

Investigation of the *In Vitro* Antibacterial Effects of Different Toothpastes and Fluoride Gels: An *In Vitro* Study

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Abstract

Background: Dentifrices from the most commonly used chemotherapeutic agents in daily plaque control are capable of preventing bacterial adhesion, colonization and metabolism, and thus affect the bacterial growth.

Aim: The aim of this *in vitro* study is to investigate the antibacterial effects of the fluoride gels and kinds of toothpaste with fluoride and nonfluoride.

Methods: Six kinds of toothpaste for children (3 nonfluoride and 3 fluoride) were tested for their antibacterial activity against five oral pathogens; *Streptococcus mutans* (ATCC 25175), *Streptococcus sobrinus* (ATCC 33478), *Lactobacillus casei* (ATCC 4646), *Actinomyces naeslundii* (ATCC 19039) and *Candida albicans* (ATCC 10231) by agar well diffusion assay and two fluoride gels were also tested by disc diffusion assay. Data were analyzed statistically by using Kruskal Wallis and Mann Whitney U tests. Test with a significance level of $p < 0.05$.

Results: Group I fluoride toothpaste was found statistically effective against only *C. albicans* ($p < 0.01$). Group II and III fluoride toothpaste were found statistically effective against all tested oral pathogens ($p < 0.05$). Group IV nonfluoride toothpaste was found statistically effective against *C. albicans*, *S. sobrinus*, *L. casei* ($p < 0.01$). Group V nonfluoride toothpaste was found statistically effective against *S. mutans*, *S. sobrinus*, *L. casei* and *A. naeslundii* ($p < 0.05$). Group VI nonfluoride toothpaste was found ineffective against all tested oral pathogens ($p > 0.05$). Group VII fluoride gel was found less effective *C. albicans* and *S. sobrinus* ($p < 0.05$). But Group VIII zinc fluoride gel was found statistically ineffective against all tested oral pathogens ($p > 0.05$).

Conclusion: Despite gels were found ineffective in this *in vitro* study, they will be given more positive results in clinical usage and *in vivo* studies. *In vivo* studies should be performed to investigate the antibacterial effects of toothpastes and fluoride gels.

Keywords: Toothpaste; *Streptococcus mutans*; *Streptococcus sobrinus*; *Lactobacillus casei*; *Actinomyces naeslundii*; *Candida albicans*

Introduction

Dental plaque is defined as "a biofilm composed of various microbial communities embedded in a matrix of host and bacterial polymer growing on tooth surfaces". Dental plaque microorganisms are often responsible for common diseases such as tooth decay and periodontal disease [1-3].

Caries begins when the acidogenic microorganisms in the bacterial plaque on the tooth metabolise and produce acid in the plaque of the individual with food and drink, and dissolve the calcium phosphate crystals that contain the teeth of the hard tissue elements in the face of these acids [4].

Everyday individual oral hygiene practices emphasize the removal of the dental plaque by dentists for a long time and are considered an important element of oral health. Many new products are being designed for oral health and the search for this issue is ongoing. The most widely used individual agents for dental plaque removal and prevention of tooth decay are toothpastes [5].

Fluoride toothpastes are widely used all over the world and extensive research has identified their ability in terms of caries resistance [6]. Fluoride in toothpastes or fluoride gels that are often used in professional applications has an important role as an antibacterial agent in the prevention of caries formation and decay progression as a result of regular use [5,7]. To maximize the beneficial effect of fluoride in toothpaste, the teeth should be brushed twice daily with 500 ppm fluoride toothpaste and 1000 ppm fluoride toothpaste between 6 and 2 years of age in children between 6 months and 2 years of age; 6 years and over (AAPD) 1450 ppm fluoride toothpaste [8,9].

Fluoride is considered as an effective antimicrobial agent due to several mechanisms of action: 1) prevention of mineral demineralization, reduction of dental minerals solubility by changing hydroxyl groups in calcium hydroxyapatite structure and the formation of more acid-resistant fluorapatite minerals; 2) promoting remineralization by continuously adsorbing to the tooth surface from the saline along with calcium and phosphate ions and thus causing the development of the fluorapatite-like substance; 3) inhibition of bacterial metabolism [10].

It is stated that the decline in the prevalence and severity of tooth decay in recent years is mainly due to the widespread use of fluoride [11]. It is useful to maintain oral health by controlling the fluoride decay mechanism used with individual applications in the provision and development of oral hygiene [4,12].

In addition to individual applications, professionally applied topical fluoride treatments are among the effective methods to reduce the prevalence of caries [13].

The most commonly used substances for fluoride treatments practiced by dentists professionally are 5 percent sodium fluoride varnish (NaF; 22,500 ppm F) and 1.23 percent acidified phosphate fluoride (APF; 12,300 ppm F).

