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Antimicrobial efficacy of antiplaque agents of common toothpastes against *Porphyromonas gingivalis* and *Streptococcus oralis*: An *in vitro* study

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ABSTRACT

Background: This study was designed to evaluate the antimicrobial activity of common gum protection and antiplaque toothpastes against *Porphyromonas gingivalis* (*P. gingivalis*) and *Streptococcus oralis* (*S. oralis*) as important periodontal pathogens.

Materials and Methods: This experimental study investigated the antimicrobial activity of 15 commonly used toothpastes from different companies on the two common types of periopathogens, *S. oralis* and *P. gingivalis*. The antimicrobial activity of toothpaste was evaluated at three concentrations of 100%, 50%, and 25% and analyzed by agar well diffusion plate method and zone of inhibition. The obtained data were compared and statistically analyzed by SPSS software using one-way ANOVA and the least significant difference *post hoc* tests ($\alpha = 0.05$).

Results: One-way ANOVA showed that the mean diameter of the two-bacterial zone of inhibition was significantly different at 100%, 50%, and 25% concentrations of toothpastes ($P < 0.001$). In general, the mean diameter of the zone of inhibition was greater at 100% concentration than the other two concentrations in all toothpastes. The highest zone of inhibition of the *S. oralis* was in the toothpastes containing tin. Further, the highest zone of inhibition of *P. gingivalis* was found in the triclosan-containing toothpastes.

Conclusion: Toothpastes containing triclosan had the most antimicrobial activity against *P. gingivalis*. Moreover, toothpastes containing tin compounds had the most antimicrobial effect against *S. oralis*.

Key Words: Bacteria, periodontal diseases, toothpastes

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INTRODUCTION

The use of toothbrushes and toothpastes is the most widely used oral hygiene method in the world.[1] So far, a range of chemical antimicrobial agents has been added to toothpastes to prevent the direct formation of plaque. The most important antiplaque compounds used in different types of toothpastes are chlorhexidine, tin salts, oil or vegetable extracts, sodium bicarbonate, and triclosan.[2] Dental plaque bacteria are divided into primary and secondary colonization classes. Primary colonizing bacteria are attached to the tooth surface through their receptors and provide receptors for binding other bacteria

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from the secondary colonizing class, which is called coadhesion. As clusters of microorganisms grow, small colonies are formed, and eventually matured biofilms are created. Each of the different species of bacteria has its own connective species.[3]

The role of *Porphyromonas gingivalis* (*P. gingivalis*) and *Streptococcus oralis* (*S. oralis*) as the major pathogens of these diseases has been documented.[4] *P. gingivalis* is an anaerobic and nonmigratory Gram-negative bacterium in the oral cavity. It is one of the important causes of periodontal disease, which is also found in the upper gastrointestinal tract and vagina in people with vaginitis. The bacterium creates black colonies in an agar blood culture.[5-7] *P. gingivalis* can produce various virulence factors, such as lipopolysaccharide, playing important roles in the pathogenesis of gingivitis and periodontitis. Within the oral biofilm, these bacteria can also trigger the host immune response and trigger the host to secrete cytokines such as interleukin (IL)-1β and IL-6.[8-10]

S. oralis, an important primary colonizing bacterium, is a nonmigratory Gram-positive bacterial α-hemolytic coinfection that belongs to the mitis group and is found in the oral cavity. *S. oralis* is a normal human oral flora and can be an opportunistic pathogen. It is one of the most abundant commensal bacteria in the oral cavity, which is considered a primary colonizer of dental plaque[11,12] and is known as one of the earliest bacteria involved in the formation of dental plaque.[13] Several studies have been conducted to investigate the antimicrobial effects of toothpastes. In an *in vitro* study, Ghapanchi *et al*. examined the cytotoxic and antibacterial effects of 16 Iranian toothpaste samples against *Streptococcus mutans*. All toothpastes showed an antistreptococcal effect, but the severity of this effect was not studied in this research.[14]

