

## Comparison of the antibacterial effects of persica mouthwash with chlorhexidine on streptococcus mutans in orthodontic patients

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### ABSTRACT

Chlorhexidine mouthwash has shown the highest antimicrobial effects in orthodontic patients, however, some complications have limited its widespread use. The goal of this study was to compare the antimicrobial effects of persica mouthwash with chlorhexidine in fixed orthodontics patients.

Sixty patients (13-18 years old) on fixed orthodontic treatment, with desirable oral health were randomly divided into three equal groups of control, chlorhexidine and persica. Patients were educated to properly use these mouthwashes. The control group was asked to wash their mouth with water twice a day. The numbers of streptococcus mutans colonies available in the elastic rings around the two bracket bases were determined in culture mediums before and immediately after a single application of water or mouthwashes and also following their daily uses for three weeks. The number of bacterial colonies then were compared at these three groups.

The use of persica resulted in a significant ( $p < 0.001$ ) reduction in the number of streptococcus mutans colonies, albeit it was not as potent as chlorhexidine. Thirteen and forty percent of patients using persica mouthwash experienced tooth discoloration and changes in taste respectively. The corresponding figures for chlorhexidine were 86% and 73% respectively.

Significant reduction of streptococcus mutans colonies by persica as well as its lower tooth discoloration effects and unpleasant taste relative to chlorhexidine might give credence to the use of complementary herbal compounds in orthodontics patients.

**Keywords:** Chlorhexidine, Persica, Antimicrobial effects

### INTRODUCTION

The presence of fixed appliances in the mouth for one to two years is usually associated with the multiplication of decaying bacteria (1-2). The presence of such bacteria leads to oral health problems such as vast primary lesions and progressive enamel loss after the removal of fixed orthodontic appliances (3).

Mouthwashes are very useful in reduction of microbial plaques. Among available mouthwashes, chlorhexidine was shown to be highly effective in reduction of dental plaques and pathogenic microorganisms including streptococcus mutans. Nowadays, in most studies on mouthwashes, chlorhexidine is used as a positive control to compare the efficacy of other products, since it is believed that chlorhexidine is a gold standard (4-7). However, the incidence of side effects such as undesirable tooth discoloration, unpleasant taste, dryness and burning sensation in the mouth discourage patients to use this mouthwash (8-9). Recently, the use of herbal mouthwashes such as

persica is increasing. Persica is prepared from *Salvadora persica* extract. It has been shown that using this herbal medicine or its extract would support periodontal health, and reduces the accumulation of microbial plaques (10), and bleeding during brushing (11,12), and controls gingivitis and periodontal diseases (11-13). Previous studies have shown that Persica decreased the number of streptococcus mutans colonies and thereby dental decay problems (12,14-16). Some studies have pointed that both persica and chlorhexidine could significantly decrease gingival inflammation, without significant differences between their effects (17). Another study did show that there was no significant differences between persica and chlorhexidine in the reduction of the number of streptococcus mutans colonies in saliva (18). Considering the side effects of chlorhexidine and promising effects of persica mouthwash, this study was designed to compare the effects of persica with those of chlorhexidine on the

colonies of streptococcus mutans in microbial plaques around the bracket bases in orthodontic patients.

#### Subjects

Sixty fixed orthodontic patients were selected from 312 orthodontic patients referring to orthodontic clinic of Shiraz University of Medical Sciences from March to September 2005. The inclusion criteria were an age between 13-18 years and a normal gingiva without inflammation, bleeding, discoloration, and microbial plaque on teeth. The exclusion criteria were prior use of any antibiotic or mouthwash for 10 consecutive days in the last three months, a history of sensitivity to any mouthwash, the use of corticosteroid in the last three months, severe gingival inflammation during the study, and poor cooperation reported by parent of subjects.

