Dental Research Journal

Original Article

The comparison of antimicrobial effects of herbal and chemical agents on toothpaste: An experimental study

Fahime Kooshki[1], Fahimeh S. Tabatabaei[2], Sahar Tajik[3], Azadeh Aayan[1]

[1]Department of Pediatric Dentistry, [2]Department of Dental Biomaterials, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [3]Dentist, Tehran, Iran

ABSTRACT

Background: Nowadays, health-care companies use different antimicrobial agents in toothpastes to reduce oral microorganisms. The aim of this study was to investigate the antimicrobial effects of one Iranian herbal toothpaste in different concentrations compared with the chemical type on oral microorganisms *in vitro*.

Materials and Methods: In this experimental study, the antimicrobial effect of one Iranian herbal toothpaste in comparison with its chemical type at three concentrations of 1, 1:1, and 1:3 on *Streptococcus mutans* (SM), *Lactobacillus* (LB), and *Candida albicans* (CA), respectively, were studied by agar disc diffusion method. The microorganisms were cultured on 21 plates. Then, four sterile paper discs were placed on each plate and the extracts were placed on them in prepared concentrations and incubated at 37°C ± 0°C for 24 h. The diameter of the inhibition zone around the discs was then measured in millimeters and recorded two-way ANOVA, one-way ANOVA tests, and regarding the difference variances, Tamhane supplementary tests were used at the significance level of $P < 0.05$.

Results: According to the results of this study, the full concentration of Iranian herbal toothpaste on SM, LB, and CA microorganisms had higher antimicrobial effect than the other two concentrations. This difference was statistically significant ($P < 0.05$). Furthermore, all the three toothpastes at full concentration had the same antimicrobial activity ($P < 0.05$). The antimicrobial effect of herbal toothpaste decreased significantly compared with the chemical toothpaste while the concentration decreased ($P < 0.05$).

Conclusion: At full concentration, herbal and chemical toothpastes have the same antimicrobial effect, but by reducing the concentration, the antimicrobial effect of herbal toothpaste is reduced compared with the chemical one.

Key Words: Ant-ibacterial, chemical, herbal, toothpaste

Received: September 2017
Accepted: February 2018

Address for correspondence:
Dr. Azadeh Aayan,
Department of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail: az.aayan68@gmail.com

INTRODUCTION

Oral and dental health is an essential and nonintegrated part of general health[1] and plays an important role in the prevention of chronic diseases such as diabetes as one of the public health hazards.[2] Tooth decay, gingivitis, and periodontitis are the most common oral and dental health diseases which can be seen frequently in different age groups and in different parts of the world.[3] In the development of these diseases, various microorganisms, such as *Streptococcus mutans* (SM),

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Kooshki F, Tabatabaei FS, Tajik S, Aayan A. The comparison of antimicrobial effects of herbal and chemical agents on toothpaste: An experimental study. Dent Res J 2018;15:289-94.

have a significant effect.[4] This microorganism plays a key role in carbohydrate fermentation resulting in lactic acid production which leads to demineralization of enamel. This bacterium results in the enhancement of cariogenicity and easy absorption of carbohydrates by production of extracellular polysaccharide that is one of the components of dental plaque.[5,6] SM has been identified as a microorganism that initiates dental caries due to its adhesion to oral and dental tissues.[7] Since plaque-associted diseases tend to be localized, the use of local antimicrobial agents may be more effective than systemic use.[8] Brushing with toothpaste is the most common form of dental disease prevention in many countries around the world.[9] Today, in toothpastes, a wide range of chemical agents such as fluoride, which are mainly antimicrobial agents, have been added to create a direct inhibitory effect on plaque formation.[9-11] Several clinical studies have shown the inhibitory effect of antimicrobial toothpastes on oral and dental bacteria,[9,11,12] as well as reduction of toothbrush's microbial contamination.[11] When these agents are added to toothpastes, they eliminate microorganisms by destroying the cell wall and disrupting enzymatic activity. They also prevent bacterial accumulation and reduce the proliferation of microorganisms and inhibit their endotoxin release.[13,14] However, in the recent years, the use of herbal products against chemical products in toothpastes has become more popular among people due to fewer side effects and greater impact.[15] *Calendula* flowers and salvia are among the medicinal herbs that have been used in ancient medicine in Iran and the rest of the world and their anti-inflammatory and antimicrobial properties have been proven.[16,17] *Calendula* extract contains flavonoids, polyphenols, coumarins, essential oils, and fatty acid, which has wound healing, anti-microbial, and anti-inflammatory properties.[18-20] Salvia extract has spasmolytic, antiviral, antifungal, antibacterial, and antioxidant properties; its antimicrobial properties are due to the presence of its hydrocarbon, monoterpene, and sesquiterpene.[21,22] In this study, the antimicrobial effect of one Iranian herbal toothpaste that contains *Calendula* and sage clay extracts in comparison with its chemical type against SM, *Lactobacillus* (LB), and *Candida albicans* (CA) was studied *in vitro*.

