ORIGINAL RESEARCH

Clinical Efficacy of Single Use of Three Different Mouthrinses on the Level of *Streptococcus mutans* in Saliva

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Abstract

Aim and objective: The aim of this cross-sectional population-based clinical study was to assess the effect of single use of three different mouthrinses on the level of salivary *Streptococcus mutans* of 8 to 10-year-old Saudi children.

Materials and methods: Convenient samples of 52 Saudi children aged 8–10 years were randomly allocated into four groups of 13 each. Saliva samples were collected to assess the level of *S. mutans* at baseline before rinsing with the assigned mouthrinse or control. Three mouthrinses, Avalon Avohex, Listerine Miswak, and Optima Aloe Dent Mouthrinse, were randomly distributed to the children. Each participant was instructed to rinse for 2 minutes using 10 mL of the assigned mouthrinse. Saliva samples were collected after rinsing and colony forming unit (CFU) of *S. mutans* per mL of saliva was calculated. Statistical analysis was performed to compare *S. mutans* count at baselines and postintervention values of each experimental group and control using paired *t*-test and one-way ANOVA. All statistical analyses were set at a significance level of $p < 0.05$.

Results: All test groups showed a reduction in salivary *S. mutans* compared to that at baseline. Statistically significant reduction ($p > 0.05$) in bacterial count was seen in Avalon Avohex group.

Conclusion: A single-time rinse of chlorhexidine extract mouthrinse for 2 minutes effectively reduced the number of *S. mutans* of 8 to 10-year-old Saudi children.

Clinical significance: Rinsing with chlorhexidine extract mouthrinse should be considered as a potential method in prevention of dental caries in children.

Keywords: Antimicrobial agent, Caries, Chlorhexidine, Clinical study, Mouthrinse, *Streptococcus mutans*

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Introduction

The dental biofilm has a main role in the etiology of dental caries and periodontal diseases.[1] Dental caries is one of the most predominant and preventable oral infectious diseases that affect the majority of the world's population and might lead to pain, tooth destruction, or loss.[2] It is a multifactorial disease that is characterized by demineralization of the dental hard tissues.[3] *Streptococcus mutans* is a gram-positive, facultative, anaerobic round-shaped bacterium, which is considered a key contributor to the formation of cariogenic plaque.[4] It is a highly acidogenic and acid-tolerant bacteria that use dietary sucrose to synthesize large amounts of extracellular polysaccharides and adheres firmly to glucan-coated surfaces.[4] Besides that, *S. mutans* has the ability to consume extra- and intracellular polysaccharides as short-term storage mixtures, which offer an extra increase in the amount of acid production and extent of acidification.[5] Genetic variability of *S. mutans* is still not well understood.[6]

A study assessed the genetic variability of *S. mutans* by extensive whole-genome sequencing revealed that the core genome size of *S. mutans* was determined to be around 1,370 genes by including 67 *S. mutans* genomes.[6] Many antiplaque agents have been in practice as additional aids.[7] It is thought that using mouthrinses might act as an effective and harmless way for the delivery of antimicrobial agents that inhibit microbial adhesion and colonization and disturb the bacterial growth.[7] Chlorhexidine is the gold standard mouthrinse due to its antimicrobial properties.[8,9] On the contrary, despite its side effects like teeth and soft tissue staining, taste alteration, and supragingival calculus formation, its continued use is supported.[9–11] Concerning dental caries, a systematic review found that no randomized clinical trials assessed chlorhexidine mouthrinses for the purpose of the prevention of dental caries in children and adolescents.[12]

Nowadays, an interest in the naturally derived biologically active compounds has been increased.[13,14] These compounds might have a helpful and therapeutic usage in medicine and dentistry fields.[13,14] Herbal extracts and plant essential oils (EOs) have the potential to be used as therapeutic agents for chronic gingivitis and periodontitis conditions that have both bacterial and inflammatory components.[15,16] These are useful, as their long-term daily usage has

