RESEARCH ARTICLE

Antimicrobial Efficacy of Chlorhexidine and Herbal Mouth Rinse on Salivary *Streptococcus mutans* in Children with Mixed Dentition: A Randomized Crossover Study

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Abstract

Aim and objective: *Streptococcus mutans* (*S. mutans*) shows increased resistance to currently available antibiotics and chemotherapeutics. The present study compares the effectiveness of chlorhexidine and Herbal mouth rinse against salivary *S. mutans* in children with mixed dentition.

Materials and methods: Subjects ($n = 60$) with mixed dentition were selected for the study. Caries status was recorded using Nyvard's criteria. Baseline saliva samples were collected and assessed for quantifying *S. mutans*. Subjects were instructed to rinse their mouths with 0.2% w/v chlorhexidine and herbal mouth rinse for 7 days. Saliva samples were collected after 7 days and assessed for *S. mutans*. After a run-in period of 21 days, both the mouth rinses were crossed over according to the Latin square design, and a similar procedure was carried out. Later, determination of mean colony-forming units (CFU/mL) from the saliva samples was done. For statistical analysis, Kolmogorov and Mann–Whitney *U* tests were applied.

Results: Both the groups showed a significant reduction in *S. mutans* count, at baseline and 7 days ($p = 0.0001$), and the reduction of *S. mutans* count in herbal mouth rinse as compared to chlorhexidine mouth rinse ($p = 0.0209$) was statistically significant.

Conclusion: Herbal mouth rinse proved to have better antimicrobial efficacy than Chlorhexidine mouth rinse.

Keywords: Antibacterial, Chlorhexidine, Dental caries, Herbal, *Streptococcus mutans*.

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Introduction

Dental caries is characterized by demineralization of calcified tissues of the teeth due to several factors.[1] Approximately, 60–65% of children in India are affected by dental caries. Several organisms are involved in the development of carious lesions.[2,3] However, the literature strongly reports *Streptococcus mutans* (*S. mutans*) as the potential organism involved in the initiation of dental caries. This is because *S. mutans* can attach to the enamel surface, produce acid metabolites, provide glycogen reserves, and has the ability to synthesize extracellular polysaccharides.[1]

Several antimicrobial agents with varying efficacy have been reported for the reduction of dental caries.[4] Preventive programs such as the use of fluoride toothpaste, community water fluoridation, and mouth rinses, focusing primarily on the reduction of caries have also been carried out to reduce the prevalence of the disease.[5] These substances inhibit the adhesion of bacteria, their colonization, and metabolic activity ultimately affecting their growth.[4] Presently, bisbiguanide being the most efficacious chemotherapeutic agent against *S. mutans* has high bactericidal activity against both gram-positive and gram-negative bacteria. But it has a few side effects such as tooth discoloration, the altered sensation of taste, and erosion of oral mucosa.[6,7] Also, due to the developing antimicrobial resistance to the currently available antibiotics and chemotherapeutics, the implementation of alternative treatment options for oral diseases that are safer, effective, and economical are needed.[8]

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Natural, organic, and herbal mouth rinses do not contain alcohol, artificial preservatives, or colors and flavors, and have unique therapeutic properties. Hence, they are attaining popularity among today's relatively more aware consumers. Research data supports their bactericidal action, antiplaque, and anti-gingivitis efficacy.[8] Moreover, studies pertaining to herbal mouth rinses and their efficacy in the reduction of dental caries are lacking; hence, it is essential to produce required evidence. The present study was therefore conducted to evaluate and compare the effectiveness of Chlorhexidine and Herbal mouth rinse on salivary *S. mutans* in children with mixed dentition.

Materials and Methods

Study Design and Setting

The current randomized crossover study was conducted at an underprivileged Government-residential school in the Northern part of Karnataka. The study was briefed to the Principal along with the guardian of the school and consent was obtained. Written informed consent was also obtained from the children before participating in the study. Procurement of ethical clearance was done by the Institutional Ethics Committee. The following formula determined the sample size: $n = 2S^2 /d^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2$, α = 5% ($Z_\alpha = 1.96$) and β = 80%, ($Z_{1-\beta} = 0.824$) where α = Probability of type I error, 1-β = Power of the study, S = 794.57, d = 575.24, Z = Standard normal deviate. As per the calculation, the required sample size was 60. Children with mixed dentition, who were free of any systemic diseases, and residing in the same geographic area were included as part of the study. Children suffering from any disease affecting the flow of saliva, children having special healthcare needs, and those showing any adverse reactions to the products used in the study or under any antibiotic therapy (1 month before the study) were excluded from the study.

