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Antimicrobial Effects of Mass and Oral-B Mouthwashes on Streptococcus mutans and Candida albicans: An In Vitro Study

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Objectives The present study aimed to compare the antimicrobial properties of Iranian Mass mouthwash and alcohol-free Oral-B mouthwash against Streptococcus mutans (S. mutans) and Candida albicans (C. albicans).
Methods In this in vitro study, S. mutans and C. albicans were separately cultured on BHI agar plates. The agar well-diffusion method was used to compare the antimicrobial properties of Mass and Oral-B mouthwashes, and 0.2% chlorhexidine (CHX) as the positive control and saline as the negative control. The diameter of growth inhibition zones was then measured. The experiment was performed in triplicate. The minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) of the two mouthwashes were determined for each microorganism using the broth micro-dilution method. Data were analyzed by the Kruskal-Wallis and Dunn's test (Benjamini-Hochberg).
Results The mean diameter of the growth inhibition zone of S. mutans was 26.33 and 27.66 mm for Mass and Oral-B mouthwashes, respectively. These values were 18 mm and 17.66 mm, respectively for C. albicans. There was no significant difference in the mean diameter of growth inhibition zones of the two mouthwashes against C. albicans (P=0.38) or S. mutans (P=0.23). The MIC of Mass and Oral-B mouthwash for S mutans was in 1/1024 dilution ratio and the MIC of Mass and Oral-B mouthwashes for C. albicans was in 1/512 and 1/256 dilution ratios, respectively. The MBC values were the same as the MIC values for both mouthwashes.
Conclusion Mass mouthwash was as effective as Oral-B mouthwash against S. mutans and C. albicans.
Keywords Anti-Infective Agents; Streptococcus mutans; Candida albicans; Mouthwashes

Introduction

Dental plaque is the main etiologic factor for tooth decay since attachment of dental plaque microorganisms to the tooth surface and gingiva is the initial stage of oral and dental diseases. [1-3]

Dental caries is caused by colonization of oral microorganisms such as Streptococcus mutans (S. mutans) and Lactobacillus. [4] S. mutans is one of the most common oral pathogens that colonizes the tooth surface and survives in the salivary pellicle and the saliva.[5]

Early childhood caries refers to the presence of one or more decayed, missing, or filled tooth surfaces in each deciduous tooth of a 5-year-old or younger child which significantly degrades the quality of life of children.[6] A recent study showed that high levels of S. mutans and C. albicans are detected in the biofilm formed on the tooth surface of children affected by early childhood caries. The synergy of these two oral pathogens amplifies biofilm maturation and its virulence.[7]

Mechanical plaque control methods such as tooth brushing and flossing are widely accepted methods for dental plaque removal. Besides, mouthwashes and oral antimicrobial sprays are considered as simple adjunctive methods to reduce the microbial load.[8]

In the past 30 years, rate of invasive fungal infections in humans has increased significantly; among which, candida species are considered as the most preeminent fungal pathogens.[9] Candida albicans (C. albicans) coexists normally in the oral cavity of 45% of infants, 45-65% of healthy children, 50-65% of people who use removable dentures, 90% of people with leukemia undergoing chemotherapy, 95% of HIV positive patients, and 30-45% of healthy adults.[10] C. albicans infections are difficult to treat. Generally, amphotericin B and azoles are clinically used. Regarding amphotericin B, due to its low permeability through the membrane, the received dosage of drug by the patient must be increased which will cause severe side effects such as renal failure. In order to reduce the severity of side effects, amphotericin B is usually mixed with other antifungal drugs such as azoles. However, the number of reports about C. albicans' resistance to azoles has recently increased dramatically; thus reducing the dosage of amphotericin B by mixing it with a new anti-fungal product e.g., a mouthwash is currently considered.[11]

Several studies have compared the antibacterial and antifungal effects of different mouthwashes. [12-14] The optimal antibacterial and antifungal efficacy of chlorohexidine (CHX) and Oral-B mouthwashes has been previously documented. [1, 14, 15] The present study aimed to compare the antimicrobial properties of the Iranian Mass (Iran Najo, Iran) mouthwash and alcohol-free Oral-B mouthwash (Protel & Gambel, USA) against S. mutans and C. albicans.

Methods and Materials

The present in vitro experimental study was approved by the ethics committee of Shahid Beheshti Dental School (IR.SBMU.URC.REC.1398.182).