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no side effects on the health of an individual.[15,16] In addition, these are more cost-effective and easily available as over-the-counter products. Aloe vera has shown antibacterial properties against a variety of bacteria mainly against *S. mutans*, which accounts for its antiplaque action.[16] Some of the components of Aloe vera like vitamin C, hyaluronic acid, and dermatan sulfate are involved in collagen synthesis and, hence, can relieve swelling and bleeding gum.[17] A study evaluated the effect of Aloe vera mouthrinse on the dental plaque in the investigational period of four days reported that Aloe vera could prove an effective mouthrinse due to its capability in minimizing dental plaque.[18] Traditional medicinal plants can show biological action that improves oral health. For example, *Salvadora persica* L., family: *Salvadoraceae* (Sp), root extract is well reported for its antibacterial effect properties against dental plaque.[19,20]

Miswak (*S. persica*) has been used as a natural way for tooth cleaning in many parts of the world for thousands of years.[21] A number of scientific studies have confirmed that the miswak (*S. persica*) possesses not only antibacterial but also antifungal, antiviral, anticariogenic, and antiplaque properties. It was stated that those using chewing sticks have saliva with a significant lower level of primary plaque colonizers (including *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus oralis*, and *Streptococcus salivarius*) as compared to those utilizing conventional toothbrushes.[22] A randomized controlled crossover clinical trial evaluation of *S. persica* L. reported an antiplaque effect for a 24-hour period.[23] A study evaluated the effectiveness of Miswak products on cariogenic bacteria in comparison with regular toothpaste reported that Miswak products, particularly mouthrinse, were more effective in minimizing the growth of cariogenic bacteria than ordinary toothpaste.[24]

Currently, a global trend has been seen for the usage of natural products due to their proven pharmacological effects on the oral environment as efficient caries-preventive agents. Therefore, clinical studies are the way to confirm the actual contributions of natural products to caries reduction in children. In summary, naturally derivative compounds appear to have potential for preventing and/or treating dental caries. However, more clinical studies in this area are therefore still warranted. Hence, exploring more caries-preventive approaches and agents is needed. Therefore, the aim of this cross-sectional population-based clinical study was to evaluate the effect of single use of three different mouthrinses, Avalon Avohex, Listerine Miswak, and Optima Aloe Dent Mouthrinses, on the level of salivary *S. mutans* of 8–10-year-old Saudi children. The null hypothesis tested was reported that there is no difference between the effect of the tested mouthrinses and the positive control mouthrinse against the level of salivary *S. mutans*.

Materials and Methods

The study was approved by the Ethical Committee of Human Studies, College of Dentistry Research Center, King Saud University, and Medical City, King Saud University.

Sample Size Calculation

The sample size was calculated using OpenEpi software (version 3.01), through the formula: $n = (Z\alpha/2 + Z\beta)2 \times 2 \times \sigma 2/d2$. The power of the sample size when the number of participants was equivalent or more than 16 was calculated. At the two-sided confidence interval = 95%, $\alpha = 0.05$, SD in each group (σ) = 100 million bacteria and the minimum acceptable difference (d) = 100 million bacteria, the power of the study = 80% ($\beta = 0.2$), and the ratio of randomization groups = 1, the sample size was ≥16. To be familiar with the study protocol, a pilot study was conducted before the start of the actual study. Operators were trained and calibrations were undertaken throughout the pilot study. Intra-examiner reproducibility of the investigators who performed the clinical investigation was assessed during the pilot study by reassessing 10% of the participants.

Inclusion and Exclusion Criteria

The inclusion criteria were healthy Saudi children aged 8–10 years, currently not under using any medication, have no active periodontal diseases, not underneath active orthodontic treatment, and with Decayed, missing due to caries, and filled teeth in the primary teeth (dmft) and Decayed, missing due to caries, and filled teeth in the permanent teeth (DMFT) equal or fewer than four.

