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RESEARCH ARTICLE

EFFICACY OF THREE COMMERCIAL MOUTH RINSES CONTAINING CHLORHEXIDINE , HERBAL & SODIUM FLUORIDE-TRICLOSAN- XYLITOL AGAINST SALIVARY BACTERIA, STREPTOCOCCUS MUTANS: A MICROSCOPIC STUDY

[1,*]Dr. Nayar Islam, [2]Dr. Nilishashukla, [3]Dr. Amyn Chamadia and [4]Dr. Sanket Deolkar

[1]Post Graduate student, Department of Prosthodontics, Saraswati Dental College, India
[2]Post Graduate student, Department of Prosthodontics, Saraswati Dental College, Lucknow, India
[3]Post Graduate student, Department of Prosthodontics, Babu Banarasi Das Dental College, Lucknow, India
[4]Post Graduate student, Department of Prosthodontics, Pacific Dental College, Udaipur, India

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ABSTRACT

Objective: The purpose of the present study is to compare the efficacy of three commercially available mouth rinses containing chlorhexidiene , herbal &sodium fluoride-triclosan- xylitol respectively against salivary bacteria, streptococcus mutans. **Materials and Methodology-** A sample of 60 subjects was selected from the single school of Jodhpur city and was randomly equally divided into 4 groups. The subjects were instructed to rinse the mouthwash 10 ml of mouthwash for one minute twice daily for fifteen days. Number of colony of Streptococcus mutans were counted by using Mitis Salivarius agar plate, at the beginning and at the end of the study period. Inter group and intra group comparisons were done. Intra group comparisison was assessed using Wilcoxon signed rank test (non-parametric equivalent to paired't' test) whereas the difference between three groups was assessed using Kruskal-Wallis Annova. **Results**: In the present study, no statistically significant difference was found between the three mouthwashes with regard to their efficacy in reducing Smutans. **Conclusion** – All the three commercial available mouthwashes are equally potent in reducing s mutans counts effectively.

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INTRODUCTION

Prevalence of dental caries in children population is increasing at an alarming rate (Hegde, 2005). The modern concepts of cariogram demonstrate micro-organisms as one of the major etiological factor in the formation of dental caries (Kulkarni, 2003). Streptococcus mutans is considered one of the most important cariogenic species of the human oral microbial flora (Paula, 2010). Therefore, targeting Streptococcus mutans forms is the most important measure for prevention of dental caries. To cut back their level in the oral cavity will provide an additional goad for the prevention of dental caries (Kulkarni, 2003). Mouthwashes have been found to be one of the safe and effective delivery system as anti-microbial agent. These mouthwashes are capable of inhibiting bacterial adhesion, colonization and metabolic activity which ultimately affects bacterial growth (Kulkarni, 2003).

Corresponding author: **Dr. Nayar Islam,**
Post Graduate student, Department of Prosthodontics, Saraswati Dental College, India.

Chlorhexidinegluconate is known as "gold standard" of all mouth rinse. It is a cationic bis-biguanide, active against an array of micro-organisms, including gram-positive and gram-negative organisms, fungi, yeasts, and viruses. It displays both anti-plaque and anti-bacterial properties. It acts by altering integrity of cell membrane of bacteria. When used as a mouthwash, its mode of action is purely topical and because it is poorly absorbed systemically, it is regarded as a relatively safe drug (Parwani, 2013). Chlorhexidine has got certain side effects like long-term use like brown discoloration of teeth, some restorative materials and dorsum of tongue; taste perturbation; oral mucosal ulcerations and paresthesia; unilateral/bilateral parotid swelling, and enhanced supra-gingival calculus formation (Flotra, 1972). In consideration of such side effect the World Health Organization (WHO) advice researchers to investigate the possible use of natural products such as herb and plant extracts (Bhat et al., 2013). Herbs and plant extract have been used in oral hygiene products for many years. Herbal medicine is both promotive and preventive in its approach.