Data Collection

Demographic data and oral findings of all the patients were recorded in a predesigned proforma. Caries status was recorded using Nyvad's criteria.[9] Baseline unstimulated whole saliva[10] of volume 1 mL was collected between 10 and 11 am[11] by a suction method using a sterile disposable syringe (Unolok Hindustan Syringes & Medical Devices Ltd., Faridabad, India) and transported immediately in thioglycolate medium for microbial assessment on the same day of sample collection.

Intervention

The intervention was carried out according to Latin square design[6] (Fig. 1) in two phases– Phase I and Phase II. In Phase I, the subjects were randomly assigned into two groups: Chlorhexidine group (Hexidine mouthwash 0.2%, ICPA Health Products Ltd., Ankleshwar, India) and herbal mouth rinse group (Hiora mouthwash, The Himalaya Drug Company, Bengaluru, India) by lottery method. The assigned mouth rinse (5 mL) was used to rinse the mouth for 1 minute and then expectorated, twice daily, for 7 days under the supervision of the primary investigator. The subjects were told not to consume anything 30 minutes after rinsing. On day 8, collection of 1 mL unstimulated whole saliva was done an hour after rinsing the mouth with the respective mouth rinse, and assessed for *S. mutans* growth as mentioned in baseline saliva evaluation.

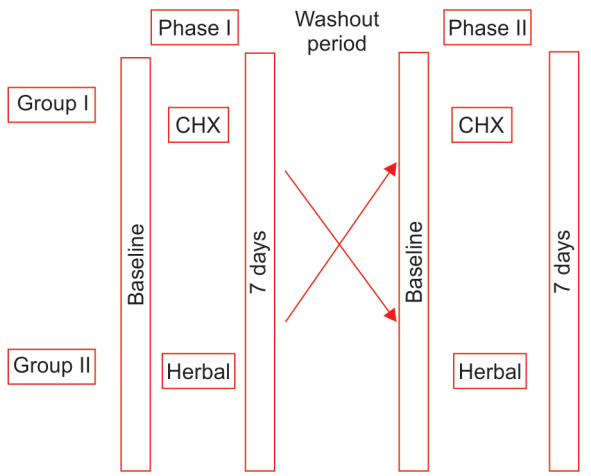


Fig. 1: Latin square design used in the study

A washout period of 21 days was given when neither of the two mouth rinses was used. The selected subjects were then assigned to Phase II in which the baseline saliva was collected with a similar protocol as followed in Phase I. The mouth rinse formulations were interchanged as per Latin square design and the subjects were told to use the assigned mouthrinse for the next 7 days. The post-test saliva was collected on Day 8 and subjected to microbiological assessment. During the entire duration of the study, the subjects were told to follow their routine oral hygiene habits.

Microbial Assessment

Dilution (1: 10,000) of the saliva samples in 0.05 M phosphate buffer (pH 7.0) was done using serial dilution method; 0.1 mL of which was inoculated on Mitis Salivarius Agar with potassium tellurite medium and bacitracin (MSBA). Incubation of the plates was done for 48 hours at 37°C in a 5–10% CO_2 jar, under a light microscope.. The number of Colony Forming Units (CFU) of *S. mutans* were determined using a stereomicroscope. Further, the determination of CFU per mL was done using the following formula:

No. of colonies × Dilution factor/Volume inoculated in mL = CFU/mL

Where, Volume inoculated = 0.1 mL and Dilution factor = 10^4

Statistical Analysis

SPSS v20 was used to analyze the data. The collected data were tabulated using Microsoft Excel 2007 and subjected to analysis statistically. Determination of distribution of the data was done using the Kolmogorov Smirnov test. Intergroup comparison was done using Mann–Whitney *U* test and intragroup comparison using Wilcoxon signed-ranks test to compare the effectiveness of chlorhexidine and herbal mouth rinse. A p value < 0.05 has proven to be statistically significant.

Results

Out of 60 children with a mean age of 8.97 + 1.91 years, 31 children (51.67%) were male and 29 children (48.33%) were female. The mean difference of *S. mutans* count, at baseline and 7 days was not significant (A group p value is 0.0880 and B group p value is 0.2590) and followed a normal distribution (Table 1).

The intragroup comparison showed that *S. mutans* count had significantly reduced in both the groups at baseline and after 7 days ($p = 0.0001$; Table 2 and Fig. 2).

A statistically significant difference in *S. mutans* count ($p < 0.0036$) was seen on the intergroup comparison at baseline; whereas a statistically insignificant difference ($p < 0.1347$) between both the groups was seen on the 7th day. However, a statistically significant overall mean difference of *S. mutans* count between both the groups was observed ($p = 0.0209$; Table 3).