MATERIALS AND METHODS

In this experimental study, the effect of two herbal and chemical toothpastes on three microorganisms of LB, SM, and CA was investigated. For better comparison and reduction of possible errors, a non-Iranian toothpaste (Crest cavity protection) was considered as a positive control and sterile distilled water as a negative control. To provide various concentrations of herbal and chemical toothpastes, three tubes were purchased from three different stores in Tehran. However, non-Iranian toothpaste was one type provided from a store. Then, from each of the toothpastes, three different concentrations of full, 1:1, and 1:3 were prepared. In order to obtain full concentration, after calibration of the scale (Sartorius, Germany) with a precision of 0.0001, 150 mg of toothpaste from each tube was transferred to a 6-cm plate and mixed with vortex mixer (Techne, South Korea) for 2 min. To prepare 1:1 concentration, 150 mg of each toothpaste with 150 ml deionized sterilized distilled water and, for 1:3 concentration, 150 mg of each toothpaste with 450 ml deionized sterilized distilled water, in a 6-cm plate, were mixed by vortex mixer for 2 min.

To incubate SM (ATCC35668), LB (ATCC39392), and CA (ATCC10231) microorganisms, brain–heart infusion broth, De Man, Rogosa and Sharpe agar (MRS). All microorganisms were incubated at 37°C ± 0.5°C for 24 h. After incubation, the concentrations of 1.5×10^8 colony-forming unit/ml (equivalent to the half-McFarland standard) were provided from broths using a spectrophotometer.

To determine the antimicrobial effect of the samples, a disc diffusion (agar) method was used.[23] Sterilized swabs were used for transferring LB, to MRS agar, SM, to the brain–heart infusion agar, and CA to the Sabouraud dextrose agar medium. The microorganisms were cultivated in their special medium near a flame. Then, in all media, the plates were divided into four equal parts, and the paper discs were stacked at the same distance. Each plate contained five discs, three discs containing 150 mg of three different concentrations of a toothpaste, a disc containing 150 ml of deionized distilled water (as a negative control), and an empty disc. Similarly, 21 plates were prepared for each microorganism. In order to grow lactobacilli and SM, the plates were incubated at 37°C ± 0.5°C for 24 h and CA plates were incubated at 37°C ± 0.5°C for 48 h. After 24 h, the LB and SM plates and, after 48 h, CA plates were removed from the jar, and the diameter of the inhibition zone (if present) around the discs was

measured by a ruler with a precision of 1 ml by a microbiologist. Results were reported as average. Data were analyzed by SPSS software version 16, two-way ANOVA, one-way ANOVA tests, and regarding the difference variances, Tamhane supplementary tests were used at the significance level of $P < 0.05$.

RESULTS

The mean and standard deviation of zone of inhibition for the microorganisms studied for the Iranian herbal and chemical toothpastes and positive control are summarized in Table 1. According to the results of this study, Iranian chemical and herbal toothpastes at full concentration had the highest mean diameter of inhibition zone for LB and CA microorganisms, while for SM, the highest mean diameter of the inhibition zone for herbal toothpaste was at full concentration and for chemical toothpaste it was at a concentration of 1:3.