A convenient sample of 52 Saudi children aged 8–10 years who visited the clinics of the dental hospital is decided to participate in this clinical study, and then, a consent form was signed by their parents or legal guardian. Study protocol was emphasized about the importance of following the instructions for the participated children before the saliva collection visit that was given within approximately 1 week after the initial visit.

Randomization and Blindness

Participants were randomly assigned into four groups of 13 each. Group 1 was assigned to use Avalon Avohex mouthrinse (Avalon Pharma, Riyadh, Kingdom of Saudi Arabia), group 2 was assigned to use Listerine Miswak Mouthrinse (Johnson and Johnson, Kingdom of Saudi Arabia), group 3 was assigned to Optima Aloe Dent Mouthrinse (Optima Naturals, Kingdom of Saudi Arabia), and group 4 assigned to distilled water mouthrinse as a negative control group. The ingredients of each mouthrinse are shown in Table 1. Allocation concealment was used in the randomization method to avoid any participant from knowing in advance which group he or she was part of. Different participants were assigned to conduct the experiment and collect the saliva. To create unbiased study environment, the participants who did the microbiological investigation did not know which mouthrinse was used for each sample; saliva samples were given different codes.

Methods for Assessment of Oral Hygiene and Caries

Demographic data and dental as well as medical history were collected from parents and subjects' files. Clinical examination was carried out for dental caries and oral hygiene status. For caries assessment, incidence of decayed, missed, and filled surfaces was documented in the primary Decayed, missing due to caries, and filled surfaces in the primary teeth (dmfs) as well as in the permanent Decayed, missing due to caries, and filled surfaces in the permanent teeth (DMFS) dentition according to the World Health Organization.[25]

Oral hygiene was measured by using DI-S score, which describes the level of soft deposits and is one of the two components of the Simplified Oral Hygiene Index (OHI-S) introduced by Green and Vermillion.[26–28]

Sample Collection

The participants did not consume food or drink any liquids (including water and soft drink) for 60 minutes before the collection of saliva. Besides that, the participants should not brush their teeth

Table 1: Ingredients and manufacturers of the mouthrinses

Mouthwash	*Ingredients*	*Manufacturer*
Avalon Avohex	Chlorhexidine gluconate 0.2% w/v.	Avalon Pharma, Riyadh, Kingdom of Saudi Arabia
Listerine Miswak	Aqua, sorbitol, propylene glycol, poloxamer 407, sodium lauryl sulfate, zinc chloride, benzoic acid, eucalyptol, aroma, sodium benzoate, methyl salicylate, thymol, sodium fluoride, menthol, sodium saccharin, caramel, sucralose, glycerin, *Salvadora persica* bark extract, sodium fluoride (220 ppm F)	Johnson and Johnson, Kingdom of Saudi Arabia
Optima Aloe Dent	Aqua, Aloe Barbadensis, sorbitol, polysorbate 20, Citrus paradisi (grapefruit seed extract), Mentha piperita, sodium lauroyl sarcosinate, aroma menthol, Melaleuca alternifolia (tea tree oil), Escin (horse chestnut), Centella asiatica (Indian pennywort), xylitol sodium hydroxymethylglycinate, sodium monofluorophosphate, citric acid	Optima Naturals, Kingdom of Saudi Arabia

for at least 8 hours before the saliva collection. The saliva was collected in an aseptic condition. Each participant was rinsed twice with 10 mL of water for 30 seconds. After 2 minutes, each child was requested to spit normal (unstimulated) saliva (2 mL) into a sterilized disposable 5-mL tube (before rinsing with the assigned mouthrinse) to use for baseline count of *S. mutans.*

