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Comparative evaluation of antimicrobial efficacy of three different brands of Silver diamine fluoride when combined with potassium iodide and glutathione against Streptococcus mutans and Lactobacillus acidophilus: An in vitro study

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ABSTRACT:
Silver diamine fluoride effectively treats dental caries by forming a protective layer on decayed surfaces, increasing mineral density and hardness, inhibiting enzyme activity, and directly attacking bacteria, but it can cause tooth discoloration, prompting research into additives like potassium iodide and glutathione to reduce staining.
Aim: *This in vitro study aimed to address the limited research available on the potential impact of adding KI and GSH to SDF by exploring their antimicrobial efficacy against Streptococcus mutans and Lactobacillus acidophilus. In addition, the study will investigate and compare the antimicrobial effects of various SDF brands, namely Advantage Arrest, Kids e-SDF, and FAgamin.*
Method: *Disk-diffusion susceptibility tests were carried out on Mueller–Hinton and sheep blood agar plates inoculated with Streptococcus mutans and Lactobacillus acidophilus, respectively—organisms recognized as cariogenic. Filter paper disks, approximately 6mm in diameter, containing 10 µL of the test solutions were positioned on the agar surface. Subsequently, the plates were anaerobically incubated at 37°C for 48 hours, and the resulting zones of inhibition were measured using a Vernier caliper. The data collected was entered in Microsoft excel, cleaned and subjected to analysis of variance (ANOVA) [SPSS 22.0, IBM Analytics, New York, U.S.A]. Pairwise p value calculated using Tukey's post hoc test.*
Results: *Showed GSH groups exhibited the highest antimicrobial efficacy, followed by SDF and KI groups. Advantage Arrest brand showed the most promising results upon comparison with other brands.*
Conclusion: *We concluded that incorporation of glutathione enhanced the antibacterial property of conventional SDF.*
Keywords*: Silver diamine fluoride; glutathione; Potassium iodide; antibacterial.*
Highlights:

- *This paper underscores the efficacy of a new method, incorporating glutathione, to mitigate tooth discoloration following the application of Silver Diamine Fluoride.*
- *Comparison of the antimicrobial efficacy of this newer method with potassium iodide and conventional Silver diamine fluoride against.*
- *Comparative analysis of readily accessible commercial Silver Diamine Fluoride (SDF) brands.*

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I. Introduction:

Oral health is fundamental to overall well-being, and dental caries, a widespread and multifactorial disease, arises from imbalances in the processes of tooth demineralization and remineralization. In 2020, global prevalence rates surged to 46.2% and 53.8% in primary and permanent teeth, respectively, impacting over 530 million children worldwide.[1] India's diverse practices, social dynamics, and imbalanced oral healthcare workforce significantly contribute to the prevalence of dental caries.[2] Pathogenic microorganisms, particularly Streptococcus mutans alongside Lactobacillus, play a crucial role in the disease's development and progression.[3] According to J. van Houte, S. mutans metabolizes sugar to produce lactic acid, demineralizing tooth structure,

while Lactobacilli exhibit high acid tolerance and acidogenicity in an acid milieu.[4] Over the last century, dental caries management has evolved from traditional surgical and irreversible approaches like the G.V. Black cavity design to the contemporary Minimal Intervention Dentistry (MID), focusing on preventing progression, maintaining pulp vitality by incorporating the principles of prevention, remineralization, and minimal intervention.[5]

In 1969, Nishino et al. attributed to this approach by describing the caries-arresting properties of silver diamine fluoride (SDF). Recognized for its non-invasiveness, cost-effectiveness, and user-friendly application, SDF serves as an effective agent for arresting caries, particularly beneficial for high-risk groups.[6] Initially approved for dentinal hypersensitivity in 2014, SDF recently gained approval (code D1354) as an improvised caries arresting medicament.[7] The synergistic antibacterial effects of silver and remineralizing properties of fluoride in SDF make it a promising therapeutic agent for managing carious lesions, especially in young children and individuals with special health care needs.

