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Research Article

Comparison of the Antibacterial Properties of Three Mouthwashes Containing Chlorhexidine Against Oral Microbial Plaques: An *in vitro* Study

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Background: The mouth provides an environment that allows the colonization and growth of a wide variety of microorganisms, especially bacteria. One of the most effective ways to reduce oral microorganisms is using mouthwashes.
Objectives: The aim of this study was to investigate the antibacterial effects of chlorhexidine mouthwashes (manufacture by Livar, Behsa, Boht) on common oral microorganisms.
Materials and Methods: In this *in vitro* study, isolated colonies of four bacteria, including *Streptococcus mutans*, *S. sanguinis*, *S. salivarius* and *Lactobacillus casei,* were prepared for an antimicrobial mouth rinse test. The tube dilution method was used for determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC).
Results: The MICs for Kin gingival, Behsa and Boht mouthwashes were 0.14, 0.48 and 1000 micrograms/mL using the tube method for *S. mutans*, respectively. The MBCs for the mentioned mouthwashes were 0.23, 1.9 and 2000 micrograms/mL for *S. mutans*, respectively. The MICs for Kin gingival, Behsa and Boht mouthwashes were 0.073, 0.48 and 250 micrograms/mL using the tube method for *S. sanguinis*, respectively. The MBCs for the mentioned mouthwashes were 0.14, 1.9 and 1000 micrograms/mL for *S. sanguinis*, respectively.
Conclusions: The Kin Gingival chlorhexidine mouthwash has a greater effect than Behsa and Boht mouthwashes on oral microorganisms and is recommended to be used for plaque chemical inhibition.

*Keywords:*Chlorhexidine; Mouthwashes; Minimum Inhibitory Concentration

1. Background

The mouth provides an environment for the colonization and growth of a wide variety of microorganisms, especially bacteria (1). The bacterial plaque is one of the influential factors in the destruction of teeth and periodontal tissues (2). The primary method of preventing disease and maintaining good oral hygiene is to control plaque and mechanically prevent its accumulation on the teeth and adjacent gingival surfaces (3). Mechanical methods for maintaining good oral hygiene include brushing and flossing, which are considered as the gold standard for plaque control (4). Despite the importance of mechanical plaque control methods, there is a high prevalence of gingival inflammation due to a lack of proper plaque control and physical disability. For this reason, other chemical methods such as the use of toothpastes and mouthwashes with anti-inflammatory and anti-plaque properties are recommended to maintain good oral hygiene (5) and enhance the effectiveness of mechanical methods.

Many clinical studies have examined the effect of mouthwashes as an anti-plaque and anti-inflammatory agent (6-8). Mouthwashes are very useful in reducing bacterial plaques. Chlorhexidine among the available mouthwashes has been found to be very effective for the reduction of bacterial plaque and pathogenicity of microorganisms, including *Streptococcus mutans,* and in many studies has been considered as a positive control for comparison with the antimicrobial effects of other substances (9-12). Despite the above advantages, this mouthwash has dental complications such as dental stain, changes in taste, irritation and dryness of mouth. However, it is regarded as the gold standard (13).

2. Objectives

Since many companies produce chlorhexidine mouthwashes with different brands and prices, the best of which have not been evaluated in terms of their effect on microorganisms and the reduction of gingival inflammation, this study compared three mouthwashes containing chlorhexidine that have a huge price difference, in terms of their antibacterial effect.

3. Materials and Methods

The minimum inhibitory concentration (MIC) was used to evaluate the inhibitory effects of chlorhexidine mouthwashes, Kin Gingival (Livar, Spain), Boht (Boht, Iran) and Behsa (Behsa, Iran), on standard strains of *Streptococcus mutans* (PTCC1683), *S. sanguinis* (PTCC1449), *S. salivarius* (PTCC1448) and *Lactobacillus casei* (PTCC1608). All bacterial strains were obtained from the Persian type culture collection. Fifteen sterile test tubes were collected and 1 mL of sterile tryptic soy broth (TSB, Merck Germany) was added to each tube. Next, 1 mL of each mouthwash with specified dilutions was prepared using the serial dilution method, and was added to the tubes. A bacterial suspension of $1.5x10^8$ cfu equal to No. 0.5 McFarland standard was prepared from the standard strains and diluted at a ratio of 1:500.

An amount of 1 mL of the dilute suspension was added to each set of 15 tubes that contained TSB medium and mouthwash (14). After the bacterial suspension was added to the test tubes, the tubes were placed in a candle jar (to provide 5% CO_2) and were incubated at 37°C for 24 - 48 hours. After this period was elapsed, the tubes were examined for the presence of turbidity, which indicates microbial growth. The last tube or the last dilution of mouthwash at which turbidity was not observed, was considered as the MIC of the respective mouthwash on certain microorganisms. Then, the MIC of each of the four mouthwashes was compared in terms of ability to inhibit microbial growth. After 24 hours of incubation, the tubes without turbidity (transparent), which indicated the inhibition of bacterial growth by the respective mouthwash, were transferred to a solid medium (Blood agar, Merck Germany) and were evaluated in terms of microbial growth to determine the MBC of mouthwashes. The last tube, which was negative in terms of culture on solid medium, indicated the minimum bactericidal concentration (MBC) of mouthwashes. This procedure was performed for all bacterial strains. All data were analyzed by Kruskal-Wallis and Chi-Square tests using the SPSS ver. 16 software (SPSS Inc., Chicago, IL, USA). A P value of < 0.5 was considered significant.

