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DIFFERENT TOPICAL PROTECTION EFFECT ON BACTERIAL COUNT: AN IN-VITRO PILOT STUDY

Sara Elhassan*, Kamaleldin Elmotayam * *and* Fatma Abdelgawad*

ABSTRACT

Objective: To evaluate the inhibition impact of various topical protection treatments on *Streptococcus mutans*. **Material and Methods**: *Streptococcus mutans* was cultured at 37ºC on selective media (*mitis salivarius* agar) anaerobically, then cultivated on brain heart infusion (BHI) media with 5% glucose, and the medium was refreshed daily without disturbing the specimen surface for 21 days, this procedure was performed at Microbiology and Immunology Department, Faculty of Medicine, Cairo University. **Results**: Statistical analysis after 48 hours revealed statistically significant variations between various groups (P<0.001). Silver diamine fluoride (SDF) /potassium iodide (KI) (2.99 ± 0.14) and silver diamine fluoride (3.21 ± 0.01) were significantly the highest with an insignificant difference between them, followed by sodium fluoride (1.43 ± 0.23), while silver nitrate (0.85 ± 0.19) was significantly the lowest. **Conclusions**: We conclude that SDF/KI and SDF 38% are useful, efficient, and widely applicable agents for bacterial inhibition based on the study's results. Potassium iodide addition reduced the action of SDF on *Streptococcus mutans*.

KEYWORDS: potassium iodide, silver diamine fluoride, silver nitrate, sodium fluoride, streptococcus mutans.

INTRODUCTION

Dental caries is a disease that affects the hard tissues of teeth and is dynamic, non-communicable, diet-modulated, biofilm-mediated, and results in net mineral loss. Environmental, behavioral, psychological, and biological factors all play a role in the development of carious lesion forms [1].

There is proof that the topical rather than the systemic effects of fluoride have a greater cariostatic effect. This impact could be enhanced if paired with regular dental care practices like brushing thoroughly with fluoride toothpaste. No matter what their caries risk, all children should use fluoridated toothpaste twice a day and follow oral hygiene guidelines [2].

* Department of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry, Cairo University, Cairo, Egypt.

The idea of using fluorides in topical application rather than ingesting considerable amounts of fluoride has gained widespread acceptance as a crucial component in the prevention of caries in a manner that does not involve heavy fluoride consumption. Additionally useful as therapeutic agents for the non-invasive treatment of dental caries, where they can inactivate or halt carious lesions [3].

Many fluoride-based treatments have been developed to combat this condition. One such treatment, Silver Diamine Fluoride (SDF), has shown particular promise. SDF is a topical solution that can be applied directly to affected teeth. Studies have indicated that a 38% concentration of SDF can effectively stop the progression of dentin caries in about 65.9% of cases [4].

The growth of bacteria that cause cavities is delayed by the presence of SDF, which also increases the remineralization of dentine and enamel while inhibiting demineralization and reduces the deterioration of collagen in dentine. SDF has been utilized at concentrations ranging from 10% to 38% for several decades in Japan, China, Brazil, and Argentina [5].

Dental caries is typically associated with *Streptococcus mutans,* a type of bacterium that has been extensively studied and proven to have a major part in the emergence of tooth cavities. *Streptococcus mutans* typically predominates in the human mouth, whereas *Streptococcus sobrinus* is more of a footnote. Most cases of *Streptococcus mutans* appear in children shortly after their first teeth erupt [6].

Applying a silver nitrate solution inhibits the growth of *Streptococcus mutans* and *Staphylococcus aureus*. Which has antibacterial properties. Additionally, silver ions can bind to DNA molecules containing phosphorus, inhibiting the process of bacterial aggregation, and interacting with intracellular sulfur-containing enzymes to decrease the metabolic processes of the bacteria [7].

Since dental caries is a polymicrobial condition, comprehending the biofilm's qualitative and quantitative characteristics is essential to figuring out how it develops. It has long been recognized in the research community that microorganisms in dental plaque are the primary causal agents of dental caries [8].

Extracellular glucans synthesized by MS promote biofilm development. MS are required for fissure caries to form, but they are inadequate. The retaining habitat for the growth of lactobacilli, and other microorganisms associated with this condition, is thought to have been created by MS, which is detected in children who had and did not have dental cavities [6].

The oral microbiota implicated in dental caries is a multifaceted community comprising numerous acidogenic species. Although dental caries is recognized as a polymicrobial disease, *Streptococcus mutans* remains a primary focus of research owing to its capacity for biofilm formation, acid production, and acid tolerance [9].

