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IN VITRO ANTIBACTERIAL ACTIVITY OF KEDO SDF SOLUTION VS SDF GEL AGAINST *ENTEROCOCCUS FAECALIS*

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Name of Author:
Srigopika T[1], Dr Lavanya Govindaraju[2]

Affiliation:[1] *Undergraduate student*
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences
Saveetha University,
Chennai-600077.
Email ID- 152001029.sdc@saveetha.com
[2]*Department of Paediatric and Preventive Dentistry,*
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai-600077.
E-mail: glaavuu@gmail.com

Corresponding Author:
Dr. Lavanya Govindaraju
Department of Paediatric and Preventive Dentistry,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai-600077.
E-mail: glaavuu@gmail.com

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Abstract: Background: Dental caries is the most common disease of the oral cavity; they are multifactorial in nature. The most common cause is the interaction between the dental plaque, the high sugar diet and the susceptibility of the host. Enterococcus faecalis is a facultative anaerobic gram-positive bacterium which has been frequently recovered from dental caries. Early Childhood Caries (ECC), is one of the most common chronic childhood diseases and is considered a worldwide health problem and prevention of this is of utmost concern. One recent advancement involves the usage of Silver Diamine Fluoride (SDF), which is a non-invasive, topical agent that has been shown to arrest caries progression in primary teeth by up to 80%, making it particularly valuable for underserved populations and children with dental anxiety or special healthcare needs.**Materials and methods** Antibacterial activity was assessed against Enterococcus faecalis, using Mueller–Hinton agar plate by agar well-diffusion technique. Inoculum containing 106 cfu/mL of the freshly prepared bacterial culture was spread onto the MHA plates. Two wells (9 mm) were punched into the agar plate medium and filled with different concentrations (100 µg, and 25 µg). After incubation, the diameter of the ZOI was recorded in each plate. The results were expressed as mean value with standard deviation. Statistical analysis was performed to compare the antibacterial efficacy of the two delivery systems. Statistical significance was set at $p < 0.05$. All calculations were performed using SPSS 23.0 software.**Results** As for antibacterial activity in SDF solution group, ZOI ranged from 32 mm to 34 mm, with a calculated mean of 32.8 ± 0.84 mm, whereas the SDF gel group produced zones of inhibition ranging from 27 mm to 30 mm, with a mean value of 28.4 ± 1.14 mm. The antibacterial activity of SDF solution was found to be significantly greater than that of SDF gel with a p-value of 0.00012 ($p < 0.001$).**Conclusion** Kedo SDF solution showed better antibacterial properties against E. faecalis.

Keywords: Antibacterial activity, Early childhood caries, Enterococcus faecalis, Innovation, In vitro , Silver diamine fluoride, Sustainability

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INTRODUCTION

Dental caries is the most common disease of the oral cavity; they are multifactorial in nature. The most common cause for its occurrence is the interaction between the dental plaque, the high sugar diet and the susceptibility of the host [1]. Dental caries is a global public health challenge, specifically in low and middle income countries and it has been linked to underlying socioeconomic and social disadvantages [2]. Caries have adverse effects on both oral and general health. This has been noted by the dental community and public health authorities and it is well recognized as a disease in both permanent and deciduous teeth [3].

Enterococcus faecalis is a facultative anaerobic gram-positive bacterium which has been frequently recovered from secondary and persistent endodontic infections and dental caries [4]. The ability of E. faecalis to form dense biofilms on root canal walls is attributed to its biofilm-associated pili (Ebp) and its collagen-binding protein (Ace), making this microorganism invade dentinal tubules and root canal complexities [5]. This microorganism is known for its high cariogenic potential, acidogenicity, and acid tolerance. It metabolizes carbohydrates and produces acids, thereby demineralizing the tooth structure. If left untreated, carious lesions penetrate the dentin and reach the pulp, leading to endodontic infections that cause pulpal and periapical alterations [6] .