#### Methods

Patients were randomly divided into three groups of control, chlorhexidine and persica. Oral B orthodontic toothbrushes and toothpaste were given to all patients after bonding. All patients were educated to brush their teeth with Horizontal-Scrub method at least twice a day for 2 to 4 minutes. The control, chlorhexidine and persica groups were instructed to use water, chlorhexidine or persica as mouthwash twice daily. They were advised to use them according to the instructions on the brochures after a thorough explanation. Persica (Poursina Company, Batch No. 80-s-p-061) was administered as 15 drops in 15 ml of water twice a day for 20 second, and chlorhexidine 0.2% (Shahr Daru, Batch No. 72-SH-019) as 15 drops for 30 second, twice a day. The participants were recommended to avoid any food or drink during one hour after using the mouthwashes.

Bacterial sampling was carried out three times. The first sampling (T1) was done four weeks after bonding. For sampling, the elastic rings around the brackets of upper left canine and premolar teeth were removed and immersed in thioglycolate media. Afterwards, the subjects were asked to wash their mouth with water or chlorhexidine or persica mouthwashes. Then the elastic rings of the right side were also removed immediately and immersed in thioglycolate media (T2). The third sampling (T3) was done 20 days after T1 following the use of persica or mouthwashes. For sampling the elastic rings of the right canine and premolar were removed and were similarly immersed in the same media. At T3, the presence or absence of any dental discoloration and change in taste or color and any burning sensation were also recorded.

#### Microbiological tests

Routine microbiological tests were performed on the elastic rings using thioglycolate media at 37°C for 24 hours. Then, the optical density was determined using a 20 spectronic system at 620 nm wave length. In order to confirm the results, pour plate method was used with different dilutions to determine the colony forming unit (CFU), in such a manner that different dilutions (1:10, 1:100) were provided from thioglycolate media. Then 0.01 ml of the dilutions were cultured on EMB, Muller Hinton agar and blood agar medias (using sheep blood). The available samples were cultured on mitis salivarius agar obtained from sheep blood and were kept at 37 °C in 10% CO<sub>2</sub> for 48 hours. Then, the streptococcus mutans colonies were diagnosed and counted using alpha hemolysis and asculin tests (19). The tests were conducted three times for each sample and the mean of CFU was determined.

The number of bacterial colonies, transferred to logarithmic (Log) scale, are presented as mean and SD. The values at T1, T2 and T3 in the control, chlorhexidine and persica groups were compared using Kruskal-Wallis followed by Dunn test. A P value < 0.05 was statistically considered significant. The rate of occurrence of tooth discoloration, and unpleasant taste and burning sensation in persica and chlorhexidine groups were compared using chi-squared tests.

### RESULTS

At T1 there was no significant differences among the number of streptococcus mutans colonies in control ( $13.210 \pm 0.30$  CFU), persica ( $12.93 \pm 0.48$  CFU) and chlorhexidine ( $13.020 \pm 0.28$  CFU) groups (table 1). There were statistically significant differences in the number of streptococcus mutans colonies between persica ( $12.08 \pm 0.16$ ) and control ( $13.27 \pm 0.21$ ) groups, and chlorhexidine ( $11.33 \pm 0.28$ ) and control ( $13.27 \pm 0.21$ ) groups at T2 step ( $p < 0.001$ ). Also, there was statistically significant difference in the number of streptococcus mutans colonies between persica and chlorhexidine groups at this step ( $p < 0.05$ ) (table 2).

There was statistically significant difference in the number of streptococcus mutans colonies between persica ( $9.26 \pm 0.26$ ) and control ( $13.27 \pm 0.20$ ) groups, and chlorhexidine ( $8.11 \pm 0.44$ ) and control ( $13.27 \pm 0.20$ ) groups at T3 step ( $p < 0.001$ ). Also, there was statistically significant difference in the number of streptococcus mutans colonies between persica and chlorhexidine groups at this step ( $p < 0.05$ ) (table 2).