Its major merit lies with the natural herbs, with no reported side effects till date (Nagappan, 2012). Herbs such as Bibhitaka (T. bellerica) one of the ingredients of highly praised Ayurvedic compound Triphala, used both, internally as well. Pilu (S. persica) shows antioxidant activity. Ela is an effective gargle in bad odor of the oral cavity and dental ailments (Aspalli et al., 2014). Fluorides are abundantly used in oral health products including mouth rinses. Sodium fluoride mouth rinses are effective in reducing caries and inhibit carbohydrate utilization of oral microorganisms by blocking enzymes involved in the bacterial gylcolytic pathway (Subramaniam, 2011). Triclosan is a non phenolic, broad spectrum antimicrobial & anti plaque agent. It is used to increase the ability of mouthwashes to bind to oral mucosa and therefore is available for longer period of time (Phan et al., 2006). Xylitol is a naturally occurring non cariogenic sugar substitute that cannot be metabolized by oral bacteria (Subramaniam, 2011). It inhibit bacterial growth through two mechanisms: direct inhibition of the glycolytic route resulting from the xylitol 5-phosphate derivative and/or indirect inhibition resulting from the competition for the HPr-P carrier between glucose and xylitol (Paula, 2010). There are numerous studies on chlorhexidine but studies on herbal and sodium-fluoride- triclosan-xylitol are scarce hence the purpose of the present study is to compare the efficacy of three commercially available mouth rinses *Rexidiene (Chlorhexidine), Kidodent (Sodium Fluoride- Xylitol- Triclosan), Hi-Ora (Herbal)* products against salivary bacteria, streptococcus mutans.

MATERIALS AND METHODOLOGY

A sample of 60 subjects was selected from the single school of Jodhpur city and wasequally divided into 4 groups.

Group A- Rexidiene mouthwash (Chlorhexidine)
Group B- Kidodent mouthwash (Sodium Fluoride- Xylitol- Triclosan)
Group C- Hi-Ora(Herbal mouthwash)
Group D- Distilled water (Positive control)

Prior to the study, written consent was obtained of school. Ethical clearance wasobtained from the ethical committee of the institution. The subject with at least one active white spot on smooth surfaces (facial or lingual) was considered a high caries activity subject. The presence of the active lesions on these surfaces and a high caries activity imply that the subject has a high infection of mutans streptococci which means that he/she belongs to high caries risk group.

Exclusion criteria

- Physical limitations, which precluded the normal tooth brushing and mouth rinsing.
- Marked intra oral soft tissue pathology.
- Medically compromised patients and subjects with history of taking antibiotics 3 months prior to or during the course of study.
- Subjects undergoing orthodontic treatment or with extensive intra prosthesis
- Children who could not brush their teeth or rinse on their own
- Presence of any intraoral soft tissue pathology

Stimulated saliva was collected for microbial analysis. The subjects were asked to simulate chewing action with sterilized cotton rolls for 4 min. At the end of 4 min, the students were made to expectorate into sterile container. The stimulated saliva was then transported for analysis within 30 min in ice boxes. Standardization of the saliva collection technique was followed and the same day all samples were cultured. The teachers were educated and trained in the use of mouthwash so that the children, under the supervision of the teachers, could use the mouthwash. Each of the groups used the respective mouthwash, as a daily, supervised rinse after lunch in the afternoon. The children were advised not to eat or rinse for the next 30 min. They were instructed to carry home the mouthwash bottles on weekends.

Incubation and Microbiological Enumeration: Saliva samples were serially diluted in 6 fold steps in normal saline. The serial dilution was carried out in pre-prepared sterile water blanks of 9ml each. 20 µl of saliva sample were spread on mitissalivarius agar (Hi Media Company, India) supplemented with 0.2 U/ml bacitracin (Hi Media Company, India) and sucrose (15% w/v) using cotton swab for *S. mutans* count. The preparation of media, required sterilization and pouring of plates were done one day before the plating of cultures. All the plates were kept in incubator for one hour before the saliva samples were spread on it. Care was taken that all this procedure of serial dilutions and plating was done in work station called as Laminar Air Flow work station, it provides work space for sterile transfers. Petri-dishes were then kept in candle jar at 37ºC for 48 hours, than the plates were kept in anaerobic chambersand then placed in incubators at 37ºC for 48 hours. The identification of colonies were done using morphology and characteristics observed under light microscope. The confirmation of MS was done under light microscopy after heat fixed smear slide. Additional confirmation was done using gram staining and catalase negative confirmation test. Microbial counts were further expressed as colony forming units (CFU) per ml of stimulated saliva.

- Score 1 < 10^4CFU/ml.
- Score 2 10^4- 10^5CFU/ml.
- Score 3 10^5 -10^6 CFU/ml.
- Score 4 > 10^6 CFU/ml.

Data was analyzed using SPSS software version 17. Intra group comparison was done using Wilcoxon signed rank test (non-parametric equivalent to paired‘t’ test) whereas the difference between three groups was assessed using Kruskal-Wallis Annova.