Table 1: Descriptive statistics regarding the diameter of the inhibition zone for microorganisms at different concentrations of toothpastes (mm)

Toothpaste type	Microorganisms (mean±SD)		
	Lactobacillus	*Streptococcus mutans*	*Candida albicans*
Iranian herbal toothpaste			
Full	1/85±11/22	0/97±14/78	1/80±15/33
1:1	2/69±1/33	5/78±7/67	2/29±7
1:3	2/40±1/56	0/72±10/44	3/34±6/22
Iranian chemical toothpaste			
Full	3/67±12/67	1/16±14/11	2/69±14/67
1:1	1/64±1/22	1/64±14/22	2/64±13
1:3	1/76±1/11	2/39±15/33	3/15±12/22
Positive control non-Iranian toothpaste			
Full	1/52±3/67	0/57±12/33	1/15±10/33
1:1	0/54±1/65	1±11	1/15±11/67
1:3	1/15±3/33	0/57±12/67	2±12

SD: Standard deviation

Based on the results, the antimicrobial effect of full-concentration herbal toothpaste on all microorganisms was significantly higher than the concentrations of 1:1 and 1:3 ($P < 0.05$). However, in the positive control toothpaste, antimicrobial effect of all the three concentrations was the same in all the three microorganisms. The antimicrobial effects of all the three concentrations of Iranian chemical toothpaste on SM and CA were similar, but the effect of full-concentration chemical toothpaste on the LB was higher than that of the other concentrations [Table 2]. As shown in Table 3, at full concentration, the herbal and chemical toothpastes have the same antimicrobial effect on SM, LB, and CA microorganisms ($P < 0.05$), while in 1:1 concentration, the chemical toothpaste in comparison with the herbal toothpaste against SM ($P < 0.028$) and CA ($P < 0.001$) microorganisms has higher antimicrobial effect. Chemical toothpaste also has higher antimicrobial effect at 1:3 concentrations on SM and CA microorganisms compared with the herbal toothpaste. Based on the results, at full concentration, the antimicrobial effect of the herbal toothpaste was higher than positive control on SM ($P < 0.005$), LB ($P < 0.005$), and CA ($P < 0.005$) microorganisms and, at 1:3 concentration, the antimicrobial effect of positive control toothpaste was higher than that of the herbal one on SM and CA microorganisms. Herbal and positive control toothpastes at the concentration of 1:1 have the same antimicrobial effect on SM and LB microorganisms. The findings also indicate that the antimicrobial

Table 2: The comparison of antimicrobial effects of different toothpaste concentrations on microorganisms

Toothpaste type	Concentrations and microorganisms				
	Concentrations		*Lactobacillus* (*P**)	*Streptococcus mutans* (*P**)	*Candida albicans* (*P**)
	Concentration (A)	Concentration (B)			
Iranian herbal toothpaste	Full	1:1	0/018	0/018	0/000
	Full	1:3	0/000	0/000	0/000
	1:1	1:3	0/468	0/468	0/544
Iranian chemical toothpaste	Full	1:1	0/000	0/998	0/496
	Full	1:3	0/00	0/479	0/262
	1:1	1:3	0/260	0/612	0/925
Positive control	Full	1:1	0/151	0/350	0/649
	Full	1:3	0/989	0/888	0/994
	1:1	1:3	0/109	0/227	0/612

*Test Tamhane

Table 3: The comparison of antimicrobial effects of toothpastes in different concentrations on the diameter of the inhibition zone of the studied microorganisms

Concentration	Microorganism	Toothpaste (A)	Toothpaste (B)	*P**
Full	SM	Herbal Iranian	Chemical Iranian	0/501
		Herbal Iranian	Positive control	0/005
		Chemical Iranian	Positive control	0/027
	LB	Herbal Iranian	Chemical Iranian	0/676
		Herbal Iranian	Positive control	0/005
		Chemical Iranian	Positive control	0/001
	CA	Herbal Iranian	Chemical Iranian	0/907
		Herbal Iranian	Positive control	0/005
		Chemical Iranian	Positive control	0/012
1.1	SM	Herbal Iranian	Chemical Iranian	0/028
		Herbal Iranian	Positive control	0/345
		Chemical Iranian	Positive control	0/020
	LB	Herbal Iranian	Chemical Iranian	0/440
		Herbal Iranian	Positive control	0/440
		Chemical Iranian	Positive control	0
	CA	Herbal Iranian	Chemical Iranian	0/000
		Herbal Iranian	Positive control	0/006
		Chemical Iranian	Positive control	0/595
1.3	SM	Herbal Iranian	Chemical Iranian	0/001
		Herbal Iranian	Positive control	0/013
		Chemical Iranian	Positive control	0/035
	LB	Herbal Iranian	Chemical Iranian	0/961
		Herbal Iranian	Positive control	0/335
		Chemical Iranian	Positive control	0/143
	CA	Herbal Iranian	Chemical Iranian	0/004
		Herbal Iranian	Positive control	0/032
		Chemical Iranian	Positive control	0/999