Other topical fluoride products, such as sodium fluoride (NaF) mouthwash (900 ppm F) and brush gellies (eg, 1.1 percent NaF; 5,000 ppm F), have also been shown to be effective in reducing tooth decay on permanent teeth [1].

Purpose of the Study

The purpose of this study was to investigate the antibacterial effects of the two fluoride gels and six toothpastes with fluoride and non-fluoride *in-vitro*.

Materials and Methods

In this study, we aimed to examine the antibacterial effects of toothpastes with different contents and fluoride gels, the contents of used materials and manufacturers are shown in table 1.

Groups	Product and manufacturer	Fluoride type and ppm
Group I	Sensodyne Pronamel (GlaxoSmithKline. USA)	Fluoride toothpaste. 1450 ppm NaF
Group II	Oxyfresh (Oxyfresh. USA)	Fluoride toothpaste. 0.235% NaF
Group III	Colgate (Herstal. Belgium)	Fluoride toothpaste. 1450 ppm NaF
Group IV	Nenedent 2-4 (Dentinox. Berlin. Germany)	Non-fluoride toothpaste
Group V	Oxyfresh (Oxyfresh. USA)	Non-fluoride toothpaste
Group VI	R.O.C.S (WDS. Swetzerland)	Non-fluoride toothpaste
Group VII	Sultan (Sultan Healthcare. Turkey)	Sodium Fluoride gel. 2%
Group VIII	Oxyfresh (Oxyfresh . USA)	Zinc fluoride gel. 22%

Table 1: Materials used in this study.

Six toothpastes (three fluoride toothpaste Group I,II,III and three nonfluoride toothpaste Group IV, V, VI) for children were tested (Table 1) for their antibacterial activity against five oral pathogens; *Streptococcus mutans* (ATCC 25175), *Streptococcus sobrinus* (ATCC 33478), *Lactobacillus casei* (ATCC 4646), *Actinomyces naeslundii* (ATCC 19039) and *Candida albicans* (ATCC 10231) by agar well diffusion assay and two fluoride gels (Group VII -Sodium fluoride gel and Group VIII zinc fluoride gel) were also tested by disc diffusion assay. The organisms were cultured in trypticase soy broth and transferred to the selective media to revive from the stock.

The microbial organisms employed in the study were cultured in their respective selective media. Mitis salivarius agar medium was used for the growth of *Streptococcus mutans* and *Streptococcus sobrinus*, the rogosa agar medium was used for the growth of *Lactobacillus casei*, the brain heart infusion agar medium was used for the growth of *Actinomyces naeslundii* and Sabouraud Dextrose agar was used for the growth of *Candida albicans*.

The bacterial strains which were lyophilized were produced carefully in sterile conditions in the Microbiology Laboratory of the Faculty of Dentistry of Istanbul University. 10 µl of bacterial suspension prepared with 24-h cultures of *Streptococcus mutans* (ATCC 25175), *Streptococcus sobrinus* (ATCC 33478), *Lactobacillus casei* (ATCC 4646) and *Actinomyces naeslundii* (ATCC 19039) resuspended in fresh brain heart infusion (BHI; Difco Laboratories, Detroit, MI) broth (approximately 10⁶ CFU/ml) was placed and incubated for 1h in a humid atmosphere at 37°C.

The microbial suspensions were added to BHI and Sabouraud agar plates with the sterile ecuvion sticks and gently stirred for 2 minutes. For agar well diffusion assay, six wells with 6 mm diameters were prepared and filled by the toothpastes. For agar disc diffusion assay, the gels were absorbed on sterile blank antimicrobial susceptibility discs and then placed on the inoculated agar plates.

Antibacterial activity was determined by measuring the inhibition zone diameters after incubation for 24 hours in the agar plate incubation conditions prepared in this way.

Data were analyzed statistically by using Kruskal Wallis, Mann Whitney U tests, Wilcoxon Sign Test, Fisher Freeman Halton Exact Test and Mc Nemar Test with a significance level of $p < 0.05$.

Results and Discussion

Results

Group I was found statistically effective against only *C. albicans*. Group II and III were found statistically effective against all tested oral pathogens ($p < 0.05$) ($p < 0.01$) (Table 2).

Group IV was found statistically effective against *S. sobrinus*, *A. naeslundii* and *C. albicans* ($p < 0.05$). Group V was found statistically effective against all tested oral pathogens. Group VI was found ineffective against all tested oral pathogens ($p > 0.05$) (Table 3).

