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Comparative Evaluation of Antimicrobial Efficacy of 12.5% Xylitol, 0.2% Chlorhexidine and Combination Mouthwash against S mutans

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ABSTRACT

Aim: To compare the antimicrobial efficacy of 12.5% Plain xylitol mouthwash with 0.2% chlorhexidine mouthwash, and combination (chlorhexidine, sodium fluoride, zinc sulphate & xylitol) mouthwash against S mutans.

Material and method: In present in-vitro study mouth rinses used were combination (MR1) of Chlorhexidine (0.2%), Sodium fluoride (NaF 0.05%), Zinc sulphate (0.01%) and xylitol, 12.5% xylitol (MR2), Chlorhexidine (0.2%) (MR3), mouthrinse and normal saline (0.89%) (MR4) as a negative control. The antimicrobial activities of mouth rinses were determined by culturing the test organisms on Agar Well Diffusion method and inhibition zone diameters (in mm) were read and statistically analyzed.

Result: The mean actual Zone of Inhibition for MRI was 14.10±1.73, MR2 was 10.40±3.31, zero for MR3 and MR4.

Conclusion: The combination mouthwash showed the highest zone of inhibition than Chlorhexidine (0.2%) mouthwash and control group.

Keywords: Antimicrobial, mouth wash, S. Mutans, xylitol.

INTRODUCTION

The oral cavity is one of the most complex parts of the human body that consists of hard and soft tissues and harbours a variety of microbial community which makes it vulnerable to infectious diseases.[1] One of the most common infectious diseases of the oral cavity is dental caries, which has multi-factorial aetiology such as diet, microflora, host and time. It has been demonstrated that micro-organisms are one of the major etiological factors. Amongst the pathogenic flora Streptococcus Mutans is considered to be the main microorganism associated with dental caries.[2] Dental plaque is of vital significance as it contributes in the initiation of dental caries and periodontal diseases and Streptococcus Mutans is considered as the main colonizer in the multispecies dental biofilm.[3] Although mechanical removal of dental plaque is still regarded as promising approach,[4] high

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incidence of dental and gingival disease makes the use of an adjunctive method for improving oral health. Use of mouthrinses has been introduced as an effective way for reducing dental plaque accumulation.[5]

Streptococcus mutans induces mineral loss due to its strong adhesion to the tooth surface and production of acid from fermentable carbohydrates, which keeps the local pH low.[6] The acidogenic and aciduric (associated with acid tolerance) properties of S. mutans, together with its ability to synthesize extracellular glucans, are the major factors for the development and establishment of cariogenic biofilms.[7] Antiseptic mouth rinse solutions with variations of the antimicrobial efficacy and kinetics of the solutions are used in clinical situations for different prophylactic and therapeutic purposes.

Chlorhexidine gluconate mouthwash has earned the eponym of the gold standard to treat and/or prevent periodontal disease. Its a cationic bisbiguanide with a very broad antibacterial, anti-cariogenic and remineralising actions and few toxic effects and is most widely used over the counter mouth rinse.[8,9] It is used as an adjunct to mechanical cleaning procedures as well as used alone. The major advantage of chlorhexidine over most other compounds lies in its substantivity. It binds to soft and hard tissues in the mouth, enabling it to act over a long period after application of a formulation. However, chlorhexidine has several side effects, such as staining and taste alteration, which limit its long term use. Therefore, chlorhexidine is used as a positive control in many clinical trials. [10]

Xylitol is a naturally occurring five-carbon polyol and is a white crystalline carbohydrate. It is found naturally in fruit, vegetables, berries and is artificially manufactured from Xylan rich plant material such as birch and beechwood. Xylitol reduces the level of Mutans Streptococci in plaque and saliva by disrupting their energy production process, leading to a futile energy cycle and cell death. It reduces the adhesion of these microorganisms to the teeth surface and also reduces acid production potential. Excess xylitol is known to induce osmotic diarrhoea, indicating that there is an upper limit to its dosage that can be tolerated.[11] Studies suggest xylitol that consistently produces positive results ranged from 4-10gm per day divided into 3 to 7 consumption period.[12]

An in vitro combination of CHX and xylitol has been studied by Modesto and Drake and Deng et al. who observed that the combined use of such substances dramatically inhibits both growth of S.mutans and formation of biofilm.[13] The xylitol and chlorhexidine combination inhibited streptococci more when compared with xylitol or chlorhexidine being used alone. This newly discovered synergistic action could be used for high-risk caries patients or for reducing MS transmission from mother to child.