*Test Tamhane. SM: *Streptococcus mutans*, LB: *Lactobacillus*, CA: *Candida albicans*

effect of the full concentration of chemical toothpaste on SM ($P < 0.027$), LB ($P < 0.001$), and CA ($P < 0.012$) microorganisms is higher than the positive control. At 1:1 and 1:3 concentrations, the antimicrobial effect of the chemical toothpaste was significantly higher than the positive control on SM microorganisms ($P < 0.05$) [Table 3].

DISCUSSION

In the present study, the antimicrobial effect of Iranian herbal, chemical, and Crest cavity protection toothpastes was compared. The results showed different responses at different concentrations against SM, LB, and CA microorganisms. Based on the results of this study, at different concentrations of herbal toothpaste, the full concentration has more antimicrobial effect on three microorganisms at concentrations of 1:1 and 1:3 ($P < 0.05$). Iranian chemical toothpaste showed the same antimicrobial effect at all concentrations against SM and CA microorganisms, while for LB, its full concentration was more effective. Positive control toothpaste also showed the same antimicrobial effect on all the three microorganisms at all the three concentrations ($P > 0.05$). According to the results of this study, at full concentration, the antimicrobial effect of the herbal and chemical Iranian toothpastes is similar on SM, LB, and CA microorganisms, but by decreasing the concentration, their antimicrobial effect decreases as well. Similar to this finding, Prasanth has reported that reducing toothpaste concentrations may reduce the antimicrobial effects of toothpastes on CA and SM.[24] In addition, in the study conducted by Sadeghi and Assar, the antimicrobial effect of toothpastes decreased significantly in fluoride-containing toothpastes while the concentration decreased.[25] However, in the study by Shaheen *et al*., the reduction in toothpaste concentration did not affect the antimicrobial properties of toothpastes.[26] The findings of this study showed that at full concentration, the antimicrobial effect of herbal and chemical toothpastes on SM, LB, and CA microorganisms is similar. While Gibraiel *et al*. showed that natural agents-containing

toothpastes have higher antimicrobial effect than triclosan chemical toothpastes against *E. coli* and CA,[3] this may be due to the herbs used. Furthermore, in a study by Bhat *et al*. on CA and *S. sanguis* microorganisms, it has been reported that herbal toothpastes have antimicrobial activity only against SM microorganisms.[27] Teh JY *et al.* also reported that there is no significant difference in antimicrobial effect between herbal mouthwashes on SM, *Streptococcus sobrinus, Lactobacillus salivarius, Staphylococcus aureus*, CA, and *Pseudomonas aeruginosa* ($P < 0.05$).[28] In this study, agar diffusion method (disc) has been used because it is the most common method to study the antimicrobial properties of oral and dental products before *in vivo* studies. In studies by Leyster,[29] Sadeghi and Assar,[25] Bhat *et al*.,[27] de Camargo Smolarek *et al*.,[30] and Shaheen *et al*.,[26] the same method was used to study the antimicrobial properties of toothpastes and mouthwashes. *In vivo* studies, oral and dental products are diluted by saliva, which may dilute their antimicrobial agents so that the concentrations of 1:1 and 1:3 in this study are similar to the oral state, in which toothpaste is diluted by saliva. Several studies have also used different concentrations of toothpastes.[25,26] In this study, by reducing the concentration of the Iranian herbal toothpaste, its antimicrobial effects are reduced as well, while in Crest toothpaste; this is not the case, which is probably due to the specific formulation of Crest toothpaste In such a way, the minimum inhibitory concentration and minimum bactericidal concentration are lower than the concentrations studied in this experiment, so it might be better to make changes in the formulation of the herbal toothpaste, which, despite its dilution, keeps its antibacterial properties. Different results from different studies indicate the effectiveness of herbal toothpastes in preventing dental decay.[26,27] In the same field, Shaheen *et al*., after studying the effect of antimicrobial activity of 12 herbal toothpastes on CA, SM, *Streptococcus sanguinis*, *Actinomyces viscosus*, *Streptococcus pyogenes,* and *S. aureus* microorganisms, suggested that herbal toothpastes can be effective in improving oral and dental health, similar to other toothpastes.[26]

