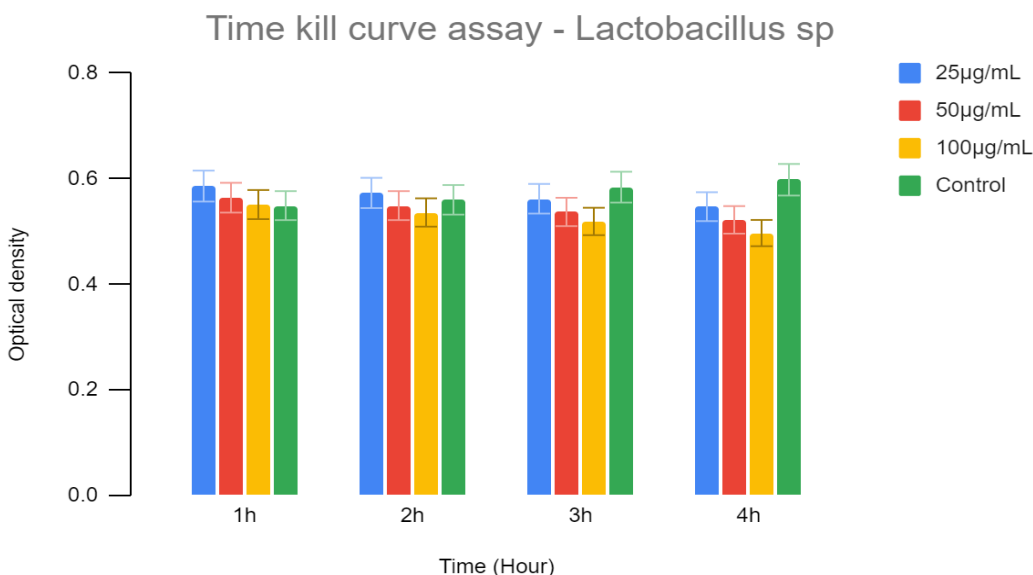


Figure 4: The time-kill curve analysis for *Lactobacillus sp.* showed a gradual decrease in optical density with increasing concentrations of SDF gel over the 4-hour observation period. The highest concentration (100 µg/mL) exhibited the greatest inhibitory effect, suggesting strong antibacterial activity. The control group demonstrated comparatively higher optical density values, indicating active bacterial growth. These findings support the effectiveness of SDF gel in inhibiting acidogenic bacteria associated with dental caries.

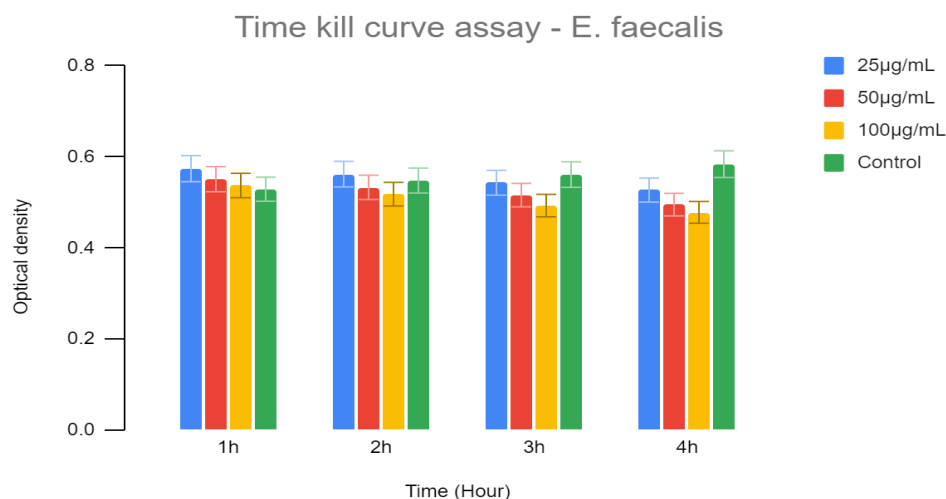


Figure 5: The time-kill assay for *Enterococcus faecalis* demonstrated a moderate reduction in optical density with increasing concentrations of SDF gel over time. Although all tested concentrations exhibited antibacterial effects, the reduction was less pronounced compared to other microorganisms. The highest concentration (100 µg/mL) showed the greatest inhibitory activity, while the control group maintained higher growth levels. These results suggest that *E. faecalis* is relatively more resistant but still susceptible to SDF in a dose-dependent manner.