Discussion

This study was performed with a crossover design known as Latin square design. This design has the advantage of subjects acting as their own controls and hence there is less variability within a subject. Also, the order in which the interventions are carried out in a crossover trial may cause errors which are named "order effects." These errors are eliminated by Latin square design.[6] However, the carryover effect is one of the biggest problems associated with Latin

square design. To eliminate this effect, a run-in period of 21 days had been used in this study, though it was more time-consuming.[12]

Children with mixed dentition with a mean age of 8.97 + 1.91 were included in the present study because of second window of infectivity. The infectivity window is mostly observed in children between 6 and 12 years, during which multiple permanent teeth erupt and tooth surfaces are exposed to caries risk until the second molars are fully developed.[13]

Stimulated and unstimulated saliva are the two different methods of saliva collection. Unstimulated saliva was used in the present study because of its lower concentration of bicarbonate ions thus reducing the bias due to the buffering action of saliva. The four most common approaches for the collection of unstimulated saliva are the draining method, suction method, spitting method, and absorbent (swab) method.[10] Due to its ease of use, a suction method was used in this study. A significant circadian rhythm in the rate of flow and in concentration of sodium and chloride was seen in unstimulated whole saliva all throughout the day.[11] With the increase in the flow rate, the pH of the saliva also increased, and thus, to standardize the procedure, saliva was collected between 10 and 11 am of the day.[11] Although there are several specific media available for isolation of *S. mutans,* MSBA is the most specific test

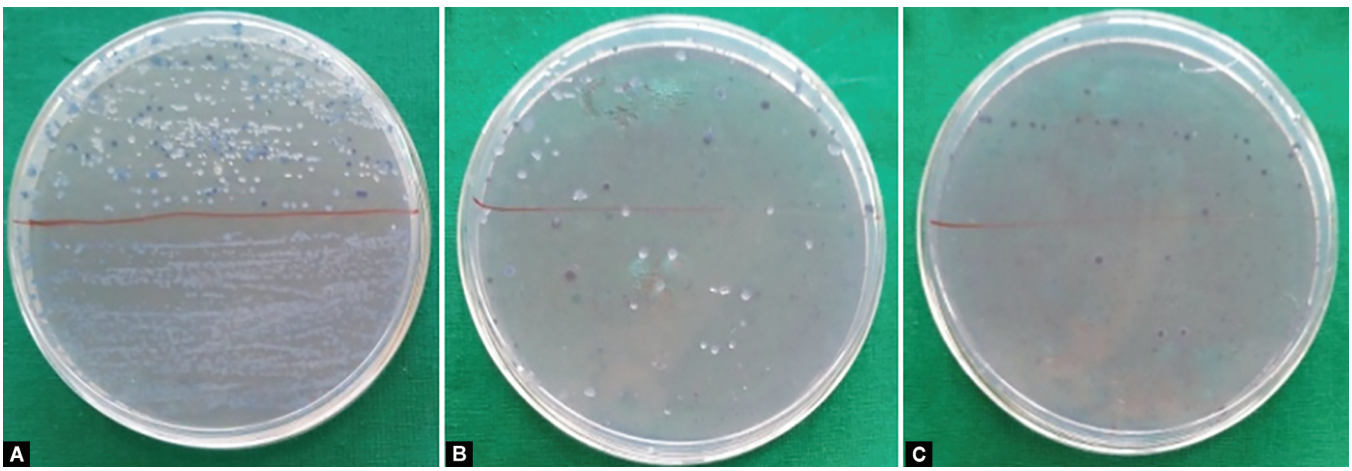
Table 1: Distribution of *S. mutans* count among the test groups at baseline and 7 days

	No. of CFU ($x10^8$)			
	Group A chlorhexidine		*Group B herbal*	
Time	*Mean ± SD*	*p-value*	*Mean ± SD*	*p-value*
Baseline	1.52 ± 1.11	0.0380*	2.23 ± 1.38	0.0360*
7 days	0.56 ± 0.40	0.0340*	0.72 ± 0.52	0.0680
Difference	0.96 ± 1.00	0.0880	1.50 ± 1.29	0.2590

Kolmogorov Smirnov test, *$p < 0.05$ indicates non-normal distribution

Table 2: Intragroup comparison of *S. mutans* count (number of CFUx10^8) *At baseline and 7 days*

	Mean ± SD, CFU/mL		
Mouth rinse	*Baseline*	*7 days*	*p-value*
Chlorhexidine Group A	1.52 ± 1.11	0.56 ± 0.40	0.0001
Herbal Group B	2.23 ± 1.38	0.72 ± 0.52	0.0001



Figs 2A to C: Photograph showing the growth and colonization of *S. mutans* at: (A) Baseline; (B) After administration of chlorhexidine mouthrinse; (C) After administration of herbal mouthrinse

Table 3: Intergroup comparison of *S. mutans* count (number of CFUx10^8) *at baseline and 7 days*

Time points	*Chlorhexidine Group A (Mean ± SD)*	*Herbal Group B (Mean ± SD)*	*p-value*
Baseline	1.52 ± 1.11	2.23 ± 1.38	0.0036*
7 days	0.56 ± 0.40	0.72 ± 0.52	0.1347
Difference	0.96 ± 1.00	1.50 ± 1.29	0.0209*

Mann–Whitney *U* test, *$p < 0.05$

for this organism.[14] Moreover, *S. mutans* is the only organism intended to be isolated in the present study.

