ORIGINAL RESEARCH

Antibacterial Efficacy against *Streptococcus mutans* of Different Desensitizing Dentifrices: A Comparison *In vitro* Study

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Abstract

Aim: To evaluate the antibacterial efficacy of desensitizing dentifrices.

Material and methods: An experimental, *in vitro*, longitudinal, analytical, and prospective study was carried out. Subsequently, the following groups were formed: *Streptococcus mutans* vs Vitis® Sensible. *S. mutans* vs Sensodyne® Repair and Protect. Also, *S. mutans* vs Colgate® Sensitive Pro-Relief™ and *S. mutans* vs Colgate Total 12® at 100, 50, 25, and 12.5%. Each Petri dish was properly labeled with the letter corresponding to the toothpaste and was placed in the incubator for 24 hours at 37°C. A 0.12% chlorhexidine solution was used as a positive control and distilled water as a negative control. The manuscript was written following the checklist for reporting *in vitro* studies (CRIS) guidelines.

Results: It was found that when comparing the inhibition halos of the desensitizing toothpaste against *S. mutans*, Colgate® Sensitive Pro-Relief™ 100% paste had the highest efficacy at 24 and 48 hours with an average of 25.2 ± 1.0 and 23.5 ± 1.1 mm, respectively. On the other way, Sensodyne paste had no efficacy at any of its concentrations 100, 50, 25, and 12.5%. Finally, it was found that there were statistically significant differences between each of the groups evaluated with a $p < 0.001$.

Conclusions: It was concluded that mainly the 100% pure concentrations of the desensitizing pastes had antibacterial efficacy against *S. mutans*. However, Sensodyne® Repair and Protect paste had no effect.

Clinical significance: This research has clinical relevance because the use of desensitizing pastes is highly frequent. Therefore, it is necessary to know if these pastes offer an efficient antibacterial effect to control the main microorganisms of the oral cavity.

Keywords: Antibacterial efficacy, Desensitizing dentifrices, *In vitro* study.

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Introduction

Dental caries is a disease characterized by the loss of non-organic elements from the hard tissues of the tooth, due to the presence of weak organic acids originated by cariogenic bacteria such as *S. mutans*. Increased acidity in the oral environment causes the dental tissue to be destroyed and this generates the spread of calcium and phosphate ions.[1] For this reason, throughout history, various strategies have been tried to reduce or eliminate the presence of dental caries. Thus, we find the use of toothpaste, which has been considered an effective and accessible vehicle that increase the strength of the enamel against acid attacks.[2]

The use of toothpaste is not only for the purpose of cleaning teeth but also to combat dental caries, gum disease, bad odor, calculus, erosion, remineralization, and dentin hypersensitivity.[3] From their appearance to the present day, toothpaste has evolved into numerous products with complex formulations. Efficient remineralization of tooth enamel has been achieved due to the advance in nanotechnology, which has made it possible to transform toothpaste with the aggregation of calcium phosphate salts. The calcium and phosphate would proceed by filling the micropores, forming crystalline nuclei, and incorporating new ions from oral saliva.[4]

Desensitizing dentifrices is part of the therapeutic plan against dentin hypersensitivity. They can be divided into two categories, those that occlude the open dentinal tubules and those that block neural transmission. In this way, they can reduce dentin sensitivity.[4–9] However, there is little literature supporting their potential as an antimicrobial agent, although some microbiological studies show the importance of controlling oral microflora.[10–12]

It is very important to know the antimicrobial efficacy of desensitizing toothpaste in contrast to commercial toothpaste

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because they have different chemical components. For this reason, this study used different kinds of toothpaste such as Vitis® Sensitive, Sensodyne® Repair and Protect, Colgate® Sensitive Pro-Relief, and Colgate® Total 12. The novelty of this study is that it simultaneously compares the antimicrobial efficacy of the main desensitizing toothpaste, which gives us an important insight to identify which of the toothpaste has the best efficacy. Therefore, the objective of this study was to evaluate the antibacterial efficacy of desensitizing dentifrices.

Materials and Methods

Study Design

An experimental, *in vitro* study was carried out. The current research was carried out from March to July 2021. The microbiological study was carried out at the Analytical Control Center of the Faculty of Pharmacy and Biochemistry of the Universidad Nacional Mayor de San Marcos, Lima, Peru – Code No. 00422-CPF-2021.

Sample Size

The sample size was calculated in relation to the data obtained in the pilot test with an Alpha of 0.5 and a beta test power of 0.8. Using the mean comparison test with Stata 15.1 software showed a sample size of $n = 36$ for each group. The manuscript was written following the CRIS guidelines.