The sterilizing effect of silver in the form of an ammoniacal silver nitrate solution on infected coronal and root dentin was reported as early as 1917 by Howe et al.[8] In recent years, the bactericidal and antifungal properties of silver diamine fluoride (SDF) have been well documented, with studies showing a significant reduction in the growth of Streptococcus mutans and Lactobacillus acidophilus, as well as the inhibition of their adherence to tooth surfaces.[9-13] The potential mechanism of action can be attributed to its strong inhibitory effect on the proteolytic activity of enzymes such as matrix metalloproteinases and cathepsins.[14,15] The bacteria destroyed by silver have high biocidal action toward active microorganisms. This phenomenon is termed as the ―zombie effect‖.[16] Silver diamine fluoride at a concentration of 38% is more effective in arresting active caries in primary teeth.[17]

While the application of Silver Diamine Fluoride (SDF) doesn't lead to sudden systemic issues, staining in arrested carious lesions is a common observation, linked to higher SDF concentrations and application frequency. To address discoloration, applying a saturated potassium iodide (KI) solution after SDF has been reported to be effective.[18] Glutathione (GSH), an antioxidant and metal chelator used to coat silver particles for improved interaction with complex bio-systems and enhance water solubility, has shown promise in minimizing tooth discoloration after SDF application in in vitro studies.[19-22]

To our knowledge, there are no previously published reports in the literature regarding the comparison of the antimicrobial efficacy of three brands of Silver Diamine Fluoride with Potassium iodide and Glutathione (20% w/v) against *Streptococcus mutans and Lactobacillus* acidophilus. The null hypothesis of the present study was that potassium iodide and gluthatione have no effect on the antibacterial properties of SDF.

II. Materials And Method:

Ethical approval was obtained from Institutional Ethical Committee, M.C.E. Society, Azam Campus, Pune (dated January 28, 2021, reference number: MCES/EC/6512021) in accordance with the Code of Ethics (Declaration of Helsinki). This study was conducted following the CRIS Guidelines (Checklist for Reporting In-vitro Studies).

Preparation of material

In this study, three different brands of SDF (Advantage Arrest, e-SDF & FAgamin) were used as controls (Table no. 1). In the experimental group, the following test materials were added to the control groups:

a. Potassium iodide - saturated solution
b. 20% Glutathione

Table no. 1: Details of the materials used

Code	Materials	Composition	Manufacturer
SDF - A	Advantage Arrest	38% Silver diamine fluoride	Elevate Oral Care, LLC, USA

SDF - B	e-SDF	38% Silver diamine fluoride	Kids e Dental LLP, India
SDF- C	FAgamin	38% Silver diamine fluoride	Tedequim, Argentina
KI	Potassium Iodide	White, crystalline powder of KI	Sisco Research Laboratories Pvt. Ltd.
GSH	Glutathione	Glutathione reduced	Sisco Research Laboratories Pvt. Ltd.

Therefore, this study consisted of nine groups as follows:

- Group 1- SDF-A; (Advantage arrest®)
- Group 2- SDF-A+KI;
- Group 3- SDF-A+GSH;
- Group 4- SDF-B; (Kids e-SDF®)
- Group 5- SDF-B+KI;
- Group 6- SDF-B+GSH;
- Group 7- SDF-C; (FAgamin®)
- Group 8- SDF-C +KI;
- Group 9- SDF-C+GSH.

Test organisms

Two microorganisms selected for the study were L. acidophilus (MTCC 10307) and S. mutans (MTCC 497) (Institute of Microbial Technology, Chandigarh, India). Inocula of S. mutans and L. acidophilus, two of the main organisms known to be involved in the caries process, were prepared from a 24-hour anaerobic incubation on blood- agar. Organisms were harvested to produce suspensions in sterile brain-heart infusion media to a MacFarland optical density of 0.5.

Antimicrobial efficacy using agar disk diffusion assay

The Mueller-Hinton and sheep blood agar was used to prepare the petri plates upon autoclaving and kept for solidifying for 10-15 minutes. 20 µl of the targeted test organism was dispersed on the agar plates (pH7) using lawn- deposition mode. Then, filter paper disks (about 6mm in diameter) containing 10 µl portion of the test solutions were placed on the agar surface. The petri dishes were incubated under anaerobic conditions for 48 hours for growth. The test solutions then diffuse into the agar and inhibits germination and growth of the microorganisms. Then, the zones of inhibition were recorded with Vernier calliper to nearest ½ mm.

Statistical analysis:

The data collected was entered in Microsoft excel and cleaned. The statistical analysis was done using ANOVA (SPSS 22.0, IBM Analytics, New York, U.S.A). All the p values less than 0.05 will be considered statistically significant and less than 0.001 will be considered highly significant. Pairwise p value calculated using Tukey's post hoc test.

III. Results:

The evaluation was performed among the groups and within the groups.