Several studies have shown that xylitol has anti-caries activity most notably in chewing gum and candy vehicles. Frequent use of xylitol chewing gum has been shown to prevent dental caries.[14,15] Nevertheless, xylitol in the forms of chewing gum and candy may not be practical for young children or elderly adults. In these situations, an aqueous solution or mouthrinse may be useful.

Xylitol has been incorporated into fluoride-containing mouthrinses. In-vitro studies have suggested that fluoride and xylitol exert an additive inhibitory effect on growth and acid fermentation by S.mutans and Streptococcus sobrinus.[16,17]

Thus there is not enough documented literature comparing the in vitro efficacy to compare the efficacy of different mouthwashes viz chlorhexidine, xylitol and combination of both against on Salivary S. Mutans. Therefore, this *in-vitro* study was aimed to compare the antimicrobial efficacy of 12.5% Plain xylitol mouthwash with 0.2% chlorhexidine mouthwash, a combination of chlorhexidine, sodium fluoride, zinc sulphate & xylitol mouthwash against S mutans.

MATERIALS AND METHODS

In the present, an *in-vitro* study mouth rinses used were Chlorhexidine (0.2%), a combination of Chlorhexidine (0.2%), Sodium fluoride (NaF 0.05%), Zinc sulphate (0.01%) and xylitol, 12.5% xylitol mouthrinse which are commercially available mouthrinse and normal saline (0.89%) as a negative control. (table-I)

Preparation of culture media was done as per manufacture's (HIMEDIA) specifications. Sterilization was done by autoclaving at 15lbs psi

(per square inch) for 15 minutes. These media were poured in disposable sterilized plastic Petri plates to obtain matrix thickness of 4 mm. Freeze-dried cultures which were kept in the refrigerator at 4°C were reconstituted by breaking the glass ampule and adding 500µl of BHI broth. Later the tube was incubated at 37ºC for 24 hours. The culture viz: Streptococcus mutans were plated on BHI agar incubated at 37ºC for 24 hours. The purity of culture was confirmed by Gram staining before use.

The antimicrobial activities of three mouthrinses were determined by culturing the test organisms on **Agar Well Diffusion method,** as depicted in the flowchart below:

The colony growth from respective cultures was passed in 4 ml of sterile normal saline in a tube. The tubes were shaken to have visible turbidity. The surface of culture media plates was dried by keeping the plates open in the incubator for 4 hrs.

The normal saline bacterial suspension was flooded on to the surface of dried plates. Extra culture inoculum was decanted off from the surface of the medium back into the culture tubes.

The plates were incubated at 37°C for about 45 minutes for the liquid culture to absorb in the matrix.

Using the borer which had been flamed red hot and subsequently cooled, wells having a diameter of 6mm and depth of 4mm were made in the plates. Four wells were punched in each Petri plate.

Each well per plate was filled up to the brim, with full strength respective mouthrinses with marking at the back of the plate. A set of 10 plates was put up for each test organism.

The plates were kept for 1 hour at room temperature for the diffusion of the agent through the medium. Afterwards, the plates were incubated at 37°C for 24 hours with the lid upwards. This work was carried throughout the laminar airflow station.

Measurement of the zone of inhibition:

Following the incubation, inhibition zone diameters (in mm) were read and measured along the most uniform diameter with the help of scale.The zone of inhibition of growth of test strains was defined by the area where visible growth had been inhibited. All the measurements of the zone of inhibition were carried out by a single examiner.