In the Chlorhexidine group, 86% of patients complained of tooth discoloration and 73% had experienced unpleasant taste and burning mouth sensation. These figures for the Persica group

**Table 1.** Comparison of the number of Streptococcus mutans colonies at T1 step among the three groups

Group	Control	Chlorhexidine	Persica	P Value
CFU* at T1	13.21 ± 0.30	13.02±0.28	12.93±0.48	P <0.064

\* Colony forming units

**Table 2.** Comparison of the number of Streptococcus mutans colonies at T2 and T3 steps among the three groups

Groups	CFU* at T2	P Value	CFU* (T3)	P Value
1	13.27±0.21	P<0.001	13.27±0.20	P<0.001
2	11.33± 0.28		8.11±0.44	
1	13.27±0.21	P<0.001	13.27±0.20	P<0.001
3	12.08±0.16		9.26±0.26	
2	11.33± 0.28	P<0.05	8.11±0.44	P<0.05
3	12.08±0.16		9.26±0.26	

\* Colony forming units 1=Control 2=Chlorhexidine 3=Persica

were 13% and 40% respectively (table 3). However chi-squared test showed that the two groups were significantly different with these respects.

### DISCUSSION

The findings of the present study indicate that there was no significant difference among the number of streptococcus mutans colonies prior to treatment. Moreover, it showed that relative to water, both mouthwashes reduced the number of bacterial colonies significantly. In addition, chlorhexidine reduced the number of streptococcus mutans colonies at T2 and T3 significantly more than persica. Moreover, side effects such as tooth discoloration, unpleasant taste and burning sensation were much lower with persica relative to chlorhexidine.

The absence of significant difference in the the number of streptococcus mutans colonies from the groups prior to treatment indicate that the change in the number of bacterial colonies were due to mouthwashes which were used. The efficacy of chlorhexidine was demonstrated in a number of previous studies, therefore it was used as a positive control in the present study. The antibacterial activities of chlorhexidine mouthwash in the present study was similar to those reported in the previous studies (4-7).

Both mouthwashes reduced the number of streptococcus mutans colonies significantly at T2 and T3, with persica being less potent than chlorhexidine. Unfortunately, as far as we know, there is no published study reporting the comparison between the effect of chlorhexidine and persica on

the number of streptococcus mutans colonies in microbial plaque. Only in one study (18) the effect of chlorhexidine and persica mouthwashes on the number of streptococcus mutans colonies in saliva has been compared and from results it was concluded that both agents could reduce the number of streptococcus mutans colonies to a insignificantly different levels. The difference in the findings of the present study with the reported study (18) might be due to the difference in the protocols employed. In that study, the method and the time of tooth brushing as well as the type of toothbrushes and toothpastes were inconsistent. In the present study all participants were trained about the methods and the times of tooth brushing, and used only one type of toothbrush and toothpaste. Additionally, in the present study, patients used mouthwash for 20 days instead of 14 days in that study.

Persica mouthwash was also shown to have antibacterial activity in vitro. In another study with 1:32 dilution it has been shown (20) that the mouthwash inhibited the bacterial growth. The main component of persica is Miswak herb extract. The reduction of micro-organisms could be related to the various agents available in which might release agents in saliva which could prevents their bindings to the tooth by interfering with bacterial function (21). Since persica is composed of three herbs extracts, the effects of milfoil and spearmint are added to the effect of miswak herb.

The findings of the present study showed that 13% of patients in the persica group complained

**Table 3.** The percent of the patients who complained of tooth discoloration, unpleasant taste and burning mouth in Chlorhexidine and Persica groups

	Chlorhexidine	Persica
Tooth discoloration	86%	13%
Unpleasant taste and Burning mouth	73%	40%

of discoloration, which was much lower when compared with discoloration of the chlor-hexidine group (86%). Similar findings have also been obtained (22). In accordance with other report (17) the rates of complaints regarding the unpleasant taste of the mouthwash in the chlorhexidine group were more than that in the persica group.

### CONCLUSION

In conclusion the present study indicates that persica mouthwash had a comparable antibacterial

activity to that of chlorhexidine. It also suggest that tooth discoloration or unpleasant taste occurred less with persica than with chlorhexidine.