RESULTS

This study was conducted to compare the effectiveness of three commercially available mouthwashes against salivary *Streptococcus mutans among* 8-12years old school children. Table 2: The above table describes the means of streptococcus mutans count of all the three test groups and control group before rinsing and after rinsing. The mean streptococcus mutans counts of Group A (chlorhexidienr group) post rinsing is 1.87, Group B (Sodium Fluoride-Xylitol- Triclosan group) is 2.00 and that of Group C (herbal group) is 1.64 Table 3 Shows comparison of streptococcus mutans count scores between Group A (Rexidine mouthwash) before rinsing and after rinsing with mouthwash ,it showed statistically significant reduction in Streptococusmutans count (P=0.002) after rinsing with 0.2% chlorhexidine. Table 4 Shows comparison of streptococcus mutans count scores between Group B (sodium fluoride triclosan-xylitolmouthwash) before rinsing and after

Table 1. Comparison of mutans streptococci counts between study groups and control group before and after rinsing

Mutans Streptococci Scores	Group A		Group B		Group C		Group D	
	Pre Rinse	Post Rinse	Pre Rinse	Post Rinse	Pre Rinse	Post Rinse	Pre Rinse	Post Rinse
1	2	6	2	5	2	3	2	3
2	2	6	3	6	3	6	3	2
3	5	2	6	3	5	3	6	6
4	6	1	4	1	5	3	4	4

Score: 1 < 10^3 Cfu/ml; Score 2 < 10^4 Cfu/ml; Score 3 = 10^4 – 10^5 Cfu/ml; Score 4 > 10^5 Cfu/ml.

Table 2. Basic Statistics – Descriptives

Group -A	n	Mean	SD	Median	Range
Pre Rinsing	15	3	1.07	3.0000	3
Post Rinsing	15	1.87	0.92	2.0000	3
Group -B					
Pre Rinsing	15	2.80	1.01	3.0000	3
Post Rinsing	15	2.00	0.96	2.0000	3
Group -C					
Pre Rinsing	15	2.87	1.06	3.0000	3
Post Rinsing	15	2.43	1.09	2.0000	3
Group -D					
Pre Rinsing	15	2.80	1.01	3.0000	3
Post Rinsing	15	2.64	1.08	3.0000	3

Table 3. Comparison of Pre-rinse and Post-rinse between Group A

Group A	n	Mean	SD	p-value
Pre- Rinse	15	3	1.07	0.002*
Post –Rinse	15	1.87	0.92	

*Wilcoxon matched pairs test

Table 4. Comparison of Pre Rinse And Post Rinse Between Group B

Group B	n	Mean	SD	p value
Pre- Rinse	15	2.80	1.01	0.001*
Post –Rinse	15	2.00	0.96	

Table 5. Comparison of Pre Rinse And Post Rinse Between Group C

Group C	n	Mean	SD	p value
Pre- Rinse	15	2.87	1.06	0.008
Post –Rinse	15	2.43	1.09	

Table 6. Comparison of Pre Rinse and Post Rinse between Group D

Group D	n	Mean	SD	p value
Pre- Rinse	15	2.80	1.01	0.08
Post –Rinse	15	2.64	1.08	

rinsing with mouthwash ,it showed statistically significant reduction in Streptococusmutans count (P=0.001). Table 5 Shows comparison of streptococcus mutans count scores between Group C (Herbal mouthwash) before rinsing and after rinsing with mouthwash ,it showed statistically significant reduction in Streptococusmutans count (P=0.008) Table 6 Shows comparison of streptococcus mutans count scores between Group D (Water) before rinsing and after rinsing with, it showed statistically non significant results (0.08)

DISCUSSION

This study was undertaken to evaluate the efficacy of four commercially available mouthwashes on salivary Streptococcus mutans on school children aged 8-12 years. These students were divided into four groups. Group A used Rexidiene (0.2% chlorhexidiene) mouthwash, Group B Kidodent (Sodium Fluoride-Triclosan-Xylitol) mouthwash, Group C used Hi–Ora (Herbal) mouthwash and Group D used Distilled water. In the present study saliva samples were used in this study to assess the microbial aspect of dental caries. According to Mundroff et al and Sullivan et al detection of Streptococci in saliva was an excellent means as compared to either pooled plaque or oral swab samples, as these samples do not explain the variation in caries better than the stimulated whole saliva (Mundorff, 1990; Sullivan, 1996). In the present study the subjects were asked to simulate chewing action with sterilized cotton rolls for 4 min. At the end of 4 min, the students were made to expectorate into sterile penicillin bottles. This was in accordance to the previous study conducted by Bajaj N and Tandon S (Bajaj et al., 2011).