Although periodontal diseases are the most common oral infections, they are curable and preventable.[15] Reducing the prevalence and incidence of periodontal diseases decreases the systematic diseases and the complications and clinical conditions associated with them. Further, reducing the burden of periodontal diseases can decrease the treatment needs and thus reduce the costs of the health system. Identifying effective methods such as using toothpastes is one of the best ways to achieve this goal. For this purpose, this study was designed to evaluate the antimicrobial activity of the antiplaque agents of common gum protective and antiplaque toothpastes against these two bacteria, *S. oralis* as a primary colonizer and *P. gingivalis* as a secondary one. The antibacterial effect of the toothpastes investigated in this study and the type of their active ingredients have not been investigated in any study so far. The sample size of this study is larger than those of similar studies and is more comprehensive. In addition, bacteria have been investigated according to their role in primary and secondary plaque formation and in terms of being aerobic or anaerobic.

MATERIALS AND METHODS

This experimental study protocol was confirmed by the Isfahan University of Medical Sciences Research Committee and conducted at the Department of Microbiology, Faculty of Medicine, Isfahan University of Medical Sciences. A total of 15 samples of common toothpastes (three tubes from each sample) from different companies were obtained from valid pharmacies, and their antibacterial effect was investigated (three times to increase the accuracy) by the agar well diffusion method on two bacterial strains, including *S. oralis* IBRC-M 10630 (Iranian Biological Resource Center) and *P. gingivalis* (ATCC 33277). Each toothpaste was evaluated at three concentrations of 25%, 50%, and 100% (diluted with distilled water). Distilled water was used as a negative control in these experiments.

Using this method, five small wells with a depth of 5 mm and a diameter of 6 mm were created by the end of the pipette in each culture medium (inside a 10 cm plate), and the bottom of the wells was filled with molten culture medium (two drops).

Each toothpaste was prepared at three concentrations of 25%, 50%, and 100% (diluted with distilled water). To prepare a toothpaste with a concentration of 50%, 2 ml of toothpaste was mixed with 2 ml of distilled water. To prepare a concentration of 25%, 200 μl of preprepared 50% toothpaste was mixed with 200 μl of distilled water (poured with a sampler on an empty plate). The dilutions obtained were made homogeneous by a sterile looper and shaker. From the three concentrations obtained, 100 μ were added to the wells for inoculation by the sampler.

For the *S. oralis* bacterium, a culture medium of Mueller–Hinton agar was used. A suspension containing $10^8 \times 1.5$ bacteria in milliliter (equal to half McFarland) was prepared and cultured in triplicate using a sterile swab, then following which the wells were created. Then, different dilutions of 25%, 50%, and 100% toothpastes were inoculated into the wells of the culture medium. Next, they were placed in an incubator at 37°C for 24–48 h. Finally, the zone of inhibition was measured by a ruler.

A suspension of $10^8 \times 3$ bacteria/ml (equivalent to one McFarland) was prepared from an anaerobic bacterium *P. gingivalis*. Colombia agar containing hemin and Vitamin K was used. The wells were first prepared due to the susceptibility of anaerobic bacteria to the workplace oxygen. Then, in the shortest possible time, the bacteria were cultured using a sterile swab in three directions.

Next, the 25%, 50%, and 100% dilutions of the prepared toothpastes were inoculated into the wells of the culture medium. In order to provide an anaerobic condition, Plates were placed in an anaerobic jar, and oxygen was immediately replaced with nitrogen (80%), hydrogen (10%), and carbon dioxide (10%). The jars were placed at 37°C for 48–72 h, after which the jars were opened, and the zone of inhibition around each well was determined by the ruler. The obtained data were analyzed by IBM SPSS Statistic Version 22.0 (IBM, Armonk, NY, United States of America) using descriptive statistics, one one-way ANOVA test, and least significant difference *post hoc* test ($\alpha = 0.05$).

RESULTS

The results of ANOVA test showed that the mean diameter of the zone of inhibition of both bacteria at the three concentrations of 100, 50, and 25 was significantly different among the different types of toothpaste ($P < 0.001$). In general, in all toothpastes, the mean diameter of the zone of inhibition of 100% concentrations was greater than those of the other two concentrations.

