Comparison of the Antibacterial Effects of Chlorhexidine Mouth washes with Jaftex Mouth wash on Some Common Oral Microorganisms (An in Vitro Study)

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Abstract

Background and Objectives: Mouth washes with anti-inflammatory and anti-plaque properties are recommended to maintain good oral hygiene. Thus the aim of this study was to compare the antibacterial effects of chlorhexidine mouth wash (CHX) with Jaftex mouth wash.

Materials and Methods: In this in vitro study, the disc diffusion method was used to measure inhibition zone on tested mouth washes on streptococus mutans, s.sanguis, s. salivarius and lactobacillus casei. The tube dilution method was used for determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). Results were analyzed by using ANOVA test. (P < 0/05 was considered significant).

Results: The CHX mouth wash significantly exhibited greater inhibition zone than Jaftex. The MICs for CHX and Jaftex were 2 and 20 micrograms/ml for S. mutans , respectively. The MBCs for the mentioned mouth washes were 20 and 200 micrograms/ml for S.mutans, respectively.

Discussion and Conclusion: Jaftex mouth wash was less potent than the CHX in inhibiting growth on oral microorganisms and it is recommended to be used for plaque inhibition.

Key words: Chlorhexidine; Jaftex; Mouth wash; Oral Microorganisms

Please cite this article as: Please cite this article as: Ebrahim Babadi, Zahra Bamzadeh, Fatemeh Babadi. Comparison of the Antibacterial Effects of Chlorhexidine Mouth washes with Jaftex Mouth wash on Some Common Oral Microorganisms (An in Vitro Study) *World Family Medicine.* 2017; 15(9):200-203
DOI: 10.5742/MEWFM.2017.93125

Background

Medicine in Iran has a history of thousands of years (1). Dentistry is one of the most favorite fields of study in Iran (2-3). There are about 500 species of bacteria in the mouth, some of which cause mouth infectious diseases. (4-6). The mouth is a perfect environment for colonization and growth of a wide range of microorganisms especially bacteria (7). The bacterial plaque is a predisposing factor in destruction of the teeth and periodontal tissue (8). Mouth rinses will reduce bacterial plaque. Chlorhexidine (CHX) has been known as a gold standard for controlling plaque (9-11). Long-term use of (CHX) causing complications such as dental stain, changes in taste and dry mouth (12). The use of herbal medicines in recent years due to antibacterial and antifungal effects and less side effects for oral health has been common (4,9,12). Herbal mouth washes, due to having natural compounds in terms of compatibility with the body's physiology and less poisoning, has a better condition than CHX and is recommended for people who do not have the possibility of using chemical mouth rinses (13-14). Persian oak is one of the medicinal plants and its many treatment effects are listed (15). Antimicrobial properties of various species of Persian oak in various studies have been mentioned (16). A thin membrane that covers the oak is called jaft. Jaft has a great effect in the treatment of bacterial and viral diseases such as oral aphthous lesions (15). Jaftex is a new herbal mouth wash that has been prepared in the pharmaceutical research center of Ahvaz Jundishapur University of medical sciences. Jaftex is a combination of extract of oak Jaft (Oak Fruit) as a basis, extracts of Zataria Multifiu and Saturej Bachtiarica.

Objectives

This study aimed to compare the antibacterial effects of Cholorhexidine mouth wash and Jaftex on some common oral microorganisms.

Materials and Methods

This study is an in vitro study. The mouth wash of CHX 2/0% (Iran Nazhvan) was used in this study. To prepare Jaftex aqueous extracts of oak, Zataria Multifiu and Saturej Bachtiarica were taken and after combining the extracts 9 grams of sodium chloride were added and with distilled water reached to one m/l. To prepare bacterial suspensions, bacterial vials were purchased from collection of fungi and bacteria Iran (Pasteur) which included: Streptococcus mutans (PTCC 1683), S. sanguinis (PTCC 1449), S. salivarius (PTCC 1448) and Lactobacillus casei (PTCC 1608). According to the manufacturer's instructions they were dissolved in sterile saline. Following that bacterial suspension was cultured on solid medium (blood agar, Merck Germany) and incubated at 37 ° C for 24 - 48 hours. A colony was isolated from fresh cultures of bacteria and was dissolved in saline until approximate concentration of 1.5 × 10^8 cfu (equal to n: 05 Mcfarland Standrd) was obtained. Suspension of any bacteria was cultured on medium (MHA). Using dilute method, 2 ml of each mouth wash was dissolved in 2 ml of distilled water until the first concentration reached 1 mg per ml. To obtain the second concentration (5.0 mg per ml), the amount of 1 cc of this solution was dissolved with 2 ml of distilled water, and so the next concentrations (0.25-0.125 - 0.0625) for both chlorhexidine mouth wash and Jaftex were obtained, respectively. The blank disks on each medium were placed in a row and mouth washes were cultured on the disks from the highest to the lowest concentration and were incubated at 37 ° C for 24 hours. After disks of bacteria Streptococcus mutans, Streptococcus sanguinis, Streptococcus salivarius and Lactobacillus casei were cultured, for each concentration of chlorhexidine and Jaftex and were evaluated, and inhibition zone was measured using Antibiogram ruler. Then the two mouth washes without dilution and with standardized dilutions (chlorhexidine 0/2% and Jaftex) were cultured on the above microorganisms similar to the above method. Then minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each mouth washes were determined. so that each tube, 8 ml of medium, 1 ml bacteria and 1 ml of mouth wash were added and then 1 ml removed from the first tube and was added to the second tube and so on until the fourth tube reached concentrations of 200-20-2-0.2) micrograms per milliliter, respectively. Then they were incubated at 37° C for 24 hours, then the transparency of the tubes was checked visually. Tubes without turbidity, indicated the inhibition of bacterial growth. The tube that showed the highest concentration of residual turbid mouth wash was MIC. The respective mouthwashes were transferred to a solid medium (blood agar, Merck Germany) and were evaluated in terms of microbial growth to determine the MBC of mouth washes. The last tube which was negative in terms of culture on solid medium, indicated the MBC of mouth washes. This procedure was performed for all bacterial strains. This test was performed for all four target bacteria. The data were analyzed with ANOVA test using SPSS software version 13.0. P value less than 0.05 was considered significant.