Effects of SDF on bacterial growth



Figure 6: The agar well diffusion assay images illustrate the antimicrobial effect of SDF gel against different microorganisms. Distinct zones of inhibition were observed surrounding the wells containing SDF at concentrations of 25, 50, and 100 µg/mL. The size of the inhibition zones increased with concentration, with 100 µg/mL producing the largest zones, indicating maximum antimicrobial efficacy. These visual findings corroborate the quantitative results, confirming the dose-dependent antibacterial and antifungal activity of SDF gel.

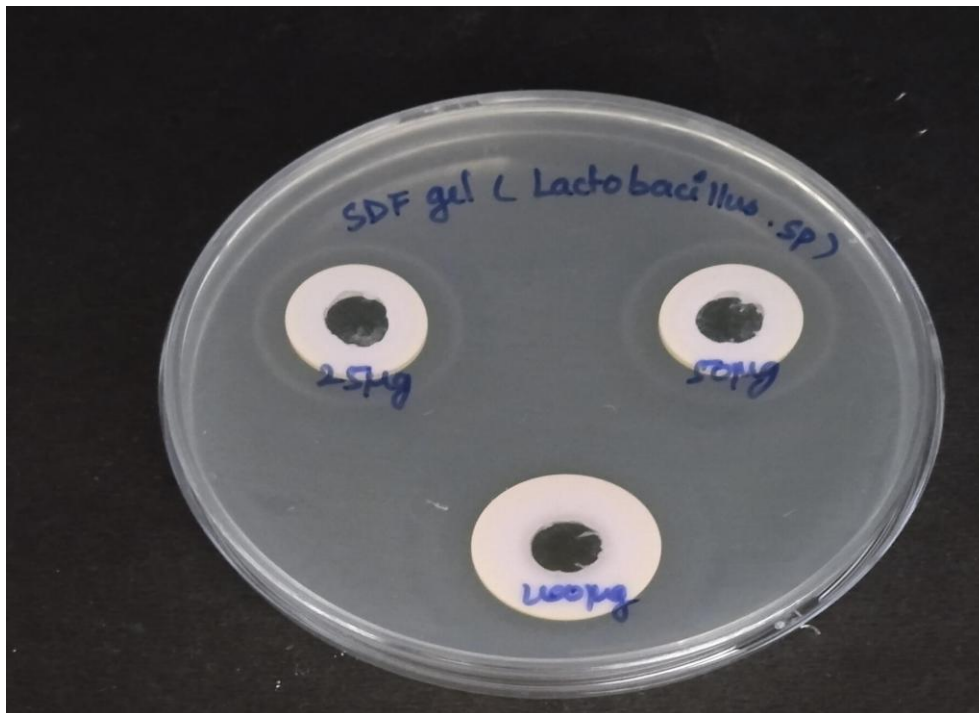


Figure 7: The agar diffusion assay for *Lactobacillus* sp. demonstrated clear zones of inhibition around discs containing SDF gel at concentrations of 25, 50, and 100 µg/mL. The diameter of the inhibition zones increased progressively with concentration, with 100 µg/mL showing the largest zone, indicating maximum antibacterial activity. These findings confirm the dose-dependent inhibitory effect of SDF gel against *Lactobacillus* sp., a microorganism implicated in the progression of dental caries.