The data was tabulated and entered into Microsoft excel. The SPSS software version 21.0 was used for statistical analysis. For each mouthrinse, the mean and standard deviation of the diameters of inhibition zones was calculated. The normality of the data was checked using the **Shapiro-Wilk test** and the data was found to follow Normal distribution. The data were analysed using one-way analysis of variance, for comparison of the mean zone of inhibition among mouth rinses. The inter-group comparison between the different mouthwashes was made using the posthoc Bonferroni test

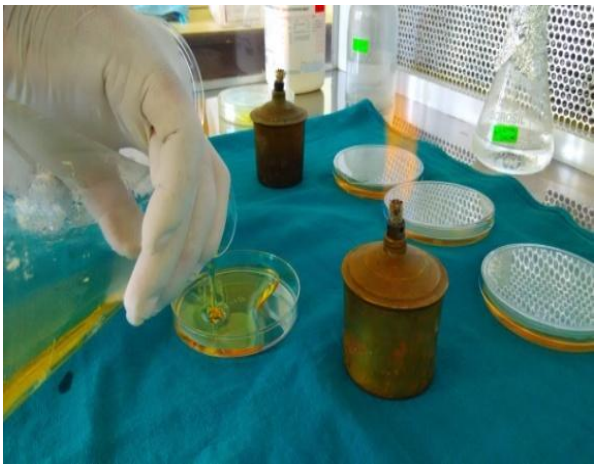


Fig 1: Flood in the broth bacterial culture in the culturing plate.

Fig 2: Cutting of 6 mm diameter wells removing the agar matrix from the well.

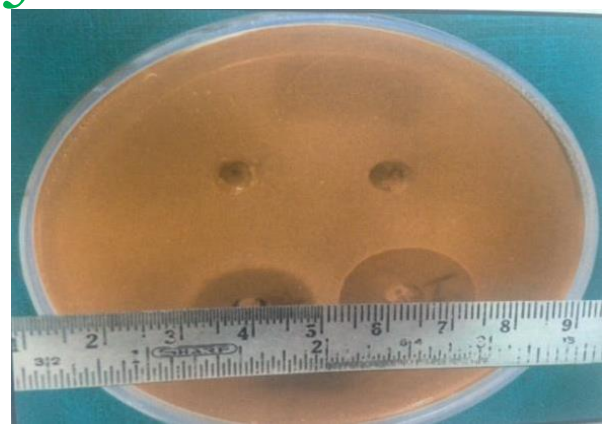


Fig 3: Measurement of the zones of inhibition using a sterilised steel scale.

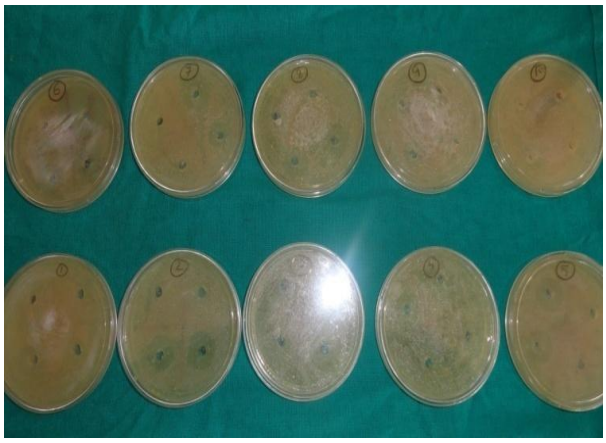


Fig 4: All the culture plates showing zone of inhibitions.

RESULTS

Combination of Chlorhexidine (0.2%), Sodium fluoride (NaF 0.05%), Zinc sulphate (0.01%) and xylitol (MR1), 12.5% xylitol mouthrinse (MR2), Chlorhexidine (0.2%) mouthwash (MR3) and Control (MR4) group.

Table 1 and Graph-1shows the comparison of the mean zone of inhibition of all the four mouth rinses against Smuts. The mean actual Zone of Inhibition for MRI was 14.10±1.73, MR2 was 10.40±3.31, zero for MR3 and MR4. The comparison of the mean zone of inhibition of 4 types of mouthwash against S mutans was made using ANOVA test. The difference between all the organisms was found to be statistically significant (p-value<0.05).

The Bonferroni post hoc test was applied for within-group comparison. The combination of Chlorhexidine (0.2%), Sodium fluoride (NaF 0.05%), Zinc sulphate (0.01%) and xylitol mouthwash showed the highest zone of inhibition which was significantly higher than Chlorhexidine (0.2%) mouthwash which was significantly (p-value < 0.05) higher in comparison to 12.5% xylitol and control group mouthwashes**(Table 2)**.