An amount of 0.2% Chlorhexidine when used as a mouth rinse was effective in reducing the salivary *S. mutans* count. Similar results have been obtained in previous studies.[4,7] Chlorhexidine is found to be effective against a wide spectrum of gram-positive and gram-negative organisms. Being bacteriostatic at lower concentrations causes cell wall leakage in the microorganisms and at higher concentrations, it is bactericidal causing precipitation of cytoplasm by protein-cross-linking.[15] Although effective, it causes a few adverse effects such as tooth discoloration, the altered sensation of taste, and erosion of oral mucosa.[7] The discoloration is mainly due to the adsorption of Chlorhexidine on the surface of hydroxyapatite crystals that alters the binding ability of the crystals.[16] There is a sodium receptor molecule in the taste buds that is unique in property and different from receptors for sweet, sour and bitter stimuli. Chlorhexidine binds to this specific receptor causing alteration of taste sensation.[6] Chlorhexidine also precipitates mucins, which can lead to a reduction in the amount of IgA available in the mouth, as IgA is found in high concentrations in the mucin layer.[15] However, it was reported that side effects were observed only when the mouth rinse is used for a long-term, that is, period of more than 1 month.[17,18] In this study, no such side effects were observed as the intervention lasted only for a week.

A significant reduction of salivary *S. mutans* count was seen after 7 days after using Chlorhexidine mouth rinse as compared to that at a baseline level in our study. Similar studies were done by Shah et al.[19] and Sharma *et al.*[20] also found significant reduction in CFU of salivary *S. mutans*, with the use of Chlorhexidine mouth rinse. Herbal mouth rinse in our study also showed a significant reduction of *S. mutans* count in saliva after 7 days compared to that at a baseline level, which is similar to study conducted by Shah et al.[19] However, the herbal mouth rinse used in the present study was found to be better than the Chlorhexidine mouth rinse in reducing salivary *S. mutans* count. Similar studies done by Shah et al.[19] and Nayak et al.[21] with different herbal products also proved better antimicrobial effect on salivary *S. mutans,* than Chlorhexidine. On the contrary, a similar study conducted by Sharma et al.[20] reported that Chlorhexidine was better as compared to herbal rinse (Hiora) in reducing *S. mutans* count.

Hiora contains *Salvadora persica* (mustard tree), *Terminalia belerica* (Baheda), *Piper betel* (Betel leaf), *Gaultheria fragrantissima* (Oil of Wintergreen), *Elettaria cardamomum* (Cardamom), *Mentha piperita* (Menthol), and *Trachyspermum ammi* (Bishop's weed). *Salvadora persica* is a medicinal plant which contains alkaloids, fluorides, glucosinolates, sulphur compounds, volatile oils such as benzyl isothiocyanate which have antimicrobial and prophylactic properties.[22] *Terminalia bellerica* contains ellagic and gallic acid. The presence of these active ingredients, of phenolic nature, is responsible for scavenging the free radicals.[23] *Piper betel* has shown to inhibit growth, acid production, cell-associated glucosyltransferase and adherence of *S. mutans*. The fatty acids present in the extract interfere with glycolytic enzymes of bacteria and thus, interfere with their acid production.[24] Other herbal mouth rinses that are also found to be effective against *S. mutans* were *Ocimum sanctum* ,(tulsi)[25] *Azadirachta indica* (neem),[26] and Triphala.[27]

Interpretation of the study results should be done observing certain limitations that include a small sample size. The result could be better relied upon if a larger sample size could have been introduced. Nonetheless, it has substantial future implications that this ayurvedic mouth rinse can be promoted by dentists as they have equal efficacy against *S. mutans* as compared to 0.2% Chlorhexidine mouth rinse with no side effects.

Conclusion

The present study demonstrated that both Chlorhexidine and herbal mouth rinses have greater antimicrobial efficacy against *S. mutans*. However, herbal mouth rinse proved to have better antimicrobial efficacy than Chlorhexidine mouth rinse. Further, long-term studies need to be done with a larger sample size to reinforce these findings.

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