For both the microbes, i.e., S. mutans and Lactobacillus spp., upon intragroup comparison (SDF vs SDF+KI vs SDF+GSH) notably, a highly significant difference ($p < 0.001$) was observed within the subgroup treated with Silver Diamine Fluoride (SDF) in conjunction with glutathione, in stark contrast to groups subjected solely to SDF or SDF combined with potassium iodide (SDF+KI). This distinction in antimicrobial efficacy was consistent across all three brands, as comprehensively elucidated in the comparative data presented in both Table no. 2 and Table no. 3. However for Lactobacillus spp., a statistically significant difference ($p < 0.05$) was observed when comparing groups treated with Silver Diamine Fluoride (SDF) alone to those treated with SDF combined with potassium iodide (SDF+KI) across the three brands.

A comparative analysis of commercially available Silver Diamine Fluoride (SDF) brands highlighted that the groups incorporating Advantage Arrest exhibited a significantly larger zone of inhibition ($p < 0.001$), followed by Kids e-SDF and FAgamin.

Table no. 2: Mean values of zones of inhibition (in mm) & Intragroup comparison of three subgroups of different brands against *Streptococcus mutans.*

	Advantage Arrest Mean (SD)	KIDS E-SDF Mean (SD)	FAGAMIN Mean (SD)
Subgroup A (ONLY SDF)	17.8 (1.03)	14.6 (2.22)	12.3 (1.76)
Subgroup B (SDF+KI)	11.6 (1.26)	10.8 (1.87)	9.9 (1.52)
Subgroup C (SDF+GSH)	23.7 (1.63)	22.7 (2.0)	20.2 (1.54)
One way Anova F test	**F = 205.5**	**F = 88.98**	**F = 111.07**
P value, Significance (overall)	**p< 0.001****	**p< 0.001****	**p< 0.001****
SDF vs SDF+KI	**p<0.001****	**p = 0.001***	**p =0.007***
SDF vs SDF+GSH	**p<0.001****	**p<0.001****	**p<0.001****
SDF+KI vs SDF+GSH	**p<0.001****	**p<0.001****	**p<0.001****

*p<0.05-significant difference **p<0.001-highly significant difference
^ Pairwise p value calculated using Tukey's post hoc test

Table no. 3: Mean values of zones of inhibition (in mm) & Intragroup comparison of three subgroups of different brands against *Lactobacillus acidophillus.*

	Advantage Arrest Mean (SD)	KIDS E-SDF Mean (SD)	FAGAMIN Mean (SD)
Subgroup A (ONLY SDF)	19.25 (0.88)	16.7 (1.22)	14.9 (1.79)
Subgroup B (SDF+KI)	18.3 (1.27)	14.65 (3.67)	13.3 (1.39)
Subgroup C (SDF+GSH)	29.4 (0.87)	27.0 (3.74)	23.5 (3.24)
One way Anova F test	**F = 357.068**	**F = 45.291**	**F =57.626**
P value, Significance (overall)	**p< 0.001****	**p< 0.001****	**p< 0.001****
SDF vs SDF+KI	**p = 0.117**	**p = 0.319**	**p =0.278**
SDF vs SDF+GSH	**p<0.001****	**p<0.001****	**p<0.001****
SDF+KI vs SDF+GSH	**p<0.001****	**p<0.001****	**p<0.001****

*p<0.05-significant difference **p<0.001-highly significant difference
^ Pairwise p value calculated using Tukey's post hoc test

III. Discussion:

Streptococcus mutans and Lactobacillus are the two principal bacteria involved in the formation of caries. S. mutans has been deeply associated with the initiation of caries, while studies have shown that lactobacilli may be more important in the advancement of caries lesions and have been found to be responsible for the onset of a low percentage of coronal caries. Reducing these microorganisms causes a significant decrease in dental caries.[11]

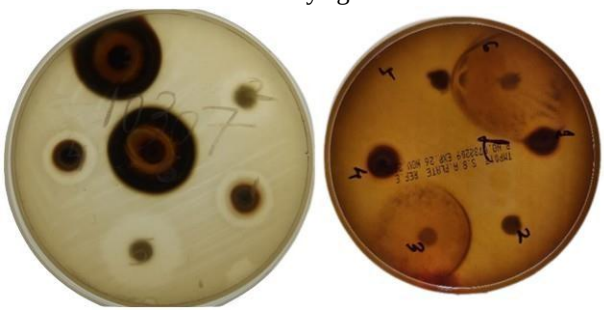
Following topical application of 38%-SDF, attachment of silver particles to biofilm-forming S. mutans resulted in tooth discoloration, growth-retardation, rupture of the bacterial cells, and the growing biofilms. The resulting discoloration has caused aesthetic concerns among practitioners and patients alike. To decrease the amount of black staining, Knight et al. reported application of potassium iodide (KI) after applying SDF.[18] The use of KI is contraindicated in expectant women and during the first 6 months for lactating women due to the possibility of iodide overload in the developing thyroid.[23] Sayed et al. introduced a newer method to reduce discoloration by using Glutathione (20% by weight).[22]