Apart from dental caries in adults, another important sub division includes Early Childhood Caries (ECC), which is also one of the most common chronic childhood diseases and is considered a worldwide health problem [7]. Early Childhood Caries (ECC) is defined as the presence of one or more decayed, non-cavitated or cavitated lesions, missing or filled due to caries surfaces, in any primary tooth of a child under six years of age [8]. This leads to pain, infection and its advancement leads to pulpal involvement and this eventually forms dental abscess [9]. If not treated at the early stage, it might even lead to loss of the tooth structure [10].

For dental caries, as well as ECC, it is crucial that the progression is cut short in the initial stages. leading to improvement in the oral hygiene of the patient. Although conventional methods such as restorations using amalgam or composite and root canal therapy exist, these methods are invasive and are the last resort to save the tooth. By application of fluorides and other gels, the treatment becomes less invasive and time consuming and the majority of the tooth structure can be saved [11].

Prevention of caries with the help of fluoride administration in a great variety of forms has been extensively studied in the past, [12] and one recent advancement involves the usage of Silver Diamine Fluoride (SDF). Countries such as Argentina, Australia, Brazil, China and Japan have used silver diamine fluoride (SDF), which is generally used as a 38% solution, as an anti-caries agent [13] and is effective in arresting cavitated carious lesions and prevents development of new caries [14] [15]. In 2014, SDF was approved by the US Food and Drug Administration as a treatment for dentinal sensitivity and from then on, it has been used extensively in preventive dentistry [16].

SDF is a colorless solution with a pH value between 9 and 10 [17] and when silver combines with fluoride in an ammonia solution, silver, which is anti-bacterial in nature, is released along with fluoride ions. Bacterial resistance against silver is hard to develop because silver kills bacteria through multiple pathways and it interferes with bacterial metabolism [18]. SDF is a non-invasive, cost-effective topical agent that has been shown to arrest caries progression in primary teeth by up to 80%, making it particularly valuable for underserved populations and children with dental anxiety or special healthcare needs [19].

SDF has better stability and less oxidizing effect in the diamine-silver complex compared to the silver fluoride, and the equilibrium lies within the diamine-silver ion [20]. For this reason, SDF is generally used in high concentrations (38%) as a means of arresting and preventing the progression of dental caries [21]. In vitro studies have demonstrated that SDF has antibacterial properties, has the potential to increase the pH of biofilm, decrease dentin demineralization, and exhibit antimicrobial action against cariogenic bacteria, thereby terminating its growth [22].

Fluoride works as a remineralisation agent reacting with hydroxyapatite to form fluoride hydroxyapatite via ion exchange [23] . Fluoride-substituted hydroxyapatite is more acid-resistant than hydroxyapatite and is thus resilient to acid attack [24]. Furthermore, fluoride can precipitate as calcium fluoride and it is said that it can act as a depot and release fluoride ions under an acidic environment to form fluorohydroxyapatite steadily. Fluoride at a higher concentration also has antimicrobial effects on cariogenic bacteria [25]. Thus, the aim of this study is to compare the in vitro antibacterial activity of kedo SDF gel and solution against E. faecalis.

Materials and Methods

Antibacterial activity of the gel and solution sample was assessed against Gram positive bacteria Enterococcus faecalis, using Mueller–Hinton agar (MHA, Himedia, Mumbai, India) plate by agar well-diffusion technique. The plates were prepared in double distilled water (pH 7.0) and sterilized in an autoclave at 121 ˚C for 15 min. Then, the sterilized MHA agar was poured into the petri plate and allowed to solidify at room temperature in laminar flow. Inoculum containing 106 cfu/mL of the freshly prepared bacterial culture was spread onto the MHA plates with a sterile cotton swab moistened with the suspension of the respective microbial culture. Then, two wells (9 mm in diameter) were punched into the agar plate medium and filled with different concentrations (100 µg, and 25 µg) of compound with the help of micropipette, and kept at room temperature for 4 hours to diffuse the gel and liquid sample into the medium. Then, the culture plates were incubated at 37 °C for 24 hours. After incubation, the diameter (mm) of the zone of inhibition was recorded in each plate. The results were expressed as mean value with standard deviation (SD).