	Group I	Group II	Group III	p
	Means ± SD (Median)	Means ± SD (Median)	Means ± SD (Median)	
<i>S. mutans</i>	0 ± 0 (0)	17.33 ± 1.15 (18)	19.33 ± 0.58 (19)	0.023*
<i>S. sobrinus</i>	0 ± 0 (0)	19.33 ± 2.08 (20)	25.33 ± 1.15 (26)	0.023*
<i>L. casei</i>	0 ± 0 (0)	17.33 ± 2.08 (18)	20.33 ± 1.53 (20)	0.028*
<i>A. naeslundii</i>	0 ± 0 (0)	34.00 ± 0 (34)	34.00 ± 0 (34)	0.018*
<i>Candida albicans</i>	3.67 ± 0.58 (4)	0 ± 0 (0)	7.67 ± 0.58 (8)	0.023*
P	0.008**	0.020*	0.011*	

Table 2: Comparisons of the fluoride toothpastes according to bacterial zone diameters (mm).

Kruskal Wallis Test; * $p < 0.05$; ** $p < 0.01$

Group VII was found less effective *C. albicans* and *S. sobrinus* ($p < 0.05$). Group VIII was found statistically ineffective against all tested oral pathogens ($p > 0.05$) (Table 4).

	Group IV	Group V	Group VI	p
	Means ± SD (Median)	Means ± SD (Median)	Means ± SD (Median)	
<i>S. mutans</i>	0 ± 0 (0)	17.33 ± 1.15 (18)	0 ± 0 (0)	0.021*
<i>S. sobrinus</i>	11 ± 1 (11)	19.67 ± 0.58 (20)	0 ± 0 (0)	0.023*
<i>L. casei</i>	0 ± 0 (0)	18 ± 1 (18)	0 ± 0 (0)	0.022*
<i>A. naeslundii</i>	20 ± 0 (20)	34 ± 0 (34)	0 ± 0 (0)	0.018*
<i>Candida albicans</i>	7 ± 2.6 (8)	4 ± 0 (0)	0 ± 0 (0)	0.030*
P	0.008**	0.011*	1.000	

Table 3: Comparisons of the non-fluoride toothpastes according to bacterial zone diameters (mm).

Kruskal Wallis Test; * $p < 0.05$; ** $p < 0.01$

Discussion

In vitro studies have shown that the presence of fluoride at constant low concentration allows *S. mutans* to produce less acid. Fluoride

	Group VII	Group VIII	p
	Means ± SD (Median)	Means ± SD (Median)	
<i>S. mutans</i>	17.33 ± 1.15 (18)	0 ± 0 (0)	0.023*
<i>S. sobrinus</i>	19.33 ± 2.08 (20)	0 ± 0 (0)	0.023*
<i>L. casei</i>	17.33 ± 2.08 (18)	0 ± 0 (0)	0.028*
<i>A. naeslundii</i>	34.00 ± 0 (34)	0 ± 0 (0)	0.018*
<i>Candida albicans</i>	0 ± 0 (0)	3.67 ± 0.58 (4)	0.023*
P	0.020*	0.008**	

Table 4: Comparisons of the fluoride gels according to bacterial zone diameters (mm).

* Mann Whitney U test; **Kruskal Wallis Test; * $p < 0.05$; ** $p < 0.01$

concentrates on the tooth plate, inhibiting carbohydrate metabolism. Thus, lactic acid production is reduced. At the same time, adhesive polysaccharides also affect the production of bacteria [14].

Malhotra., *et al.* in 2017, examined the effects of NaF, sodium MFB (0.35% and 0.38% concentration), AmF and calcium phosphate-containing pediatric toothpaste solutions on *S. mutans in vitro*. In the study results it was reported that the antibacterial properties of fluoride-containing toothpastes are better than those of fluoride-free toothpastes [14,15]. Our *in vitro* study also yielded consistent results with this study. Fluoride-containing dentifrices are effective in all oral pathogens tested, while fluoride-free dentifrices are either effective against several pathogens or are not effective against any pathogens.

Patil., *et al.* examined the effects of the toothpastes with the fluoride on oral microorganisms, especially on the reduction of *S. mutans*. All toothpastes used in the study have been reported to have antibacterial activity and it is stated that the presence of fluoride provides antimicrobial effects [16]. It appears that, fluoride-containing toothpastes show more antibacterial properties in our study.

Ostela., *et al.* were examined the antibacterial activity of dental gels containing combinations of amine fluoride, stannous fluoride, and chlorhexidine against *Streptococcus mutans*, *S. sobrinus* and *Lactobacillus casei*. At the results of the study, the most statistically significant antibacterial effect against *S. mutans* and *S. sobrinus* was observed with gel combination of AmF and SnF₂ (total fluoride content 1.2%). When compared to chlorhexidine alone, AmF as such or combined with SnF₂ was highly significantly less effective against cariogenic streptococci and the two gels did not differ significantly from each other [17].