DISCUSSION

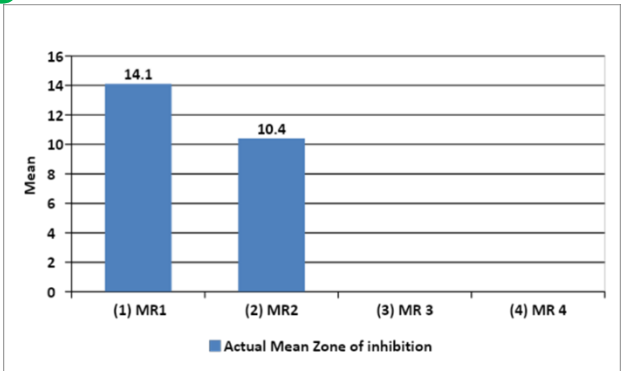
The oral cavity represents a dynamic ecosystem; therefore, it would not be totally advantageous to eliminate all elements of the oral microflora in an effort to control dental plaque-associated infections. Instead, it may be more ideal to remove only most cariogenic and periodontopathic aspects of the dental plaque microflora while permitting the more innocuous elements to remain.[18]The primary etiological factor for dental diseases is dental plaque. The formation of plaque on the tooth surface is characterized by the progression from a limited number of pioneer microbial species to the complex flora of mature dental plaque.[19]

Table 1: Details of the three test mouth rinses used.

Mouthrinse Code	**Ingredients as listed in Package**
MR1	• Chlorhexidine Gluconate solution 0.2% w/v • Sodiun fluoride IP 0.05% w/v • Zinc sulphate IP 0.01% w/v • Xylitol
MR2	12.5% Plain xylitol solution
MR3	Chlorhexidine Gluconate solution 0.2% w/v
MR4	0.89% Normal saline

Table 2: Comparison of mean actualZone of Inhibition (in mm) produced by 4 Mouthrinses against Streptococcus Mutans.

ZONE OF INHIBITION			
Mouthwashes	**Mean**	**SD**	**p-value**
(1) MR1	14.10	1.73	**<0.001***
(2) MR2	10.40	3.31	
(3) MR 3	0.00	0.00	
(4) MR 4	0.00	0.00	



Graph 1: Actual Zone of Inhibition (in mm) produced by 4 Mouthrinses against Streptococcus Mutans.

The activities of oral microflora being responsible for mouth odour and most oral diseases are not in doubt. The need to keep these oral organisms to a level consistent with oral health by antimicrobial agent inclusion in mouthrinses has been stressed.[20] These substances act by killing the microorganisms by disrupting their cell walls and inhibiting their enzymatic activity.[21] They also prevent bacterial aggregation, slow multiplication and release endotoxins. Several clinical studies have demonstrated the inhibitory effects of antimicrobial mouthrinses on oral bacteria and gingiva.[22]

Furthermore, S. mutans is able to demineralize hydroxyapatite, thus demonstrating its relevance in the development of caries.[12,23] Therefore, reducing the level of this microorganism in the oral cavity seems to be crucial for controlling caries in general.

A thorough literature search was carried out to search the in-vitro studies for the comparison of anti-microbial efficacy between Chlorhexidine, Plain xylitol, and a combination of xylitol and chlorhexidine mouthwash. Since minimal literature could be found regarding the in- vitro studies though there was an abundance of clinical trials. So, the present discussion will be quoting mostly the clinical trials.

The present in-vitro study has shown that combination of chlorhexidine, sodium fluoride, Zinc sulphate, and xylitol mouthwash has the maximum anti-bacterial action against S. mutans followed by CHX alone as depicted by the higher zone of inhibition. Whereas, plain xylitol and normal saline mouthwashes did not produce any zone of inhibition demonstrating no anti-microbial action against S mutans.

Lobo[24], in an in-vitro study, demonstrated that Xylitol was the most effective against the viability of S. mutans and in reducing the formed biofilms, followed by Chlorhexidine, Cinnamon oil and Sodium benzoate mouthrinses. Comparison between various antimicrobial agents used in this study showed that Xylitol sugar had a maximum bactericidal effect against the plaque-causing S.mutans. Hence it can be used considerably in order to prevent as well as treat plaque formation. Also, the effectiveness of Chlorhexidine was also commendable, suggesting that it, along with Xylitol in combination, is worthy of further study and commercial application.