Subsequently, the following groups were formed:

- Group of *S. mutans* vs Vitis® Sensitive at 100, 50, 25, and 12.5%.
- Group of *S. mutans* vs Sensodyne® Repair and Protect at 100, 50, 25, and 12.5%.
- Group of *S. mutans* vs Colgate® Sensitive Pro-Relief™ at 100, 50, 25, and 12.5%.
- Group of *S. mutans* vs Colgate Total 12® at 100, 50, 25, and 12.5%.

Preparation of the Culture Medium

A total of 20 mL of brain heart infusion (BHI) broth was prepared in two test tubes and autoclaved. The agar was cooled in a water bath at 45–50°C and then poured into sterile Petri dishes. Next, 1200 L of Mueller agar was prepared and poured into sterile Petri dishes to obtain a homogeneous distribution of 4 mm thickness. The plated agar was condensed at room temperature. Each batch of agar maintained a pH between 7.0 and 7.6.

Strain Activation

S. mutans ATCC 25175 strains were refrigerated at 4–8°C on BHI agar plates. A colony was taken with the bacteriological loop and seeded in tubes with sterile BHI broth and placed in the incubator for 24 hours at 37°C. Subsequently, turbidity was formed which showed the growth of the strains. It was seeded from the BHI broth and incubated for 24 hours at 37°C.

Preparation of the Inoculum

The pure strain of *S. mutans* ATCC 25175 was taken and placed in test tubes containing 10 mL of sterile saline for dilution so that the resulting solutions had turbidity like the McFarland scale tube 0.5. From these last solutions, dilutions of 1 in 3 were made, for this purpose, 3 mL of these prepared solutions were taken and dissolved to a total volume of 9 mL with physiological serum in tubes with screw caps. The resulting solutions had a concentration of 1×10^8 CFU/mL.

Preparation of the Toothpastes

The toothpastes were delivered to the laboratory fully wrapped and labeled with the symbols A, B, C, and D so that the brands studied could not be identified. Thus, in the end we obtain: A = Vitis® Sensible, B = Sensodyne® Repairs and Protects, C = Colgate® Sensitive Pro-relief, D = Colgate® Total 12. Toothpastes A, B, C, and D were worked at the following four concentrations: 100, 50, 25, and 12.5%. To obtain the dilutions of these toothpastes, distilled water was used as follows: The 100% concentration is the toothpaste as is, without any dilution. For the 50% concentration, 5 gm was placed in a tube and 10 mL of sterile distilled water was added (1:2 dilution). For the 25% concentration, 5 mL of the 1:2 dilution was taken in a tube and 5 mL of sterile distilled water was added. For the 12.5% concentration, from the above 25% dilution, 5 mL was taken in a tube and 5 mL of sterile distilled water was added. All dilutions were performed for each of the four toothpastes A, B, C, and D. The concentrations were centrifuged at 3,500 rpm for 15 minutes, to work with the supernatant.

Seeding of Samples and Controls

The agar well diffusion method was used to evaluate antimicrobial efficacy. To each of the 6 mm diameter wells, 40 µL of the dilutions of each toothpaste concentration were added. For each toothpaste group, the following eight plates were used: Plates for each dilution at 100%, plates at 50%, plates at 25%, and plates at 12.5%. Each Petri dish was duly labeled with the letter corresponding to the toothpaste and was placed in the incubator for 24 hours at 37°C. A 0.12% chlorhexidine solution was used as a positive and distilled water as a negative control.

Statistical Analysis

The statistical software Stata 15.1 was used. Descriptive statistics (mean, standard deviation) were calculated for each dentifrice. To compare between groups, the Kruskall–Wallis test was used. All analyses were performed at a significance level of 0.05 ($p < 0.05$).

Results

It was found that when comparing the inhibition halos of the desensitizing toothpastes against *S. mutans*, the Colgate® Sensitive Pro-Relief™ 100% paste had the highest efficacy at 24 and 48 hours with an average of 25.2 ± 1.0 and 23.5 ± 1.1 mm, respectively (Table 1; Fig. 1).

For the Vitis® Sensitive group, only the 100% concentration had antibacterial efficacy with an average of 7.6 ± 0.1 and 6.9 ± 0.4 mm at 24 and 48 hours, respectively (Table 1).

For the Colgate Total 12® group, the 100% concentration also had the highest efficacy with an average of 18.6 ± 0.2 and 17.6 ± 0.2 mm at 24 and 48 hours, respectively (Table 1).

On the other hand, Sensodyne® Repair and Protect paste had no efficacy in any of its concentrations 100, 50, 25, and 12.5% (Table 1).

Finally, the inferential analysis evidenced that there were significant differences between each of the groups evaluated with a $p < 0.001$.