Each child was asked to rinse with 10 mL of the assigned mouthrinse or control for 2 minutes according to their assigned group. Saliva samples were collected after 15 minutes using the same procedures for the baseline. Each tube was then labeled with a unique identifier. The key of the unique identifier was known to the investigators who collected the samples but not to the laboratory personnel. Saliva samples were transported within 1 hour to the microbiology laboratory for preparation and analysis of salivary levels of *S. mutans*. At the end of the research, any leftover saliva samples were discarded. Ten tubes, which contain 4.5 mL sterile 0.9% (w/v) sodium chloride, were used for every sample. The tubes were labeled from 1–10. A 0.5 mL of saliva was added to the first tube to a variety of 10-fold dilution. The solution was mixed vigorously by vortex mixer (VWR International Global Exports, Arlington Heights, Illinois, USA). A 0.5 mL from the primary tube was moved into the second tube and mixed vigorously by vortex mixer (VWR International Global Exports, Arlington Heights, Illinois, USA). The process was repeated down to the tenth tube. A 100-µL volume from each dilution was plated into TYCBS agar plate and evenly distributed on the agar surface using sterile glass rod spreaders (Thomas Scientific, Swedesboro, New Jersey, USA). Each dilution was done in duplicate. The plates were incubated aerobically (Memmert 854; Memmert GmbH + Co.KG; Schwabach, Germany) for 48 hours at 37°C. After the incubation period, the colonies on the plates were calculated using a digital colony counter (Colony Counter, Gallenkamp, Co. Ltd., England), and the colony forming unit (CFU) of *S. mutans* per mL of saliva was calculated.

Statistical Analysis

Data were analyzed using SPSS version 20.0 statistical software (IBM, SPSS Inc., Chicago, Illinois). The mean and standard deviation of *S. mutans* count between baseline and postintervention (after rinsing) values of every experimental group and control were recorded and compared using paired *t*-test and one-way ANOVA. The level of significance was set at a *p*-value of <0.05.

Results

For intra-examiner reliability of the investigators who performed the clinical examination, kappa was 0.93 which indicates a very good agreement in doing the clinical examination. The mean and standard deviation of DMFS for Avalon Avohex, Optima Aloe Dent, and Listerine Miswak groups were 5.00, 4.92, and 4.30, respectively. None of the participant was plaque free.

Table 2: Mean and standard deviation of the number of *Streptococcus mutans* at baseline and after (postintervention) rinsing for the four mouthrinse groups

	Mean (SD)		
Groups	*Baseline*	*Postintervention*	*p value*
Avalon Avohex	1.65×10^8 (1.41×10^8)	3.62×10^7 (4.44×10^7)	0.004*
Optima Aloe Dent	9.02×10^7 (1.63×10^8)	1.50×10^7 (1.08×10^7)	0.109
Listerine Miswak	1.03×10^8 (2.11×10^8)	3.01×10^7 (3.79×10^7)	0.215
Distilled water	8.10×10^7 (1.37×10^8)	2.54×10^7 (3.30×10^7)	0.089

*Significant—Wilcoxon signed-rank test

The mean and standard deviation of the number of *S. mutans* at baseline and after rinsing for all mouthrinse groups are shown in Table 2. There was evidence of bacterial reduction of *S. mutans* rinsing with all of the mouthrinses. To assess whether the reduction in the bacterial count was significantly different within the same group, paired *t*-test was performed and the results showed that there was a statistically significant reduction in the mean number of *S. mutans* in the children who were assigned to Avalon Avohex in group 1 (p <0.004) (Table 2).

A one-way ANOVA test was performed to evaluate whether the difference of the baseline and postintervention of the quantity of *S. mutans* was statistically significant among the four groups. The results showed that the mean difference in the reduction of the number of *S. mutans* was not statistically significant among the groups (p >0.05). Figure 1 shows the rank differences of the baseline and postintervention of the quantity of *S. mutans* in all the groups. Figure 2 shows the culture at baseline and after rinsing with the assigned mouthrinses.