Methodology for statistical analysis

The data collected from the five replicates of each group (Kedo SDF solution and SDF gel) were tabulated. Statistical analysis was performed to compare the antibacterial efficacy of the two delivery systems against Enterococcus faecalis. Data was analyzed to compare the mean zones of inhibition between the liquid and gel groups. Statistical significance was set at $p < 0.05$. All calculations were performed using SPSS 23.0 software.

Results

Figure 1 shows the antibacterial activity of Kedo SDF solution and SDF gel against Enterococcus faecalis was evaluated by measuring the zones of inhibition (ZOI) across five experimental replicates. Both formulations demonstrated distinct and measurable antibacterial properties. The Kedo SDF solution consistently exhibited larger zones of inhibition compared to the SDF gel. Figure 2 shows the zones of inhibition where it can be observed that for the SDF liquid group, ZOI ranged from 32 mm to 34 mm, with a calculated mean of 32.8 ± 0.84 mm, whereas the SDF gel group produced zones of inhibition ranging from 27 mm to 30 mm, with a mean value of 28.4 ± 1.14 mm. SDF solution formulation showed a statistically superior inhibitory effect (based on the higher mean) compared to the gel. This difference in efficacy may be attributed to the lower viscosity of the solution , potentially allowing for faster and more extensive diffusion of the silver and fluoride ions through the agar medium. Figure 3 shows the results of the independent t - test, where it can be observed that the antibacterial activity of SDF solution was found to be significantly greater than that of SDF gel with a p-value of 0.00012 ($p < 0.001$). These results suggest a superior inhibitory effect of the Kedo SDF solution over the SDF gel against *E. faecalis.*

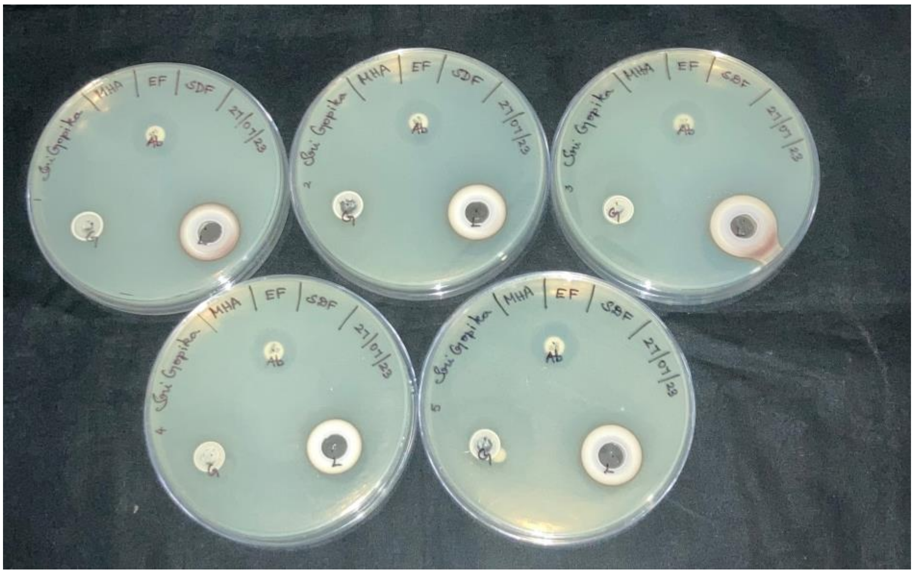

Figure 1 - Antibacterial activity (ZOI) of SDF solution and SDF gel against *E. faecalis*