Culture of microorganisms and preparation of microbial

suspensions:
Strains of S. mutans (ATCC: 35668, PTCC: 1683) and C. albicans (ATCC: 10231, PTCC: 5027) were purchased from the Iranian Research Organization for Science and Technology (Persian Type Culture Collection) in lyophilized vials. The contents of the vials were separately cultured in brain heart infusion (BHI) broth. Then, they were sub-cultured separately in BHI agar medium, and the plates were incubated at 37℃ and 90% humidity for 24 hours for C. albicans and 48 hours for S. mutans to isolate single colonies. Next, 2-3 colonies of each microorganism were separately immersed in sterile saline. The concentration of the microbial suspension was adjusted at 0.5 McFarland standard by adjusting the optical density between 0.1-0.8 in 600 nm wavelength using a spectrophotometer (Unico, USA). All stages were performed in sterile conditions, under a class II laminar flow hood.[16]
Assessment of antibacterial effects by the agar well-diffusion method:
The S. mutans suspension was inoculated as lawn culture by a sterile swab on BHI agar in three plates. After that, four wells were punched in equal diameters and equal distances in each plate, and agar was applied to the bottom of the wells. Then, 50 µL of Mass (Iran Najo,Iran) and alcohol-free Oral-B (Protel & Gambel ,USA) mouthwashes, 50 µL of 0.2% CHX as the positive control and 50 µL of saline as the negative control were poured into the related wells in each plate. All stages were performed in sterile conditions under a class II laminar flow hood. Then, the plates were incubated in an incubator (Memmert, Germany) at 37℃ for 48 hours. Afterwards, the inhibition zone diameters were measured in millimeters (mm). The same procedure was performed to evaluate the antifungal effects of mouthwashes on C. albicans, except that the incubation time was 24 hours. The experiments were performed in triplicate.[17, 18]
Determination of minimum inhibitory concentration (MIC) by the broth micro-dilution method:
The MIC assay was performed according to the Clinical and Laboratory Standards Institute protocol.[16] In order to perform the MIC assay for S. mutans, 100 µL of BHI broth (Merck, Germany) medium was added to the wells of 96-well sterile plates by a multichannel pipette. The first 12-well row was allocated to the negative control and the second 12-well row was allocated to the positive control; 100 µL of Oral-B mouthwash was poured into the first well of the third row and was serially diluted in BHI broth in 1/2, 1/4, etc. ratios until the 12th well. This was repeated for the next two rows; 100 µL of Mass mouthwash was poured into the first well of the 6th row and was serially diluted in BHI broth as 1/2, 1/4, and so on until the 12th well. This was repeated for the next two rows. Each well had a final volume of 100 µL. Then, S. mutans suspension adjusted to 0.5 McFarland standard was diluted in 1/10 ratio and 10 µL of it was added to all wells with the exception of the negative control row. Then, 96-well plate was incubated at 37℃ for 24 hours. After 24 hours of incubation, the turbid wells indicated bacterial growth while clear wells indicated no bacterial growth. To increase the accuracy of the results, 20 µL of 0.015 Resazurin dye (Sigma USA) solution was added to all wells and the plate was incubated for 2 hours at the same temperature. The latest concentration of mouthwash that had no change in color was reported as the MIC of the respective mouthwash against the respective microorganism.[16]
The same procedure was performed to evaluate the MIC of the two mouthwashes against C. albicans except that the 0.5 McFarland standard cell suspension concentration was used without any dilution.
Determination of minimum bactericidal concentration (MBC):
The MBC of the mouthwashes for S. mutans was determined by sub-culturing a sample from each well of the determined MIC on BHI agar, according to the Clinical and Laboratory Standards Institute protocol.[16] Then, the plates were incubated at 37℃ and 9% humidity for 48 hours. The MBC was determined as the lowest concentration of the mouthwash at which no colony formation occurred. This experiment was carried out in three plates. The same procedure was performed to evaluate the MBC of the mouthwashes against C. albicans, except that the incubation time was 24 hours. [16]
Statistical analysis:
The Kruskal-Wallis test was used to compare the inhibition zone diameters of S. mutans and C, albicans caused by Mass and Oral-B mouthwashes and also positive and negative controls. The Dunn's pairwise comparison statistical test (Benjamini-Hochberg) was used for pairwise comparisons of the inhibition zone diameters of the mouthwashes and the positive control. P-value less than 0.05 was considered statistically significant.