Limitations

One of the limitations of this study is its implementation in laboratory conditions. Therefore, the results cannot be generalized to clinical conditions (*in vivo*).

CONCLUSION

According to the results of this study, all the three herbal, chemical, and Crest cavity protection toothpastes have antimicrobial activity against all the three SM, LB, and CA microorganisms. In addition, the findings show that at full concentration herbal toothpastes have the most antimicrobial effect, while decreasing the concentration decreases its antimicrobial effect when compared with chemical toothpastes. This study shows that the mere presence of these toothpastes inhibits the growth of SM, lactobacilli, and CA, which leads to the reduction of biofilm formation.

Financial support and sponsorship

Nil.

Conflicts of interest

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

REFERENCES

1. Fernando S, Bakr MM, Kanthi RF. Improving maternal oral health knowledge and practices: An intervention from Sri Lanka. J Res Med Dent Sci 2015;3:249-55.
2. U.S. Department of Health and Human Services. Healthy People 2020: Understanding and Improving Health. Washington, DC: U.S. Government Printing Office (GPO); 2011.
3. Gibraiel F, Rajput M, Rajput MS, Singh M, Saxena N, Vishal A, Kumar A. *In vitro* study to investigate the antimicrobial efficacy of different toothpastes and mouthrinses. Res J Pharm Biol Chem Sci 2014;5:245-57.
4. Chatterjee T, Das SM. Antimicrobial efficacy of some medicinal plant extract against *Streptococcus mutans* causing dental caries. J Med Plants 2017;5:315-7.
5. Oztan MD, Kiyan M, Gerçeker D. Antimicrobial effect, *in vitro*, of gutta-percha points containing root canal medications against yeasts and *Enterococcus faecalis*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:410-6.
6. Garcia SS, Blackledge MS, Michalek S, Su L, Ptacek T, Eipers P, *et al.* Targeting of *Streptococcus mutans* biofilms by a novel small molecule prevents dental caries and preserves the oral microbiome. J Dent Res 2017;96:807-14.
7. van Houte J. Role of micro-organisms in caries etiology. J Dent Res 1994;73:672-81.
8. Lobo PL, de Carvalho CB, Fonseca SG, de Castro RS, Monteiro AJ, Fonteles MC, *et al.* Sodium fluoride and chlorhexidine effect in the inhibition of mutans streptococci in children with dental caries: A randomized, double-blind clinical trial. Oral Microbiol Immunol 2008;23:486-91.
9. Pannuti CM, Mattos JP, Ranoya PN, Jesus AM, Lotufo RF, Romito GA, *et al.* Clinical effect of a herbal dentifrice on the control of plaque and gingivitis: A double-blind study. Pesqui