Dilip., *et al.* were examined the antimicrobial activity of one aloe vera tooth gel and two commercial toothpastes. They demonstrated that aloe vera tooth gel without fluoride was equally effective as commercial toothpastes for controlling all of the organisms in their study. In our study different results were found with fluoride gels and fluoride gels showed less antimicrobial activity than toothpastes [6].

Wade., *et al.* compared the antimicrobial effects of two SF toothpaste products, two experimental SF plus stannous pyrophosphate toothpastes (SPSF1, SPSF2) an SF gel and a NaF toothpaste. In the study results, it was reported that all of the test pastes showed good activity against the bacteria except SnF gel [18]. In the present study, fluoride gels showed less antimicrobial activity than toothpastes and similar results were found with fluoride gels.

Malhotra., *et al.* in 2017, examined the effects of NaF, sodium MFB (0.35% and 0.38% concentration), AmF and calcium phosphate-containing pediatric toothpaste solutions on *S. mutans in vitro*. In the study results it was reported that the antibacterial properties of fluoride-containing toothpastes are better than those of fluoride-free toothpastes [14,15]. Our *in vitro* study also yielded consistent results with this study. Fluoride containing dentifrices are effective in all oral pathogens tested, while fluoride-free dentifrices are either effective against several pathogens or are not effective against any pathogens.

Randall., *et al.* examined *in vitro* the effect of 7 conventional dentifrices containing fluoride, 7 dentifrices containing herbal components and 3 different fluoride components (NaF, sodium MFP, stannous fluoride) on *S. mutans* in 2015. While all conventional dentifrices inhibit the development of *S. mutans*, only two of the plant-based toothpastes (SLS-free, fluoride-free) were found to be antibacterial. In addition, there was no bacterial inhibition zone in fluoride-containing vegetable toothpaste (containing fluoride). Only stannous fluoride antibacterial effect is found only in fluoride components. In our study, the bacterial efficacy of fluoride-containing toothpastes was found to be more effective than fluoride-free toothpastes. When we compare these two studies, different results can emerge due to the fact that the components in the dentifrices are not the same. In addition, the antibacterial activity of the fluoride gels used alone in our *in vitro* study shows similar results with this study. In both studies, the antibacterial activity of NAF alone was found to be inadequate [19].

Rossi., *et al.* natural extracts, chlorhexidine, triclosan and fluoride-containing toothpastes were examined for their activity on yeast, Gram-positive, Gram-negative bacteria using disk diffusion method. it has been reported that all toothpaste formulations except conven-

tional toothpaste (containing fluoride) exhibit antimicrobial activity against Gram-positive bacterial and yeast [20]. When we compare our study with our *in vitro* study, some differences are multiplied. In our *in vitro* study, fluoride containing dentifrices were found to be more effective against oral pathogens in working. The difference between these two studies is thought to be due to differences in other antimicrobial agents (SLS, chlorhexidine, triclosan, etc.) in toothpaste.

Adwan., *et al.* investigated the antifungal effects of dentifrices containing sodium fluoride, sodium monofluorophosphate and vegetable extract on *C. albicans in vitro*. According to the study results, they showed that all toothpastes inhibit *C. albicans* growth. They reported that toothpastes with herbal extract content had the same activity as toothpaste containing sodium fluoride. But both toothpastes containing both sodium fluoride and herbal extract had the highest anticandidal activity [21]. In our *in vitro* study, a single fluoride-containing toothpaste appears to be highly effective against *C. albicans*. Fluoride gels have been found to have no effect on *C. albicans*.

In this study fluoride-free and containing toothpastes were used. In addition, the efficacy of fluoride gels in addition to toothpastes was also included among the experimental groups *in vitro* study.

When the limitations of this *in vitro* study are emphasized, it should be kept in mind that the state of the oral cavity is different from the *in vitro* state. Many factors such as saliva, mucus layer, creatine levels, blood flow and normal flora can affect the protection of the oral cavity from harmful substances.

These limitations, as well as the differences in the other ingredients in toothpaste affect the results of the experiment. Studies have indicated that fluoride toothpaste results from the combined effect of fluoride-free components on a significant portion of the antimicrobial activity against *S. mutans* [19].

Thus, there is a need for more studies to standardize the differences of these differences in order to demonstrate the antimicrobial activity of toothpastes and fluoride gels.

Conclusion

This *in vitro* study demonstrated that tooth gel was as not effective as two commercially popular toothpastes in controlling all of the organisms used the study. Despite gels were found ineffective in this *in vitro* study, they will be given more positive results in clinical usage and *in vivo* studies. In addition, the non-fluoride toothpastes demonstrated less antimicrobial effect than fluoride gels. However, to guarantee these results and the effectiveness of these tooth care products, additional long-term clinical trials should be performed that incorporate more isolates from clinical samples.

Conflict of Interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

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