According to the review by Lemos-Junior CA and Villoria GE ingestion of large amount of alcohol in mouthwash affect normal glycogenolysis and glyconeogenesis, causing hypoglycemia (Lemos Junior, 2008). The extra hepatic metabolism of alcohol in oral tissue has been testified. In the human mouth, aldehyde dehydrogenase (ALDH), an enzyme that converts acetaldehyde into a nontoxic acetate compound, occurs less frequently than alcohol dehydrogenase (ADH). This imbalance allows for the accumulation in oral tissues of a toxic, reactive and irritating acetaldehyde. The continuous use of mouthrinses containing alcohol should be avoided. Due to a point mutation, aldehyde dehydrogenase 2(ALDH2) isoenzyme is deficient in 30–50% of Asians. These individuals have a genetic inability to remove acetaldehyde and consequently have very high salivary acetaldehyde levels after moderate dose of alcohol (Subramaniam, 2011). Therefore all the mouthwash selected for this study been alcohol free. Children in Group A used commercially available mouthwash Rexidiene, containing 0.2% chlorhexidiene**.** Ernst stated that the increase in concentration of chlorhexidine from 0.1 to 0.2% provided no clinical advantages or disadvantages.[15]An another study also states that the chlorhexidine used in different concentrations (0.02%, 0.06%, 0.12%) efficiently reduced the S mutans count and also by reducing the concentration of chlorhexidine, the bitter taste sensation is also reduced, making it more acceptable to children (Jayaprakash, 2010). Hence, in this study 0.2% concentration was used. In the present study 0.2% chlorhexidine has shown significant reduction in the mutans streptococci count (Table 3).This observation adds to the earlier studies (Agarwal, 2010; Spets-Happonen, 1985; Heffi, 1987; Malhotra, 2011). Children in Group B used commercially available mouthwash Kidodent containing Sodium Fluoride-Triclosan-Xylitol as main ingredients. According to a study conducted (Clarkson, 2000), the use of fluoride mouthwash seems to be effective in both large group and individual studies. 0.05% sodium fluoride mouthwash is a weak solution which can be used daily, whereas 0.2% sodium fluoride mouthwash is stronger and should be used once a week. Xylitol has been incorporated into fluoride–containing mouthwashes. In vitro studies have suggested that fluoride and xylitol exert an additive inhibitory effect on growth and acid fermentation by S.mutans (Maehara, 2005; Petin et al., 2008).

In the present study there was a significant reduction in the S.mutans count scores within the group after using mouthwash containing Sodium Fluoride-Triclosan-Xylitol (Table 4) The reduction in S.mutans count in Group B can be due to combined effect of sodium fluoride, xylitol and triclosan. Fluoride has direct and indirect effects on bacterial cell and is also a powerful inhibitor of acid formation by plaque microorganisms. Triclosan, possesses antimicrobial action. It has got far-out hydrophobic and lipophilic nature, it adsorbs to lipid portion of the bacterial cell membrane and in low concentrations it interferes with vital transport mechanism. xyiltol as non-fermentability and non-cariogenicity as passive effects, whereas active caries prevention effects as bacteriostatic and cariostatic (Lakade, 2014). Students in Group C used commercially available mouthwash Hi-Oraaherbal mouthwash containing herbal preparation, made from natural herbs. In the present study within the group, herbal mouthwash showed statistical difference in reducing S mutans count scores two weeks (Table 5). The antibacterial mechanism of Hi-Ora is due to its synergistic action of its potent herbs by virtue of their components which individually exert their antimicrobial activities.

The result of the present study are in agreement with previous studies (Bhat, 2013; Mehta, 2013; Shetty, 2013). However other studies (Kaim, 1998; Sher, 2011) reported that their mixed herbal extract mouthwash showed a weak antibacterial effect against oral bacteria on their experimental and dental plaque model study, is unlike our result and the different results is probably because of different conditions of the oral cavity and the in vitro media. However, when inter group comparison were done, they showed no statistical significant difference between the groups post rinsing (p=0.16). Our study shows that all the three mouthwash used were effective in reducing the streptococcus mutans count. Since, we found no significant difference between the three mouthwashes, with regard to their efficacy in reducing *S.*mutans, the use of a low fluoride–xylitol and herbal based mouth wash appears to be a suitable choice for regular use in children.

Conclusion

Within the limits of the study following conclusions were drawn from this study.

- Group A, using Rexidine mouthwash (0.2% Chlorhexidiene) showed significant reduction in reducing streptococcus mutans count. (p= 0.002)
- Group B, using Kidodoent mouthwash (Sodium Fluoride-Xylitol-Triclosan) showed significant reduction in reducing streptococcus mutans count. (p=0.001)
- Group C, using Hi-Ora mouthwash (Herbal) showed significant reduction in reducing streptococcus mutans count. (0.008)
- When all the three mouthwash were compared there was no significant difference in reduction of streptococcus mutans count.(p= 0.16)

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