Therefore, the purpose of this study was to evaluate the inhibition impact of various topical protection treatments on *Streptococcus mutans*.

MATERIAL AND METHODS

Study registration and design

This in-vitro study was conducted at Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Cairo University, and Microbiology and Immunology Department, Faculty of Medicine, Cairo University, Egypt.

Bacterial culturing

Streptococcus mutans was cultured at 37ºC on blood agar plates on selective media (*mitis salivarius* agar) anaerobically, then cultivated on brain heart infusion (BHI) media with 5% glucose, and the medium was refreshed daily without disturbing the specimen surface for 21 days, this procedure was performed at Microbiology and Immunology Department, Faculty of Medicine, Cairo University.

Sample Grouping

ELISA plate was divided into four groups, each of six wells.

- ***Group I:*** Treated with 5% Sodium Fluoride varnish.
- ***Group II:*** Treated with 38% Silver Diamine Fluoride liquid.
- ***Group III:*** Treated with 38% Silver Diamine Fluoride followed immediately by application of potassium-iodide liquid.
- ***Group IV:*** Treated with 0.05 N silver nitrate liquid.

Bacterial count

ELISA plate was used, rows A, B, C, and D, six wells in each row with each well filled with 100 µL BHI. Each well was encountered with each one of the four intervention materials according to manufacturer instructions. 5% sodium fluoride varnish (22.600 ppm fluoride, xylitol flavors. VOCO Profluoride varnish, Germany), 38% Silver diamine fluoride (44.800 ppm fluoride,25% silver, 8 % ammonia and 62% water. SDI, Riva star step one, Australia) 38% Silver diamine fluoride /Potassium iodide (Potassium iodide solution. SDI, Riva star step two, Australia),0.05 N Silver nitrite (8.495 ±0.0001 Silver nitrate powder in 1 L of purified water. EMSURE, Merck, Germany). It was then incubated for 18, 24, and 48 hours at 37°C.

The bacterial count was assessed using the ELISA test (a stat Fax 2100 Microplate Reader Awareness Technology, Inc). An automatic ELISA tray reader was used to measure the absorbance of each well at 630 nm as a baseline prior to the interventions and after interventions and incubation at different times (18, 24, and 48h) according to Sruthi study [10].

Statistical analysis

The numerical data presented using the mean and standard deviation numbers. Shapiro-Wilk's test used to determine whether they were normal. They were normally distributed, for intergroup comparisons, one-way ANOVA, Tukey's post hoc test, and repeated measurements were utilized. ANOVA used to compare intragroup, followed by the Bonferroni post hoc test. A significant level of $p \leq 0.05$ was established within all tests. The statistical analysis was conducted using the R statistical analysis program, version 4.3.0 for Windows.

RESULTS

Intergroup comparisons

The bacterial count (CFU) mean and standard deviation (SD) data and comparison between groups are presented in Table (I).

Table (I) shows intergroup comparisons, mean and SD values of the bacterial count reduction (CFU).

TABLE (I) Intergroup comparisons, mean and SD values of the bacterial count reduction (CFU)

Group / Interval	Bacterial count (CFU) (Mean±SD)				p-value
	NaF	SDF	SDF/KI	Silver nitrite	
Baseline	0.38 ± 0.01^{B}	0.37 ± 0.01^{C}	0.40 ± 0.01^{A}	0.36 ± 0.01^{D}	<0.001*
18 hours	0.93 ± 0.17^{B}	3.20 ± 0.10^{A}	3.01 ± 0.14^{A}	0.73 ± 0.04^{C}	<0.001*
24 hours	1.04 ± 0.02^{C}	3.19 ± 0.10^{A}	3.00 ± 0.14^{B}	0.85 ± 0.09^{D}	<0.001*
48 hours	1.43 ± 0.23^{B}	3.21 ± 0.10^{A}	2.99 ± 0.14^{A}	0.85 ± 0.19^{C}	<0.001*

Within a single horizontal row, means denoted by distinct superscript letters indicate significant differences ($p \leq 0.05$) or non-significant differences ($p>0.05$).

Intragroup comparisons

The bacterial count reduction (CFU) mean and (SD) values, together with intragroup comparisons, are displayed in Table (II).

Table (II) illustrates values measured across several groups at various intervals showing a statistically significant variation.

Intergroup comparisons of percentage change

Table (III) displays intergroup comparisons as well as bacterial count percentage change (%), along with its mean and standard deviation.