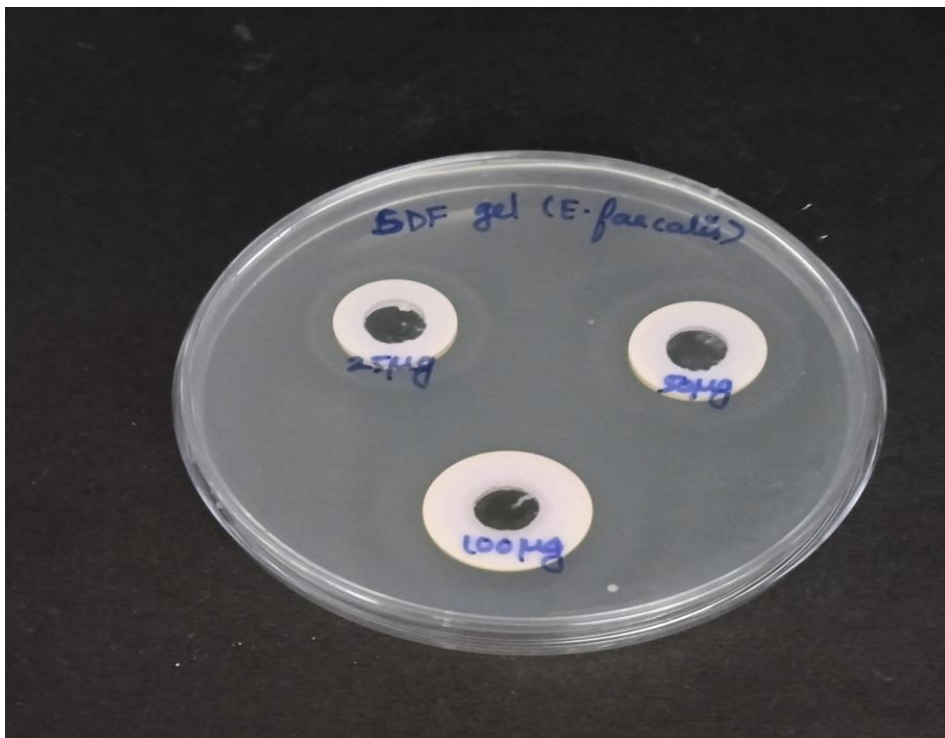


Figure 8: The agar diffusion assay for *Enterococcus faecalis* revealed the formation of inhibition zones around SDF gel discs at all tested concentrations. Although the zones were smaller compared to other tested microorganisms, a

gradual increase in diameter was observed with increasing concentration, with 100 µg/mL showing the highest inhibitory effect. These results suggest that *E. faecalis* exhibits relative resistance but remains susceptible to SDF gel in a concentration-dependent manner.