Discussion

From their appearance to the present day, toothpaste has evolved to obtain different products with complex formulations. Efficient remineralization of tooth enamel has been achieved due to

Table 1: Comparison of inhibition halos of desensitizing dentifrices against the *S. mutans* strain

	24 hours		*48 hours*		
	Mean	*SD*	*Mean*	*SD*	*p-value*
Vitis® Sensitive 100%	7.6	0.1	6.9	0.4	<0.001
Vitis® Sensitive 50%	0.0	0.0	0.0	0.0	
Vitis® Sensitive 25%	0.0	0.0	0.0	0.0	
Vitis® Sensitive 12.5%	0.0	0.0	0.0	0.0	
Colgate® Sensitive Pro-Relief™ 100%	25.2	1.0	23.5	1.1	<0.001
Colgate® Sensitive Pro-Relief™ 50%	21.2	0.2	19.7	0.4	
Colgate® Sensitive Pro-Relief™ 25%	11.1	0.4	11.4	1.0	
Colgate® Sensitive Pro-Relief™ 12.5%	0.0	0.0	0.0	0.0	
Colgate Total 12® 100%	18.6	0.2	17.6	0.2	<0.001
Colgate Total 12® 50%	11.6	0.4	10.7	1.0	
Colgate Total 12® 25%	0.0	0.0	0.0	0.0	
Colgate Total 12® 12.5%	0.0	0.0	0.0	0.0	
Sensodyne® Repair and Protect 100%	0.0	0.0	0.0	0.0	—*
Sensodyne® Repair and Protect 50%	0.0	0.0	0.0	0.0	
Sensodyne® Repair and Protect 25%	0.0	0.0	0.0	0.0	
Sensodyne® Repair and Protect 12.5%	0.0	0.0	0.0	0.0	

*Values were expressed in mm and those with no antibacterial effect (0.0) were excluded from any inferential statistical analysis

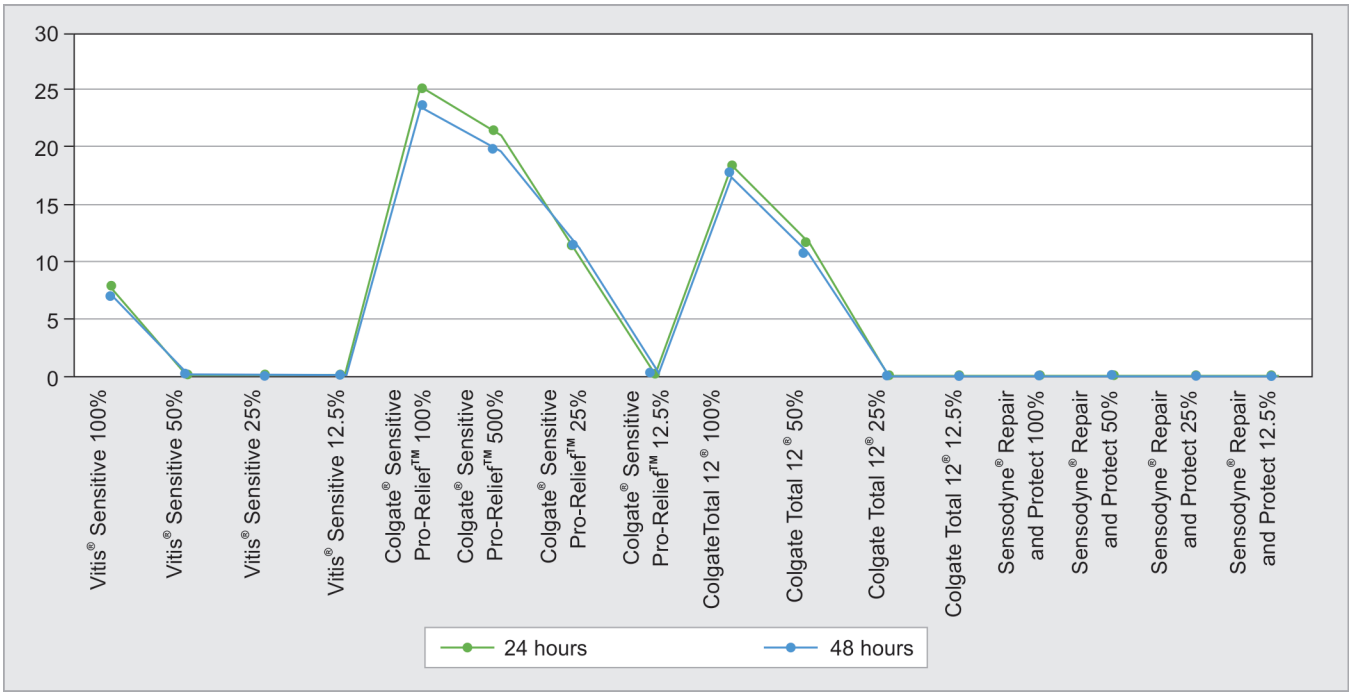


Fig. 1: Inhibition halos of desensitizing dentifrices

the advance in nanotechnology, which has made it possible to transform toothpaste with the aggregation of calcium phosphate salts. The calcium and phosphate would proceed by filling the micropores, forming crystalline nuclei, and incorporating new ions from the saliva.[13]