Several in vitro and clinical studies have proven the effectiveness of silver diamine fluoride and its superiority over other agents as caries arresting and preventing agent. This study provides essential information on the anti-cariogenic effects of SDF with potassium iodide and glutathione on *S. mutans* and *L. acidophilus.* The result showed that the groups with glutathione, i.e. group 3,6 and group 9 showed the highest antibacterial property against both; *S. mutans* and *Lactobacillus spp.* (Figure no. 1 and 2). Highly significant difference was seen when compared to only SDF and SDF with potassium iodide groups. This is in accordance with the study conducted by Zorraquín- Peña et al. (2020), where they found that GSH coated silver nanoparticles produced their highest antimicrobial activity against the oral *Streptococcus* strain when compared with *F. nucleatum* and

P. gingivalis.[24] This may be related to the unique wall component of S. mutans, which is thought to be susceptible to the effects of GSH-AgNPs, most likely through membrane-associated adenosine triphosphate ATPases.[25]

Figure no. 1: Antimicrobial efficacy against *Streptococcus mutans*

Figure no. 2: Antimicrobial efficacy against *Lactobacillus acidophillus*



On contrary, Asghar et al. (2021) found that GSH coated silver particals showed lower antimicrobial action than plain SDF. The incorporation of 5, 10, and 15% of GSH did not seem to have a significant effect on the MIC values of SDF against S mutans. The possible explanation for this could be that the antibacterial mechanisms of the effect of silver ions is by binding with the thiol (SH) groups in the bacteria, which results in the disruption of bacterial enzymes and affects their metabolic activities. Since, the silver particles in SDF were already capped with the SH groups in GSH, this antibacterial mechanism was probably not available.[26] The presence of glutathione in saliva can be described as a defensive reagent against the action of toxic xenobiotics. It was reported that, in the presence of GSH, *Lactobacillus spp.* exhibited increased growth rates and GSH content, which protects against the toxic effects of stressing agents such as acidic pH.[27]

Upon comparison between only SDF and SDF with potassium iodide; only SDF showed better antimicrobial activity against *S. mutans*. When compared against Lactobacillus spp. there was no significant difference seen amongst subgroup A and B. This finding is in accordance with Vinson et al. (2018) where they found that the reduction in the CFU count of *S. mutans* with SDF group was seven folds whereas it was just four folds with SDF plus KI group. Furthermore, there was no antibacterial action seen with potassium iodide alone.[28] Previous in vitro study done by Karched et al. (2019) showed that the growth inhibition of S. mutans was higher with SDF alone than with SDF + KI.[29] The addition of potassium iodide reduced the release of silver ions from SDF, leading to a reduction in its antimicrobial efficacy.

When compared among different brands of SDF, Advantage arrest® showed significantly better results when compared with Kids e-SDF® and FAgamin® with only SDF and SDF with glutathione experimental groups (Figure no. 3 and 4). This finding was similar with both the test organisms *(S. mutans* and *Lactobacillus spp.)*.

Figure no. 3: Intragroup comparison of three subgroups of different brands against *Streptococcus mutans.*