Discussion

The null hypothesis tested in the current study was accepted as there was no significant difference in the effect of the tested mouthrinses and the positive control group against the level of salivary *S. mutans*. The results of our study showed that there was a reduction in the number of *S. mutans* after using mouthrinses in all groups compared with the numbers at baseline. However,

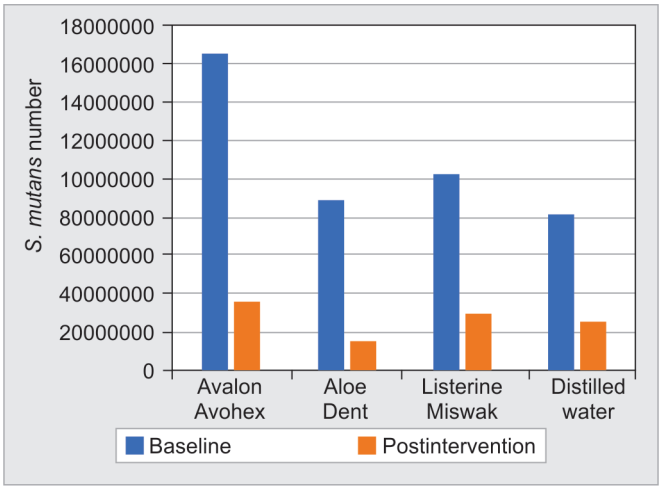


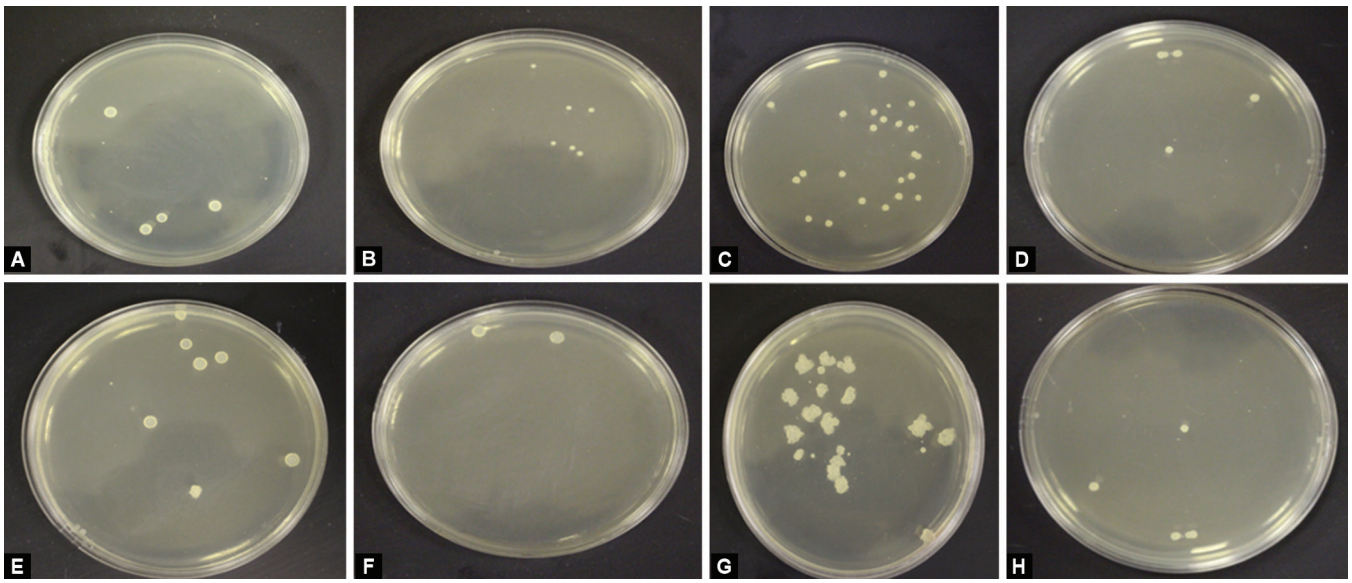
Fig. 1: Ranks of the difference in the baseline and postintervention of the number of *Streptococcus mutans* in all the groups