According to Table 1, the highest mean diameter of the zone of inhibition of *S. oralis* was found in the Oral B All-Around Protection® toothpaste, and the lowest mean diameter was determined in the Kin Gingival® toothpaste. Furthermore, the highest mean diameter of the zone of inhibition of *P. gingivalis* was detected in Pooneh 3® toothpaste, and the lowest mean diameter of the zone of inhibition was found in Darugar 2® toothpaste.

The mean diameter of the zone of inhibition of both bacteria in the control group was the same and equal to zero. Further, according to Table 2, the mean diameter of the zone of inhibition of *S. oralis* was significantly higher in the toothpaste containing the effective tin material than in other toothpastes, and the mean diameter of the zone of inhibition of this bacterium was significantly lower in toothpastes containing chlorhexidine than in other toothpastes.

Moreover, the mean diameter of the zone of inhibition of *P. gingivalis* was significantly higher in toothpastes containing triclosan and tin than in other toothpastes, and the mean diameter of the zone of inhibition of this bacterium was significantly lower in toothpastes containing fluoride alone than in other toothpastes. The diameter of the zone of inhibition of other dilutions is given in Table 2.

DISCUSSION

The results of this study showed that toothpastes containing triclosan and tin had the highest antimicrobial activity against *P. gingivalis*. In a clinical trial, Seymour *et al*. studied the effect of triclosan toothpaste on periopathogenic bacteria and the progression of periodontitis in patients with cardiovascular problems. They reported that triclosan/copolymer slowed down the progression of periodontal disease in these patients but had little effect on the major subgingival periopathogens. They attributed this to the topical anti-inflammatory effects of toothpaste. This discrepancy between the findings of this study and those of the present study may be due to different concentrations and combinations of triclosan in the toothpastes of this study.[16] In this study, the agar well diffusion method was used to evaluate the microbial activity of the toothpaste. This method is used as a preliminary method to evaluate the microbial activity of various ingredients and compounds. Since the diffusion phenomenon depends on the structure and physical and chemical properties of the substance, such as the emission factor of the material, this method can qualitatively examine the antimicrobial activity.[17]

In research, Roopavathi *et al*. investigated the antimicrobial effects of seven common toothpastes at different concentrations against *S. mutans*, *Escherichia coli*, and *Candida albicans* and found that toothpastes containing the main constituents of triclosan and zinc sulfate had the highest zone of inhibition for *E. coli*. They concluded that toothpastes containing triclosan were more effective in controlling oral microflora. The

Table 1: The mean diameter of the zone of inhibition (mm) of both bacteria at 100%, 50%, and 25% concentrations in the examined toothpastes

Toothpaste	*Streptococcus oralis* (mean±SD)			*Porphyromonas gingivalis* (mean±SD)		
	100%	50%	25%	100%	50%	25%
2080 Proclinic®	15.56±1.13	12.33±1.22	11.44±1.88	14.78±2.17	12.00±0.71	10.89±0.60
2080 Promax®	15.44±0.88	13.44±1.88	11.33±1.00	13.89±1.69	10.78±0.44	11.11±0.93
Bass Complete®	16.78±2.95	14.11±2.37	14.78±1.92	19.89±1.36	20.78±1.09	30.11±0.60
Close up Fire-Freeze®	18.56±2.96	13.11±2.62	13.44±2.74	20.22±1.09	14.56±0.73	12.89±0.33
Crest Complete 7®	20.33±0.50	13.44±2.07	13.22±2.86	12.78±0.83	10.00±0.71	0
Darugar 2®	15.56±3.47	12.00±7.26	13.89±3.55	0	0	0
Himalaya Complete Care®	23.44±5.13	13.332.60	11.67±1.50	11.55±1.33	10.56±0.73	10.56±0.73
Kin Gingival®	12.11±6.86	0	0	11.89±0.93	10.22±1.10	10.89±0.60
Nasim®	15.89±1.27	13.33±5.22	14.11±2.80	10.11±0.33	10.00±00	10.44±0.73
Oral B Gum Protecion®	23.56±2.30	15.33±2.12	15.67±1.32	20.67±1.00	0	0
Oral-B All-Around Protection®	30.00±0.50	18.75±3.31	18.89±1.27	17.44±1.01	0	0
Pooneh 3®	21.56±2.35	19.78±0.44	15.11±2.98	28.44±1.51	20.67±0.87	20.56±0.73
Misswake total 8®	16.78±2.81	14.22±5.43	10.67±1.22	10.44±0.53	0	0
Signal complete 8®	23.22±1.92	13.22±2.11	10.89±1.36	12.44±1.51	13.89±0.60	10.56±0.73
Colgate total 12®	17.78±1.79	17.78±1.30	15.00±00	27.44±2.30	20.22±0.67	21.67±0.87
Negative control	0	0	0	0	0	0