Odontol Bras 2003;17:314-8.
10. Nóbrega DF, Fernández CE, Del Bel Cury AA, Tenuta LM, Cury JA. Frequency of fluoride dentifrice use and caries lesions inhibition and repair. Caries Res 2016;50:133-40.
11. Fine DH, Furgang D, Markowitz K, Sreenivasan PK, Klimpel K, De Vizio W, *et al*. The antimicrobial effect of a triclosan/copolymer dentifrice on oral microorganisms *in vivo*. J Am Dent Assoc 2006;137:1406-13.
12. Efstratiou M, Papaioannou W, Nakou M, Ktenas E, Vrotsos IA, Panis V. Contamination of a toothbrush with antibacterial properties by oral microorganisms. J Dent 2007;35:331-7.
13. Listgarten MA. The role of dental plaque in gingivitis and periodontitis. J Clin Periodontol 1988;15:485-7.
14. Ozaki F, Pannuti CM, Imbronito AV, Pessotti W, Saraiva L, de Freitas NM, *et al.* Efficacy of a herbal toothpaste on patients with established gingivitis – A randomized controlled trial. Braz Oral Res 2006;20:172-7.
15. Smullen J, Finney M, Storey DM, Foster HA. Prevention of artificial dental plaque formation *in vitro* by plant extracts. J Appl Microbiol 2012;113:964-73.
16. Lauten JD, Boyd L, Hanson MB, Lillie D, Gullion C, Madden TE, *et al.* A clinical study: *Melaleuca*, *Manuka*, *Calendula* and green tea mouth rinse. Phytother Res 2005;19:951-7.
17. Kermavshah H, Hashemi Kamangar S, Arami S, Mirsalehian A, Kamalinejad M, Karimi M, *et al*. *In vitro* evaluation of antibacterial activity of hydroalcoholic extract of *Salvia officinalis* and *Pimpinella anisum* against cariogenic bacteria. JIDA 2009;22:149-54.
18. Saini P, Al-Shibani N, Sun J, Zhang W, Song F, Gregson KS, *et al.* Effects of *Calendula* officinalis on human gingival fibroblasts. Homeopathy 2012;101:92-8.
19. Faria RL, Cardoso LM, Kisue G, Pereir CA, Junqueira JC, Jorge O, *et al*. Antimicrobial activity of *Calendula officinalis*, *Camellia sinensis*, and chlorhexidine against the adherence of microorganisms to sutures after extraction of unerupted third molars. J Appl Oral Sci 2011;19:476-82.
20. Amoian B, Moghadamnia AA, Mazandarani M, Amoian MM, Mehrmanesh S. The effect of *Calendula* extract toothpaste on the plaque index and bleeding in gingivitis. Res J Med Plant 2010;4:132-40.
21. Beheshti-Rouy M, Azarsina M, Rezaie-Soufi L, Alikhani MY, Roshanaie G, Komaki S, *et al.* The antibacterial effect of sage extract (*Salvia officinalis*) mouthwash against *Streptococcus mutans* in dental plaque: A randomized clinical trial. Iran J Microbiol 2015;7:173-7.
22. Sookto T, Srithavaj T, Thaweboon S, Thaweboon B, Shrestha B. *In vitro* effects of *Salvia officinalis* L. essential oil on *Candida albicans*. Asian Pac J Trop Biomed 2013;3:376-80.
23. Teke GN, Enongene NG, Tiagha AR. *In vitro* Antimicrobial Activity of Some Commercial Toothpastes. Int J Curr Microbiol App Sci. 2017;6(1):433-46.
24. Prasanth M. Antimicrobial efficacy of different toothpastes and mouthrinses: An *in vitro* study. Dent Res J (Isfahan) 2011;8:85-94.
25. Sadeghi M, Assar S. An *in vitro* antimicrobial activity of ten Iranian-made toothpastes. Dent Res J (Isfahan) 2009;6:87-92.
26. Shaheen SS, Reddy P, Reddy S, Doshi D, Kulkarni S, Kumar M. Antimicrobial efficacy of ten commercially available herbal dentifrices against specific oral microflora – *In vitro* study. J Clin Diagn Res 2015;9:42-6.
27. Bhat SS, Sundeep HK, Ratheesh MS. Comparison of antimicrobial potential of various herbal dentifrices. Int J Dent Med Res 2014;1:41-5.
28. Ju Ying Teh, Rabiah R, Siti Suraiya N, Haslina T, Suharni M. *In-vitro* antimicrobial effectiveness of herbal-based mouthrinses against oral microorganisms. Asian Pac J Trop Biomed 2015;5:370-4.
29. Leyster CW. AN investigation of the levels of antimicrobial efficacy in commercial dentifrices on *Streptococcus mutans* and *Lactobacillus*. Sain Martin's Univ Biol J 2006;1:155-68.
30. de Camargo Smolarek P, Esmerino LA, Chibinski AC, Bortoluzzi MC, Dos Santos EB, Kozlowski VA Jr., *et al. In vitro* antimicrobial evaluation of toothpastes with natural compounds. Eur J Dent 2015;9:580-6.