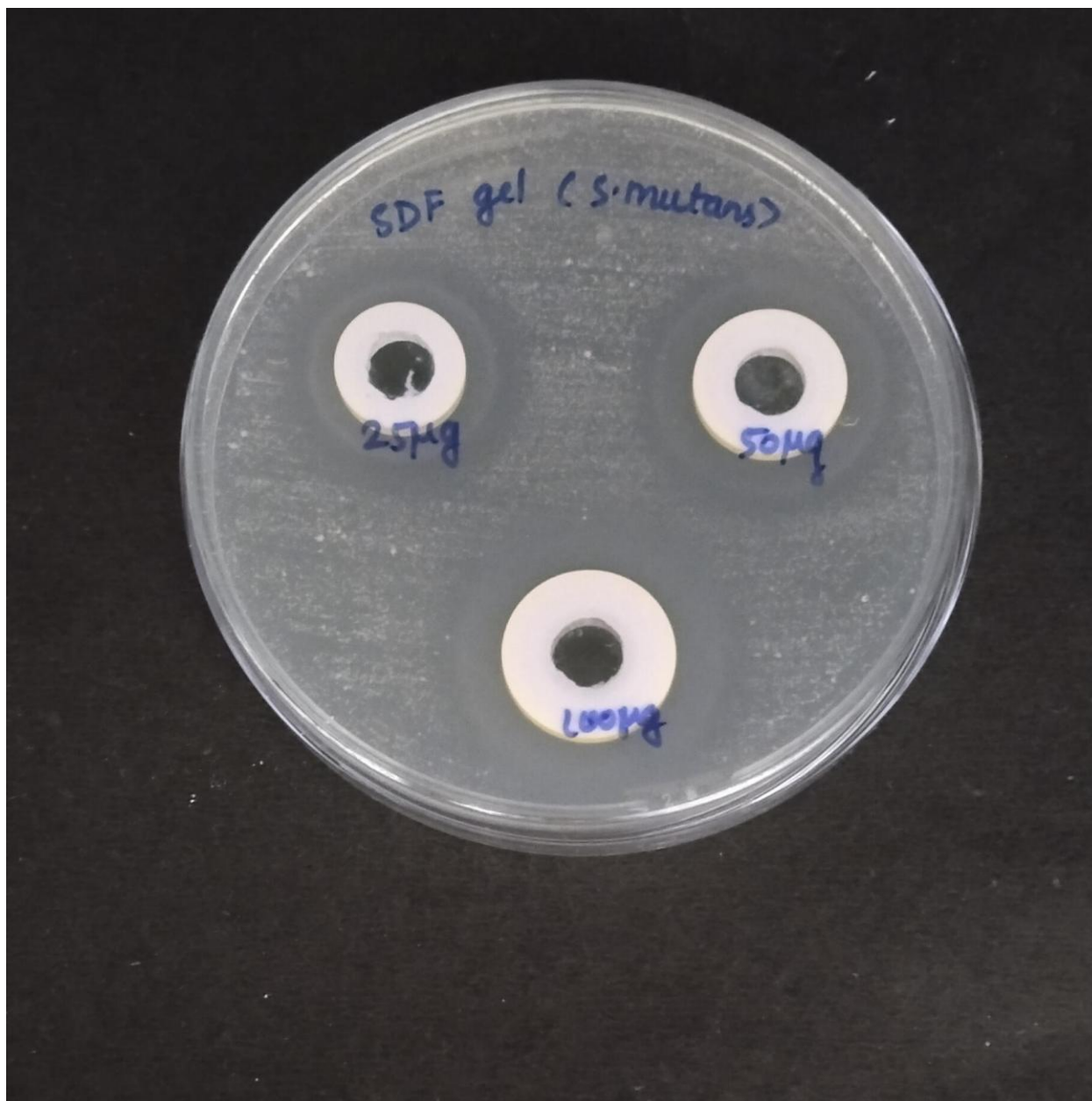


Figure 9: The agar diffusion assay for *Streptococcus mutans* showed distinct zones of inhibition around discs containing SDF gel at concentrations of 25, 50, and 100 µg/mL. A concentration-dependent increase in the diameter of inhibition zones was observed, with the highest concentration exhibiting the greatest antibacterial effect. Compared to *E. faecalis*, *S. mutans* demonstrated greater susceptibility to SDF gel, highlighting its effectiveness against primary cariogenic bacteria.

DISCUSSION

The use of Silver Diamine Fluoride (SDF) in dentistry has primarily been associated with arresting dental caries. However,

recent interest has emerged in exploring its potential application in managing dental caries. In this discussion, we delve into the findings of our in vitro pilot study and consider the implications of SDF on caries causing pathogens (Bora, Saxena and Goswami, 2023).

Our study has demonstrated varying effects of SDF on carious pathogens. It is crucial to note that these effects can be pathogen-specific. While SDF exhibited antibacterial properties against some pathogens, its impact on others appeared less pronounced. For instance, *Streptococcus mutans*, known for its role in caries, exhibited reduced viability when exposed to SDF. This suggests that SDF may have potential as an antimicrobial agent against this pathogen (Ito, 2017; Bora, Saxena and Goswami, 2023).

Microscopic examination revealed noteworthy morphological alterations in pathogens following SDF exposure. These changes could be indicative of cellular stress and damage. It is worth mentioning that the extent of these morphological changes varied among different pathogens. *Candida albicans* showed considerable morphological alterations, while *Lactobacillus* displayed fewer changes (Duangthip, 2017). The observed morphological effects might be associated with the antibacterial properties of SDF and its potential to disrupt the integrity of bacterial cell walls or membranes.

Caries causing pathogens often form biofilms, which can complicate their removal and contribute to the progression of caries. Our study also examined the influence of SDF on biofilm formation (Xu *et al.*, 2023). The results indicated that SDF had a varying impact on biofilm formation among different pathogens. For example, *C. albicans* biofilm formation was significantly inhibited, suggesting a potential role for SDF in disrupting these harmful bacterial communities.

The findings of our pilot study suggest that SDF may hold promise as an antimicrobial agent in the context of oral health (Abdellatif, El Kashlan and El Tantawi, 2023). Its ability to reduce the viability of caries causing pathogens and inhibit biofilm formation on certain pathogens may be leveraged to combat carious diseases. However, it is essential to emphasize that the effects of SDF were pathogen-dependent, and its efficacy may not be universal against all caries causing pathogens.

CONCLUSION

In conclusion, our study offers valuable insights into the potential of SDF as an antimicrobial agent against caries causing pathogens. While our findings are encouraging, more research is needed to determine the precise applications, concentrations, and safety profiles of SDF in the management of dental caries. Ultimately, SDF may become a valuable addition to the arsenal of tools used in the battle against caries and the promotion of oral health.

CONFLICT OF INTEREST

The article has been read and approved by all the contributors and there isn't any conflict of interest.

ACKNOWLEDGEMENT

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