SD: Standard deviation

Table 2: The mean diameter of the zone of inhibition (mm) of both bacteria at 100%, 50%, and 25% concentrations in the examined toothpastes with specific ingredients

Ingredients	*Streptococcus oralis* (mean±SD)			*Porphyromonas gingivalis* (mean±SD)		
	100%	50%	25%	100%	50%	25%
Triclosan	18.00±3.02	16.25±3.89	14.75±2.19	21.47±7.59	17.92±4.70	20.69±7.11
Chlorhexidine	12.11±6.86	0	0	11.89±0.93	10.22±1.09	10.89±0.60
Stannous	26.78±3.69	17.05±3.23	17.28±2.08	19.06±1.92	0	0
Zinc	20.89±3.41	13.17±2.31	12.17±2.48	16.33±4.20	14.22±0.73	11.72±1.32
Stannous and zinc	20.33±0.50	13.44±2.07	13.22±2.86	12.78±0.83	10.00±0.71	0
Fluoride	15.83±2.31	13.00±4.55	11.83±2.41	9.78±6.10	5.69±5.80	5.50±5.60
Essential oil	23.44±5.13	13.33±2.60	11.67±1.50	11.56±1.33	10.56±0.73	10.56±0.73

SD: Standard deviation

findings of this study are consistent with the results of the present study on the efficacy of triclosan.[18] In their study on the effect of active ingredients in oral detergents on salivary biofilms, Ledder *et al*. found that triclosan was the most potent active antimicrobial agent.[19]

In another *in vitro* study on the effect of toothpaste formulations on oral microflora, Ledder and McBain found that the toothpaste containing triclosan had the most antimicrobial effect on streptococcal and anaerobic species in growing plates and on Gram-negative anaerobic species and *Streptococci* in subgingival plaque.[20]

Jongsma *et al*. also found that toothpastes containing stannous fluoride reduced the incidence of *lactobacilli*, *S. oralis*, *Streptococcus mitis*, and *Streptococcus sangius* compared with other toothpastes.[21]

In the present study, the antimicrobial effects of triclosan-containing toothpastes and tin-containing toothpastes on *P. gingivalis* were significantly higher than those of other toothpastes, and the effect of fluoride-containing toothpaste was significantly lower. The toothpastes containing tin compounds had a significantly higher effect on *S. oralis* than other toothpastes, and toothpastes containing chlorhexidine had a significantly lower effect than others. Although chlorhexidine-containing mouthwashes are used as the gold standard for antimicrobial agents, the use of these toothpastes is difficult due to the inactivation of this compound in the presence of anionic components.[22]

The difference between the most effective and least active components of toothpastes in the two aerobic Gram-positive bacteria and Gram-negative anaerobic bacteria may be related to the inherent characteristics of bacterial species. The limitations and problems of the implementation of this study were the lack of access to other species, including other periopathogenic species. As a limitation of this study, the laboratory environment provides different bacterial growth conditions from the oral cavity; therefore, further *in vivo* studies are warranted to validate the results.

CONCLUSION

The comparison of the zone of inhibition in the agar well diffusion method indicated toothpastes containing triclosan and tin compounds had the most antimicrobial activity against *P. gingivalis*. Moreover, the results of this study indicated that toothpastes containing tin compounds had the most antimicrobial activity against *S. oralis*.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, and financial or non-financial in this article.

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