4. Results

The Mouthwashes stopped all tested microorganisms, and had bactericidal effects. The MICs of Kin Gingival, Behsa and Boht mouthwashes for *S. sanguinis* were 0.14, 0.48 and 1000 µg/mL, respectively (Table 1). The differences between mouthwashes were significant ($P < 0.5$). The MBCs of Kin Gingival, Behsa and Boht mouthwashes for *S. sanguinis* were 0.23, 1.9 and 2000 µg/mL respectively (Table 1). The differences between mouthwashes were significant ($P < 0.5$). The MICs and MBCs against the other bacterial strains are shown in Table 1. The lowest level of MICs for all bacterial strains was related to Kin gingival. Among the examined mouthwashes, the Kin gingival chlorhexidine mouthwash was found to have the greatest effect on *S. mutans*, *S. salivarius*, *L. casei* and *S. sanguinis;* and this difference with other mouthwashes was significant ($P < 0.5$). Among bacterial strains the *S. mutans* showed the highest resistance to chlorhexidine mouthwashes.

5. Discussion

The results of the present study, showed that all three chlorhexidine mouthwashes can cause inhibition of bacterial growth, and that there was a significant difference between the antimicrobial effects of Boht, Behsa and Kin gingival mouthwashes. Bacterial plaques have been proven to have a role in the etiology of dental caries and periodontal diseases. The mechanical methods of plaque inhibition have some limitations, for solving this problem, chemical methods are proposed for plaque inhibition. Therefore, the use of mouthwashes as disinfectants can help mechanical methods to reduce plaques (15). Mouthwashes with antimicrobial effects perform this task using three methods, which include apoptosis, inhibition of bacterial growth and/or cell metabolic inhibition; and depending on their concentration their bactericidal and/or bacteriostatic properties vary (16).

Table 1. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (µg/mL) of Kin gingival, Behsa and Boht chlorhexidine Mouthwashes Against oral Pathogenic Bacteria Determined by the Tube Dilution Method [a]

Bacteria	Mouth Washes					
	Kin Gingival		Behsa		Boht	
	MIC	MBC	MIC	MBC	MIC	MBC
Streptococcus mutans	0.14	0.23	0.48	1.9	1000	2000
Streptococcus sanguinis	0.073	0.14	0.48	1.9	250	1000
Streptococcus salivarius	0.073	0.14	0.24	0.97	250	1000
Lactobacillus casei	0.036	0.073	0.12	0.48	500	2000

[a] Abbreviations; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

According to many studies that have been conducted on the effects of mouthwashes on oral microorganisms (17, 18), the chlorhexidine mouthwash is the most superior amongst all mouthwashes. Most studies comparing chlorhexidine and other mouthwashes have shown the superiority of chlorhexidine, and only a few studied products have been able to compete with chlorhexidine in terms of antibacterial properties (19). Streptococci are the main etiological agents of dental caries. *Streptococci* bind to the acquired pellicle to form first stage of plaque formation. The removal of *streptococci* prevent plaque formation and disease extension (20). Jarvinen et al. in their study on the susceptibility of *S. mutans* to chlorhexidine and six other antimicrobial agents showed that *S. mutans* is resistant to antimicrobial agents (21). Emilson et al. explained that *S. mutans* had the greatest resistance to chlorhexidine mouthwash and even more resistance to the varnish mode of chlorhexidine (22).

Our study also confirmed that S. mutans was somewhat resistant to chlorhexidine. A study comparing between the polyphenol extracts of green tea and a mouthwash containing 0.05% fluoride and 0.2% chlorhexidine showed that fluoride-chlorhexidine solution had the greatest anti caries effect, which could indicate the synergistic effect of the substance on microorganisms (23). A study by Mozaffari et al. found that Persica mouthwash with a concentration of 50% had weak and transient bactericidal effects against *S. sanguis* and *S. mutans*, while chlorhexidine showed very effective bactericidal effects against bacteria (24). Salehi et al. noted that the chlorhexidine mouthwashes were more effective on *streptococcus* than Persica mouthwashes. Similar to the described studies, this study showed that chlorhexidine is able to eliminate streptococci, and has absolute antibacterial effects (25). One study demonstrated that green tea mouthwash could reduce oral microorganisms due to tannins, and there is no significant difference between chlorhexidine mouthwashes and green tea extracts so that both material have the same antimicrobial effects (26).

A study that compared Oral-B and chlorhexidine mouthwashes showed that a chlorhexidine mouthwash is more effective in reducing *S. mutans* in plaques around orthodontic brackets, which also indicates the high antimicrobial activity of chlorhexidine mouthwashes (27). Kin gingival chlorhexidine effectively eliminates streptococci that cause dental decay, and since these microorganisms support initial plaques, this mouthwash has beneficial antimicrobial and anti-gingival effects (28, 29). Some studies emphasized that the antimicrobial effect of the 0.12% concentration is better than the 0.2% concentration (30). Kin gingival mouthwash has the greatest effect on microorganisms, which may be due to its 0.12% concentration.

The first microorganisms that caused dental caries development were lactobacilli and chemical or mechanical removal were important in caries prevention (31). Kohler et al. evaluated the effects of chlorhexidine on streptococci and lactobacilli and they stated that the use of chlorhexidine mouthwash may reduce these microorganisms (32). Consist with this study, Lundstrom et al. evaluated the effect of chlorhexidine on *streptococci* and lactobacilli in orthodontic patients and stated that chlorhexidine mouthwashes had no effect on lactobacilli (33). The difference in results of previous studies could be due to differences in the chlorhexidine-containing compounds, as different combinations have been shown to have different effects (34). This study helps clinicians choose the best antimicrobial agent that is available on the market. According to the findings of this study, Kin gingival chlorhexidine mouthwash is more effective on oral microorganisms than the Boht and Behsa mouthwashes yet further clinical trials are required to confirm our results.

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