Results

The CHX mouth wash significantly exhibited greater inhibition zone than the Jaftex mouth wash (P = 0/010). CHX mouth wash at all dilutions showed antibacterial effects. But Jaftex mouth wash in concentrations of 0/0625 and 0/125 didn't have antibacterial effects on S.Salivarius and for Jaftex in 0/0625 dilution, its inhibition zone on L.casei was zero. The MICs of CHX and Jaftex for S.mutans were 2 and 20 micrograms/ ml, respectively (Tables 1 and 2). The differences between mouth washes were significant (P value = 0.005). The MBCs of CHX and Jaftex for S. mutans, were 20 and 200 micrograms /ml, respectively (Tables 1 and 2). The differences between mouth wash were significant (P value = 0.005). The MICs and MBCs against the other bacterial microorganisms are shown in (Table 1 and 2). The lowest level of MICs for all bacteria was related to CHX. Among the above microorganisms, S. mutans showed the highest resistance to CHX and Jaftex mouth wash. The MICs and MBCs of Jaftex for L.casei were zero (Table 2).

Table 1: Comparison of the Levels of MIC and MBC (micrograms per ml) of Chlorhexidine on Oral Microorganisms

MIC	MBC	Bacteria
2	20	S. mutans
0/2	2	S. sanguinis
0/2	2	S. salivarius

Table 2: Comparison of the Levels of MIC and MBC (micrograms per ml) of Jaftex on Oral Microorganisms

MIC	MBC	Bacteria
20	200	S. mutans
0/2	20	S. sanguinis
0/2	2	S. salivarius
0/2	0	L. casei

Discussion and Conclusion

The present study showed that CHX mouthwash and Jaftex inhibit bacterial growth, and also there were significant statistical differences between the two mouth washes. The findings of the present study are similar to other studies that have been done in this field. According to some studies, the CHX mouth wash has shown greatest antibacterial effects on oral microorganisms than the other mouth washes (17, 18). Based on inhibition zone, CHX mouth wash has most antibacterial effects on bacteria of S. mutans, S. sanguinis and S. salivarius. Sadeghi et al. reported that CHX 0.2% had most effects on S. sanguinis, S. sobrinos, S. mutans and S. salivarius and it had least effects on pseudomonas aeruginosa (19). In this study, according to the MICs and MBCs, S. mutans had most resistance to CHX and Jaftex mouth washes. Review of literature show conflicting results about the antibacterial effects of CHX on S. mutans. The results listed below are similar to our results. Jarvinen et al. in a study examined the effectiveness of CHX on S. mutans and reported that S. mutans was more resistant to antimicrobial agents (20). Yousefimanesh et al. also confirmed that S. mutans showed resistance to CHX (21). Salehi et al .reported that CHX mouth washes were more effective on S. mutans than Persica mouth washes (22). Lactobacilluses are microorganisms that play a role in the pathogenesis of dental caries and mechanical or chemical elimination of them is effective in prevention of dental decay (23). Kohler et al. examined the effects of CHX on streptococci and Lactobacillus, and concluded that CHX mouthwash may reduce dental plaque microorganisms and antibacterial effects on streptococci are more effective compared with Lactobacillus (24). Our study also confirmed results of Kohlre et al. The results of this study showed that the Jaftex mouth wash has antibacterial effects on microorganisms of S. mutans, s. anguis, salivarius, but not Lactobacillus casei. But the antibacterial effects of Jaftex are less than CHX mouth wash. The present study is the first research which surveys the antimicrobial effects of Jaftex (as a mouth wash) on common oral microorganisms. But antibacterial effects of oak and its fruit have been proven. Ebrahimi et al. conducted a study to evaluate the effects of antibacterial hydroxy extract of oak (Jaft) compared to a number of antibiotics on four bacteria: staphylococcus aureus, epidermidis, Escherichia coli and saprophyticus and reported the Persian oak has compounds with antibacterial properties and its inhibitory effects on bacteria is concentration-dependent (25). Ebraham et al. noted that Persian oak has antibacterial effects and antibacterial properties of it are due to tannins present in the extract (26). Hefaji et al. reported that herbal mouth washes have less antimicrobial effects on microorganisms than the CHX mouth wash. But the components of the herbal mouth wash are effective in preventing the growth of bacteria in the mouth and can be helpful in controlling dental plaque and inflammation (9). In this study two methods (Disc Diffusion and Tube dilution) simultaneously used to investigate antibacterial effects two mouth wash on oral microorganisms. The outstanding point in the present study was the above methods.

Acknowledgements

This project was conducted by financial support from the Vice-Chancellor for Research Affairs of Ahvaz Jundishapur University of Medical Sciences; thereby authors express thanks for the financial support. (No: GP95031)

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