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### REFERENCES

1. Chany H.S, Walsh L.J, Freer T.J. The effect of orthodontic treatment on salivary flow PH, buffer capacity and levels of Streptococcus mutans and Lactobacilli. Aust. Orthod. J. 1999; 15: 229-234.
2. Scheie A, Arneberg P, Krogstad O. Effect of orthodontic treatment on prevalence of Streptococcus mutans in plaque and saliva. Scand. J. Dent. Res. 1984; 92: 211-217.
3. Beyed, R.L., Loggatt, P.J., Robertson, P.B. Effect on gingivitis of two different 4% SnF<sub>2</sub> gels. J. Dent. Res., 1988; 67: 503-507.
4. Rosin M, Welk A, Bernhardt O, Ruhnau M, Pitten FA, Kocher T, Kramer A. Effect of a Polyhexamethylene Biguanide Mouthrinse on Bacterial Counts and Plaque. J Clin Periodontol, 2001; 28: 1121-1126.
5. Renton-Harper P, Addy M, Mora NJ, Doherty FM, Newcombe RG. A Comparison of Chlorhexidine, Cetylpyridinium Chloride, Triclosan and C31G Mouthrinse Products for Plaque Inhabitation. J Periodontol, 1996; 67: 486-89.
6. Almas K. The Effect of Salvadora Persica extract (Miswak) and chlorhexidine gluconate on human dentin: A SEM Study. J. Contemp. Dent. Pract. 2002; 3: 27-35.
7. Anderson G.B., Bowden J, Marrison E.C., Caffesse R. Clinical effect of chlorhexidine mouthwashes on patients undergoing orthodontic treatment. Am. J. Orthod Dentofac Orthop , 1997; 111: 606-612.
8. Barrajo J.L., Varela L.G. Efficacy of chlorhexidine mouthrinses with and without alcohol: A clinical study. J Periodontol 2002; 73: 317-21.
9. Bishara S.E., Damon P.L., Olsen M.E., Jakobsen J.R. Effect of applying chlorhexidine antibacterial agent on the shear bond strengths of orthodontic brackets. Angle Orthod 1991; 66: 313-316.
10. Al-Otaibi M., Al-Harthy M., Gustafsson A., Classon R., Angmar-Mansson B. Subgingival plaque microbiota in Saudi Arabians after use of Miswak chewing stick and tooth brush. J. Clin. Periodontal. 2004; 31: 1048-53.
11. Darout I.A., Albandar J.M., Skaug N. Periodontal status of adult sundanese habitual users of Miswak chewing sticks or tooth brushes. Acta Odontol Scand 2000; 58: 25-29.
12. Kalessi A.M., Pack A.R., Thomson W.M., Tomkins G.R. An in vitro study of the plaque control efficacy of Persica: A commercially available herbal mouthwash containing extracts of Salvadora Persica. Int. Dent. J. 2004; 54: 279-83.
13. Al-Otaibi M. The Miswak (Chewing Stick) and Oral Health. Studies on oral hygiene practices of urban Saudi Arabians. Swed. Dent. J. suppl. 2004; 167: 2-75.
14. Darout I.A., Skaug N., Albander J.M. Subgingival microbial level and their associations with periodontal status at the sampled sites in an adult sudanese population using Miswak or tooth brush regularly. Acta Odontol. Scand 2003; 61: 115-22.

15. Almas K., Al-zeid Z. The immediate antimicrobial effect of tooth brush and Miswak on cryogenic bacteria. A clinical study. *J. Conemp. Dent. Pract.* 2004; 5: 105-114.
16. Darout I.A., Albandar J.M., Skaug A., Ali R.W. Salivary microbial in relation to periodontal status, experience of Caries and Miswak Use in sudanese adults. *J. Clin. Peridontol.* 2002; 29: 411-20.
17. Ebrahimnejad Z., Taghavi M. Clinical comparison between the effect of persica and chlorhexidine 0.2% on the dental plaque and periodontal inflammatim. *J of Med. Sci. Shah. Beh Univ.* 2002; 20: 3, 370-378.
18. Mortazavi M., Balali N. Intra oral effect of persica mouthwash, Irsha, Poidone Iodine 1%, chlohixidine and hypertonic chloride sodium on the rate of mutants streptococci of saliva. School of Dentistry, Shiraz Univ. of Med. Sci. 2001; Dentistry Doctoral Dissertation, 797.