Results

In this study, the antimicrobial effects of Mass and Oral-B mouthwashes on S. mutans and C. albicans were investigated by the agar well-diffusion method (Figure 1). The inhibition zone diameters were measured in millimeters. The positive control (0.2% CHX) caused the highest mean diameter of S. mutans inhibition zone. The mean diameter of S. mutans inhibition zones caused by Mass and Oral-B mouthwashes was close (Table 1).

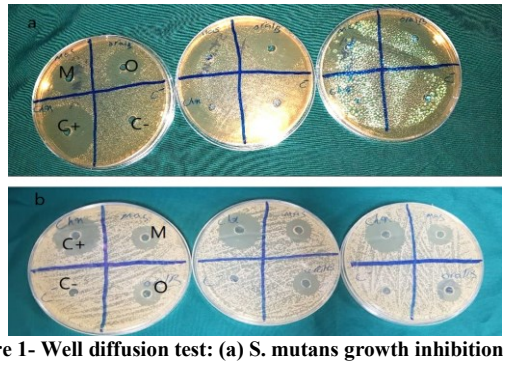

Figure 1- Well diffusion test: (a) S. mutans growth inhibition zones caused by the mouthwashes and positive and negative controls, (b) C. albicans growth inhibition zones caused by the mouthwashes and positive and negative controls (M= Mass mouthwash, O=Oral-B mouthwash, C + = Positive control and C- = Negative control)

Table 1- Mean diameter (mm) of S. mutans growth inhibition zones (n=3)

Materials	Mean (mm)	Standard deviation	Mean Rank*
Oral-B mouthwash	27.67	1.53	7.33
Mass mouthwash	26.63	1.15	5.67
Saline	0	0	2
0.2% chlorohexidine	42	5.29	11

*According to the Kruskal-Wallis test, there was a significant difference in the mean diameter of S. mutans growth inhibition zones among the groups (P= 0.021).

Similarly, 0.2% CHX showed the largest mean diameter of C. albicans growth inhibition zone, and the mean diameter of C. albicans growth inhibition zones caused by Mass and Oral-B mouthwashes was close (Table 2).

Table 2- Mean diameter (mm) of C. albicans growth inhibition zones (n=3)

Materials	Mean (mm)	Standard deviation	Mean Rank*
Oral-B mouthwash	17.66	0.58	6.17
Mass mouthwash	18	1	6.86
Normal Saline	0	0	2
Chlorohexidine 0.2%	27.66	0.58	11

*According to the Kruskal-Wallis test, there was a significant difference in the mean diameter of C. albicans growth inhibition zones among the groups (P=0.02).

The Dunn's pairwise comparisons test (Benjamini-Hochberg) was used for pairwise comparisons of the mouthwashes and positive control (0.2% CHX), which revealed that there was no difference between the mean diameter of S. mutans or C. albicans growth inhibition zones caused by Oral-B and Mass mouthwashes (Table 3).

Table 3- P values for pairwise comparisons of the inhibition zones caused by the mouthwashes and 0.2% CHX

Microorganisms	Materials	Mass mouthwash	Oral-B Mouthwash
S. mutans	0.2% chlorohexidine	0.02	0.07
	Mass mouthwash	-	0.23
C. albicans	0.2% chlorohexidine	0.04	0.04
	Mass mouthwash	-	0.38

The MIC of Mass mouthwash for S. mutans was in 1/1024 dilution ratio similar to Oral-B. The MIC of Mass and Oral-B mouthwashes for C. albicans was in 1/512 dilution ratio and 1/256 dilution ratio, respectively.

The MBC of the two mouthwashes against S. mutans was in 1/1024 dilution ratio and the MBC test results of Mass and Oral-B mouthwashes against C. albicans were in 1/512 dilution ratio and 1/256 dilution ratio, respectively (Figure 2).