Similar findings have been reported in some other previous studies, which showed that individuals using chlorhexidine had the best results regarding biofilm reduction compared to those using either fluoride or only xylitol. [25,26]

In a clinical study by Hildebrandt et al. .[25,] there was no significant difference between xylitol crystals group, xylitol chewing gums group, and a negative control group. However, MS levels tended to be lower in the Xylitol crystal group and xylitol chewing gum group.Xylitol rinse and chewing gum caused a similar but statistically insignificant reduction in MS levels in the mouth.

Arunakul et al.[27] demonstrated a significant reduction in MS count were observed in subjects using 0.05% NaF + 12.5% xylitol mouthrinse compared to0.05% (w/v) sodium fluoride, 12.5% (w/v) xylitol and distilled water (control) groups providing evidence for the inhibitory effects of xylitol, used in combination with fluoride.

Meurman (1988) studied the ultrastructure, growth, and adherence of Streptococcus mutans ATCC 27351 to hydroxyapatite, after treating bacterial suspensions for 1 hour with 0.1% chlorhexidine gluconate (CHX), 0.1% sodium fluoride (F), and a combination of the two. The fluoride-treated specimens appeared the same as the controls, where the ultrastructure was mostly normal. Treatment with fluoride alone did not cause alterations in the ultrastructure or reduction in adsorption of S. mutans.[28]

An intensely researched preventive agent in dentistry has been chlorhexidine. It is an effective anti-plaque agent and is widely available as a mouth rinse. Chlorhexidine is a bis-biguanide which is effective against Gram-positive bacteria, Gram-negative bacteria and yeast. At relatively high concentration, chlorhexidine is bactericidal; but at low concentration, it is bacteriostatic.[29] The positively charged chlorhexidine binds readily to the negatively charged microbial cell surface. This is followed by disorganization of the cytoplasmic membrane. Low concentrations of chlorhexidine allow cytoplasmic constituents to leak out, while a high concentration coagulates them. It inhibits the membrane ATPase and anaerobic process.[30, 31]

The efficacy of the Sodium Fluoride mouthrinse was in combination with Chlorhexidine against S Mutans. As mentioned earlier, the main mechanism of the action of Sodium Fluoride is to maintain equilibrium between the demineralization and remineralisation of dental hard tissues rather than antimicrobial action.[32] Most of the in vivo studies have shown a cariostatic effect of fluoride gels or mouthrinses at 1% or 2% Fluoride concentration. Thus, the observed lower efficacy of Fluoride mouthrinse could also be attributed to the lower percentage of Fluoride (0.2% of NaF) used in this study.

In recent times, various polyalcohols, particularly xylitol, have been incorporated in a number of different products for children, including mouth rinses. Xylitol is a non-nutritive sweetener that has demonstrated effectiveness for preventing caries. The nonspecific effect of xylitol is due to its non-fermentability by the plaque bacteria and thus does not encourage bacterial growth.[33]

The ability of plaque to produce acids by the metabolism of sugars is reduced by xylitol. This is explained by a selective decrease in mutans streptococci in plaque exposed to xylitol. Xylitol appears to have a unique effect in reducing adhesion and it is expected that other polyols might not show this clinical effect. The concentrations of ammonia and basic amino acids increase when plaque is exposed to xylitol, resulting in neutralization of plaque acids.[34]

In vitro studies have demonstrated that MS are target organisms of xylitol. The inhibition effect of xylitol varies among MS strains. It is reported that the ability of xylitol to act as an anti-cariogenic agent is most likely due to its ability to be transported into caries-causing oral bacteria, inducible fructose phosphotransferase system (PTS) and inhibiting fermentation either by depleting the cell of high-energy phosphate or by poisoning the glycolytic system.[35-38]

The present study demonstrated that a combination of mouth rinse is more effective than a single use of commercial mouth rinse. The studies have demonstrated the effectiveness of rinsing with an antimicrobial mouthrinse in significantly reducing both salivary and mucosal levels of bacteria. But anti-microbial efficacy was checked in vitro, so it cannot be assumed that the results of antimicrobial efficacy could be proportional or transferable to the oral cavity and translated into clinical effectiveness.

Hence further clinical studies are needed to prove the efficacy of a combination of mouth rinse.

CONCLUSION

Thus, from the results of the present study, it can be concluded that xylitol added to other mouthwashes will have a synergistic effect against the S. mutans but Plain xylitol is not effective as a mouth-rinsing agent.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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