	Gel	Liquid
	27	32
	28	33
	30	34
	29	32
	28	33
Mean	28.4	32.8
SD	1.140175425	0.836660027
Mean ±SD	28.4 ±1.14	32.8 ±0.84

Figure 2 - Zone of Inhibition of SDF gel and SDF solution

Study group	**ZOI (in mm)**	**P value**
SDF solution	32.8 ± 0.84 mm	0.00012
SDF gel	28.4 ± 1.14	

Figure 3 - Statistical analysis of SDF gel and SDF solution

DISCUSSION

Enterococcus faecalis is a gram positive facultative anaerobic bacteria associated with endodontic infections and periapical periodontitis and implicated in reinfection of endodontically treated root canals [26]. In a recent study conducted in 2022, it was observed that, apart from preventing caries progression, SDF may be effective against E. faecalis and can be used as an intracanal medicament and/or irrigant to prevent reinfection of the root canals and improve the outcomes of endodontic treatment [27].

In a study that wanted to check the efficacy of SDF in arresting cavitated caries lesions in primary molars, it was noted that SDF application was found to be overall effective in arresting caries progression (ES = 0.35, $p < 0.01$). In a total of 622 arrested lesions, out of 1205 considered, the caries arrest rate was 51.62% ± 27.40% using SDF ≥ 38% applied annually or biannually [28].

Another study concluded that SDF + NaF had significantly greater caries arrest effect than SDF alone ($p = 0.03$) with significant difference ($p = 0.03$) between moderate ($p = 0.005$) and advanced ($p = 0.08$) lesions [29]. While comparing the effectiveness of using SDF or ART in arresting caries lesions, it was noted that out the 118 participants who received treatment (SDF, n = 59; ART, n = 59), after 12-month follow-up, 91.5% of caries lesions in the SDF and 90.2% in the ART group were arrested ($p = 1.000$). After 24 months, 72% were arrested in the SDF group and 95.2% in the ART group [30].

The MIC and MBC of SDF for S. mutans are lower than that of silver ammonium nitrate and sodium fluoride, showing that SDF is more effective compared the two in inhibiting bacterial growth. SDF also possesses strong antimicrobial action against S. mutans, A. naeslundii and L. acidophilus [31], as well as E. faecalis biofilm on root canals [32]. Studies have also observed that SDF works against a wide variety of bacteria as well. One such study noted that both gel and solution forms exhibit antimicrobial efficacy against Streptococcus mutans and Lactobacillus acidophilus and that, the incorporation of potassium iodide (KI) significantly enhances the antimicrobial activity of SDF [15].

As for the frequency of SDF application, a randomised control trial confirmed from their observations that semiannual application of SDF is a highly effective and practical method for managing early and moderate caries in primary teeth, particularly in uncooperative children with ECC [33]. SDF, apart from working as an antibacterial agent, a study has also proven its antifungal activity. In a study, it was observed that disc-diffusion and time-kill assays as well as MIC50 and MIC90 evaluations against therapeutic concentrations confirmed the broad-spectrum anti-candidal potency of SDF. SDF appears to effectively abrogate filamentation of C. albicans even at very low concentrations [34].

In a recent study conducted in 2023, while comparing the antibacterial activity of silver diamine fluoride liquid and gel against organisms causing dental caries, the anticariogenic activity of SDF preparations were similar and not statistically significant. Overall performance of FAgamin SDF solution was better than the other two. It was noted that against

S.mutans, Kidz-e SDF solution performed well in 25 µl and 50 µl preparations and Fagamin SDF solution was better in 100 µl concentration. Similar results were obtained against Lactobacilli i.e, Kidz-e SDF solution performed well in 25 µl and 50 µl preparations and Fagamin SDF solution was better in 100 µl concentration [35].

CONCLUSION

From this study, it can be concluded that Kedo SDF solution showed better antibacterial properties against *E. faecalis*. However, in vivo studies also have to be carried out in order to understand the full extent of its activity and its limitations as well.

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Conflict of Interest

Nil.

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