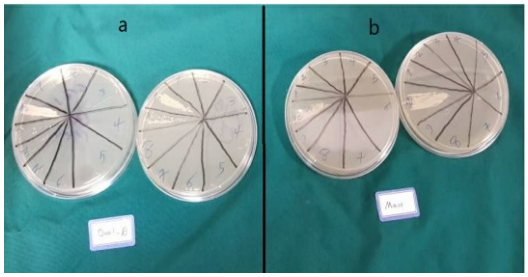

Figure 2- BHI agar plates showing the MBC of mouthwashes for C. albicans. (a) Oral-B mouthwash (b) Mass mouthwash

Discussion

Despite being preventable, dental caries is one of the most common chronic diseases worldwide and its microbial etiology is correlated with microbial factors causing dental infections. [19] S. mutans is the main culprit responsible for dental caries. [5] Besides, microbiological data indicate that dental caries is correlated with fungal microorganisms like C. albicans as well. [5]

The present study compared the antimicrobial activity of Mass and alcohol-free Oral-B mouthwashes against S. mutans and C. albicans in vitro. Given that the efficacy of Mass and Oral-B mouthwashes in inhibition of S. mutans and C. albicans is the same, this Iranian mouthwash may serve as a suitable alternative to Oral-B mouthwash. In this study, 2% CHX was used as the positive control and saline as the negative control. Barasch et al.[20] showed the effectiveness of CHX mouthwash in prevention and treatment of Candida infections in HIV patients. It was further revealed that CHX was effective in treatment of oral diseases in cancer patients undergoing radiotherapy. [21] Guarnelli et al. stated that CHX mouthwash was effective against different species of C. albicans and concluded that it can cause a 99% reduction in C. albicans colony count. [22] The antibacterial effect of CHX mouthwash on S. mutans has been previously documented as well. [1, 21, 23]

In this study, comparison of C. albicans growth inhibition zone diameters caused by Oral-B and Mass mouthwashes revealed no significant difference. However, the inhibition zone diameter caused by CHX was significantly larger than that caused by Oral-B and Mass mouthwashes. The MIC and MBC of Mass mouthwash were in 1/512 dilution ratio for C. albicans while the MIC and MBC of Oral-B mouthwash were both in 1/256 dilution ratio for C. albicans. The results showed that the antifungal effect of Mass mouthwash was higher than that of alcohol-free Oral-B mouthwash.

Talebi et al. investigated the efficacy of Iranian and foreign-made mouthwashes against C. albicans and reported that Oral-B was the most effective among the foreign-made mouthwashes, while Vi-One and Persica were the most effective among the Iranian mouthwashes. They did not observe any significant difference in the efficacy of Iranian and foreign-made mouthwashes.[24] The present study demonstrated that the efficacy of Oral-B mouthwash against C. albicans was similar to that of Iranian Mass mouthwash. Contrary to most studies, Talebi et al. showed that the effects of 0.2% chlorohexidine on C. albicans were lower than other mouthwashes including Oral-B in agar diffusion method and MIC assay.[25]

Reduction of S. mutans colony count was observed after using CHX mouthwash in several studies (26-28). Ghapanchi et al. concluded that Oral-B and CHX mouthwashes were effective, even if diluted, in controlling the proliferation of S. mutans while Persica mouthwash had no antibacterial activity. [23]

The present study showed that the inhibition zone diameters for both Oral-B and Mass mouthwashes against S. mutans

and C. albicans were similar, but the inhibition zone diameter of CHX was larger than the other two mouthwashes. Higher antimicrobial efficacy of CHX mouthwash in comparison with Oral-B and Mass mouthwashes may be due to the presence of chlorohexidine gluconate in CHX mouthwash as opposed to cetylpyridinium chloride present in the other two mouthwashes. [29]

The present study results showed that MBC and MIC values were similar for both mouthwashes against S. mutans but these concentrations were dissimilar against C. albicans. The MIC and MBC of Mass mouthwash were lower than Oral-B (in 1/512 to 1/256 dilution ratios). This difference can be due to different levels of cetylpyridinium chloride concentration in the two mouthwashes, which is 0.1 g per 100 mL in Mass mouthwash, while its concentration in Oral-B mouthwash has not been disclosed.

According to the present findings regarding the comparison of anti-fungal and anti-bacterial properties of the mouthwashes, Oral-B, and Mass mouthwashes were similar in spite of subtle differences. Thus, using Mass mouthwash may be advised to patients as a reliable and promising adjunct to mechanical plaque control. Moreover, it is further recommended to conduct more studies to evaluate the efficacy of Mass mouthwash against other microorganisms.

Conclusion

Mass mouthwash had similar effectiveness against S. mutans and C. albicans as Oral-B mouthwash. Due to this similarity, Mass mouthwash may be used to decrease the count of S. mutans and C. albicans in the oral cavity.

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Conflict of Interest

No Conflict of Interest Declared ■

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