Science has made it possible to enrich the characteristics of toothpaste, with the aggregation of nanohydroxyapatite, silver nanoparticles, calcium phosphate, nanocalcium, among others. All this has improved the qualities of toothpaste, helping to paralyze caries lesions, reduce tooth sensitivity, and

provide minerals to restore pH control or control bacterial growth.[14]

Some studies evaluating the antibacterial activity of toothpaste, given that oral microflora biofilms are at the core of dental caries and periodontal disease, it is important to control biofilms through mechanical debridement and the use of adjuvant antibacterial in dentifrices.[15] The *in vitro* anti-microbial effects of commercially available toothpaste indicates that antimicrobial activity is not only dependent on fluoride but also on the synergy with other chemical or natural components.[8]

It must be considered that laboratory research describes *S. mutans* as an initial etiologic agent of dental caries, as it can change the local environment by forming a medium rich in carbohydrate macromolecules and a low pH, forming a hospitable site for other species to thrive.[16] The present investigation evaluated the antibacterial activity of four dentifrices against *S. mutans* ATCC 25175. The dentifrices were used at concentrations of 100, 50, 25, and 12.5% on cultures of the bacteria. It was verified that only Sensodyne® dentifrice did not show any antibacterial effect at any dilution level. The other three toothpastes did show an effect to counteract the growth of the bacteria at least in some concentration, however, with significant differences among them.

On the other hand, Guven et al.[7] analyzed the antimicrobial effect of Sensodyne® Repara and Protege toothpaste finding inhibition halos of an average of 20 mm against *S. mutans*, these results disagree with the present study, such activity could be due to the ingredient sodium lauryl sulfate, which has been attributed some antibacterial properties and be responsible for the formation of the inhibitory halo. While Randall et al.[8] investigated ten fluoride toothpastes antibacterial action against *S. mutans*, where Colgate® Total showed the largest growth inhibition zone (38.3 mm), considering that within its ingredients there was also fluoride, sodium lauryl sulfate, and triclosan, which in several investigations are recognized for their antimicrobial effects. These results disagree with ours since Colgate® Total 12 obtained the second highest bacterial inhibition halo, this could be explained by the fact that Colgate withdrew triclosan from all its toothpastes in 2019 and this could lessen the antimicrobial effect of Colgate® Total 12 toothpaste.

In addition, Monterubbianesi et al.[9] analyzed a toothpaste based on zinc carbonate-hydroxyapatite nanoparticles, where they found no antimicrobial activity in the toothpaste. These results coincide with those found in the present investigation in relation to Vitis® Sensible toothpaste. This may be because the development of products based on calcium phosphates such as hydroxyapatite is not simple due to the interaction between various active components of the formulation and many of these ingredients could be inhibited and not be available in the toothpaste. Whereas Randall et al.[8] found that 0.25% sodium lauryl sulfate concentration barely showed inhibition halos of 10.5 mm, in contrast at 1% concentrations it formed inhibition zones of 23.9 mm against *S. mutans*. This could explain why Vitis® Sensible toothpaste, despite containing sodium lauryl sulfate, could be found in low concentrations and show very low antibacterial activity against *S. mutans*.

The main limitation of this research was that desensitizing pastes used mostly contain hydroxyapatite, and sodium lauryl sulfate, which may have lower antimicrobial properties compared to other natural product-based pastes,[11,17] however, there is a dose-response relationship between the concentration of sodium lauryl sulfate and growth inhibition on *S. mutans*. Another point to consider is the pH during bacterial growth, as this can alter microbial multiplication. Mueller–Hinton agar has a pH of 7.0–7.6 which does not reflect the acidic pH conditions that can be found in the oral cavity, making *S. mutans* sensitive to the effects of fluoride ions.

Conclusion

Within the limitations of this *in vitro* study, it was concluded that mainly 100% pure concentrations of the desensitizing pastes had antibacterial efficacy against *S. mutans*. However, Sensodyne® Repair and Protect paste had no effect. Finally, it was found that Colgate® Sensitive Pro-Relief™ 100% paste had the greatest antibacterial effect, followed by Colgate Total 12® 100% and Vitis® Sensible 100%. Finally, the clinical application of these results may indicate the use of the most effective desensitizing paste to better control the pathological oral microbiota.

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