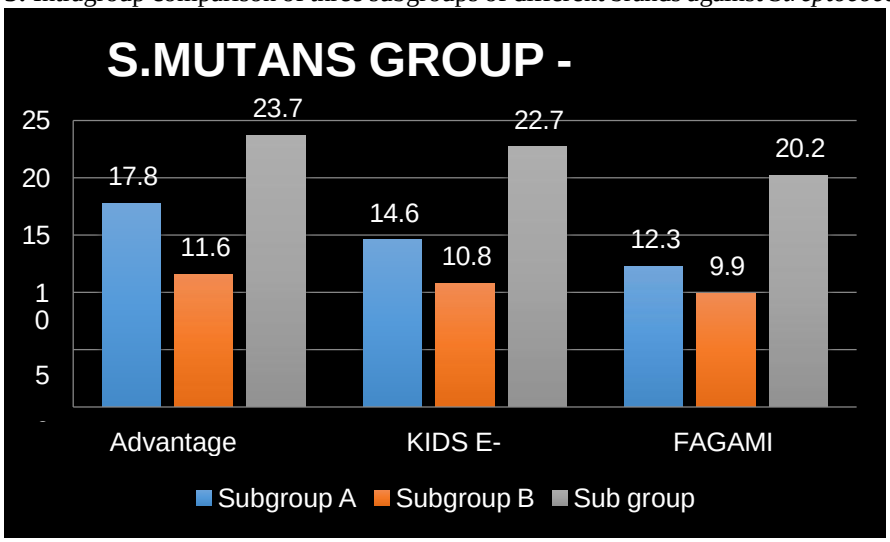
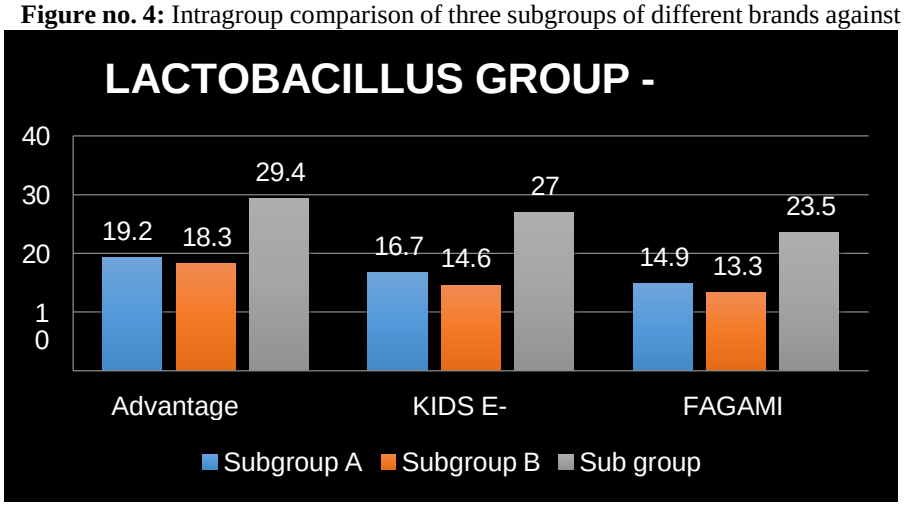


Figure no. 4: Intragroup comparison of three subgroups of different brands against *Lactobacillus acidophilus.*



Agar disk diffusion method was employed in this study as it is a widely accepted assay to assess the antibacterial properties of dental materials. Because silver from the SDF solution forms an insoluble precipitate with components of the liquid culture media, the bactericidal activity of SDF, KI and GSH could not be determined in this investigation using a test tube dilution test. The agar diffusion test is an appropriate technique for such investigation.[12] Moreover, its simplicity, low cost, ability to test many specimens and ease to interpret results provided are advantages of this method.[13] This technique has been used by Marroquín et al. (2021) in their study to compare the antibacterial effect of silver diamine fluoride and potassium against *E. faecalis, A. naeslundii and P. micra.*[30] Fakhruddin et al. (2020) in order to test the antimicrobial ability of SDF against fungi at different volumes has also been investigated using this method against four different *Candida spp.*[13]

We only utilized individual organisms, *S. mutans* and *Lactobacillus acidophilus*, which are the most common cariogenic pathogens. Understanding how these medications interact with these bacterial strains is crucial since caries is considerably more complex than a disease of one or more specific species. Furthermore, salivary conditions were not replicated in this in vitro study. It is important to note that, in this study we grew the biofilm on

tissue culture plates and not on enamel or dentin. The results of this study are not directly related to in vivo conditions, and no direct interpretation or extrapolation to any given clinical protocols should be made based on the results of this single study. Nevertheless, this study is valuable because it provides concrete evidence and support of the disrupting effect of each medicament namely silver diamine fluoride, potassium iodide and glutathione in combinations; on the viability of an established biofilm of *S. mutans* as well as *Lactobacillus spp*.

IV. Conclusion:

Within the limitations of this study, the following conclusion can be derived:
The groups containing GSH showed highest amount of antimicrobial efficacy followed by only SDF followed by KI groups.
When compared among different brands, Advantage arrest showed significantly better results.
Further research is required to fully investigate the interactions between KI and GSH with SDF, their impact on its effectiveness, and the mechanisms behind their action.

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