Between groups, A statistically significant difference ($p<0.001$) was observed. SDF possessed the highest value (765.03±20.26), followed by SDF/KI (649.35±37.00), then NaF (276.05±60.45), while the lowest value was found in silver nitrite (137.14±52.74). Every pairwise comparison was statistically significant ($p<0.001$).

TABLE (II) Intragroup comparisons, mean and SD values of the bacterial count reduction (CFU)

Group \ Interval	Bacterial count (CFU) (Mean±SD)				p-value
	Baseline	18 hours	24 hours	48 hours	
NaF	0.38 ± 0.01^{C}	0.93 ± 0.17^{B}	1.04 ± 0.02^{B}	1.43 ± 0.23^{A}	<0.001*
SDF	0.37 ± 0.01^{B}	3.20 ± 0.10^{A}	3.19 ± 0.10^{A}	3.21 ± 0.10^{A}	<0.001*
SDF/KI	0.40 ± 0.01^{B}	3.01 ± 0.14^{A}	3.00 ± 0.14^{A}	2.99 ± 0.14^{A}	<0.001*
Silver nitrite	0.36 ± 0.01^{B}	0.73 ± 0.04^{A}	0.85 ± 0.09^{A}	0.85 ± 0.19^{A}	<0.001*

Within a single horizontal row, means denoted by distinct superscript letters indicate significant differences ($p \leq 0.05$) or non-significant differences ($p>0.05$).

TABLE (III) Intergroup comparisons, mean and SD values of the bacterial count reduction percentage change (%)

Bacterial count percentage change (%) (Mean±SD)				p-value
NaF	SDF	SDF/KI	Silver nitrite	
276.05 ± 60.45^{C}	765.03 ± 20.26^{A}	649.35 ± 37.00^{B}	137.14 ± 52.74^{D}	<0.001*

Within a single horizontal row, means denoted by distinct superscript letters indicate significant differences ($p \leq 0.05$) or non-significant differences ($p>0.05$).

DISCUSSION

One of the most common infectious diseases, dental caries, arises from an imbalance between the processes of demineralization and remineralization. This imbalance encourages the development of cariogenic bacteria, which produce toxic compounds that breakdown the hard tissues of teeth and necessitate invasive intervention therapy. Minimally invasive techniques for halting and preventing carious lesions offer accessible, cost-effective solutions to the caries issue that do not require expensive machinery [11-12].

It is known that early childhood caries has a particular pattern linked to the emergence of caries in primary anterior teeth. One of the major significant odontopathogens implicated in the emergence and advancement of dental caries, *Streptococcus mutants*, are used in the current study to examine the bacterial count. The primary pathogen to be responsible for the start and advancement of tooth decay is *Streptococcus mutans*, and larger bacterial counts are linked to a higher incidence of this condition [13].

The findings demonstrated that SDF and SDF/KI were significantly the highest with insignificant differences between them, then NaF, while silver nitrate was significantly the lowest. SDF and SDF/KI possessed greater antibacterial activity than NaF, and silver nitrate. SDF was more effective than SDF/KI, but there was no statistically significant change. The breakdown of the cytoplasmic extrusion and bacterial cell wall, the result of the bacterial enzymes' high reactivity to silver ions, which are components of sulfur and phosphorus, could be the cause of the disparate results. Beyond the effects of ionic silver, fluoride, in SDF, can stop biofilms from forming by attaching to the parts of bacterial cells and modifying their enzymes. Fluoride hinders the metabolism of bacteria, whereas silver dissolves bacterial membranes. These two substances kill bacteria in different ways [14].

Applying silver nitrate solution topically produced no noticeable results, this may be due to the silver nitrate being washed away without shielding of fluoride varnish layer. Furthermore, in this study, a lower concentration of 0.05 N silver nitrate was employed.

These outcomes were consistent with Sorkhdini et al study [15], they concluded that silver nitrate was inferior to SDF & SDF/KI in terms of avoiding the formation of carious lesions. Additionally, another study by Vinson et al [16]. Reported that an established *Streptococcus mutans* biofilm was disturbed by SDF alone, SDF + SSKI, and SSKI. SDF solely caused the most significant disturbance.

CONCLUSION

From the findings of the present study, we conclude that the use of 38% SDF and 38% SDF+KI was an effective, efficient, and easily applicable agent in bacterial inhibition. The addition of KI lowered the effect of SDF ability on *Streptococcus mutans*. It is recommended to investigate SDF +KI and SDF effect on other cariogenic bacteria other than *Streptococcus mutans*.

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CONFLICT OF INTEREST

The authors of the current study declare that there is no conflict of interest

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