a statistically significant reduction was only seen in Avalon mouthrinse group. These findings were in agreement with the findings of another randomized clinical trial which showed that chlorhexidine extract mouthrinses were effective in the reduction of *S. mutans*.[23] The active ingredients in Avalon mouthrinse are chlorhexidine gluconate. Chlorhexidine is the gold standard mouthrinse due to its antimicrobial properties.[8,9] It was surprising that tested mouthrinse products did not show a statistically significant reduction of the number of *S. mutans* in comparison with the control group. This result is not expected but what is expected is to have more positive effect on the bacterial count. The findings are inconsistent with the findings of a study that showed Aloe vera mouthrinse can be an effective mouthrinse to reduce dental plaque.[18] In addition, our findings are not in agreement with a study that demonstrated that Miswak products, especially mouthrinse, have a significant effect in reducing the growth of cariogenic bacteria.[24] There are several possible explanations for these unexpected findings. One explanation could be the frequency of the exposure; the present study used a single use of mouthrinses. Several studies have reported significant effects when used more frequently over a longer duration.[18,23] Another possible explanation could be related to the composition of the mouthrinses used in this study. In our study, we used alcohol-free mouthrinses. Several clinical studies demonstrated that alcohol-containing chlorhexidine mouthrinses showed a significant effect in preventing plaque regrowth and reducing bacterial activity when compared to alcohol-free chlorhexidine mouthrinses.[29,30]

The present study used an *in vivo* model to assess the ability of the tested mouthrinses to reduce the number of *S. mutans* in saliva. The model has several advantages. The main advantage of using this model is that it uses the natural oral environment that makes the findings more relevant. However, this model has some limitations. The main disadvantage is that it depends mainly on participants' compliance. In addition, standardization is required to minimize the variations between the participants. In the current study, specific inclusion and exclusion criteria were applied to standardize the selection of the participants.

Several methods and techniques can be used for oral bacterial and sample collection. Saliva sample collection and processing are important factors that might have an effect on the observed values. Therefore, these factors should be taken into account when comparing the results of different studies. Microbiologic quantification has been shown to be the most accurate bacterial test. In the current study, bacterial culture from saliva at baseline and after rinsing with the assigned mouthrinses were obtained and used as microbiologic quantification. The technique showed the number of *S. mutans* within a reasonable time.

In the present study, mouth rinsing was performed for 2 minutes only. This protocol was used in a previous study and showed significant reduction of *S. mutans* after the use of tea mouthrinse.[31] However, other study showed positive correlation between increased exposure time and lower level of *S. mutans*.[32]



Figs 2A to H: Culture showing results: (A) At baseline in distilled water group; (B) After rinsing with distilled water mouthrinse; (C) At baseline in Aloe Dent group; (D) After rinsing with Aloe Dent mouthrinse; (E) At baseline in Avalon Avohex group; (F) After rinsing with Avalon Avohex mouthrinse; (G) At baseline in Listerine Miswak group; (H) After rinsing with Listerine Miswak mouthrinse

There are some limitations to this study. The first limitation is that due to the current pandemic of coronavirus disease-2019 (COVID-19) and other unavoidable circumstances, we could not reach the calculated sample size. Future studies with a larger sample size would strengthen the research. In the present study, single use of assigned mouthrinse was used. It would be interesting to see the long-term effect of the assigned mouthrinses. We suggest further randomized studies with more extended period and repeated use rather than single use to evaluate the long-term effect of herbal extract mouthrinses on reducing bacterial biofilm.

Conclusions

Within the limitations of this study, it was concluded that in all groups, there was evidence of reduction of the number of *S. mutans* when compared to that at baseline. A statistically significant difference in terms of the number of *S. mutans* was only seen in the Avalon Avohex group. No statistically significant differences in the mean difference in bacterial reduction were found between the tested mouthrinses.

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