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S Kumar
S Patel
J Tadakamadla
H Tibdewal
P Duraiswamy
S Kulkarni

Authors' affiliations:
S Kumar, Department of Preventive Dental Sciences, College of Dentistry, Jazan University, Jazan, Saudi Arabia
S Patel, Department of Public Health Dentistry, Darshan Dental College and Hospital, Udaipur, India
J Tadakamadla, Department of Maxillofacial Surgery and Diagnostic Sciences, College of Dentistry, Jazan University, Jazan, Saudi Arabia
H Tibdewal, Department of Public Health Dentistry, Vidarbha Youth Welfare Society's Dental College & Hospital, Amravathi, Maharashtra, India
P Duraiswamy, Department of Public Health Dentistry, Sriramachandra Dental College and Hospital, Chennai, India
S Kulkarni, Department of Public Health Dentistry, Paninneya Mahavidyalaya Dental College, Hyderabad, India

Correspondence to:
Dr S. Kumar
Department of Preventive Dental Sciences
College of Dentistry Jazan University
PO Box 114
Jazan, Saudi Arabia
Tel.: +966 565767332
Fax: +91 8457222337
E-mail: santosh_dentist@yahoo.com

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Effectiveness of a mouthrinse containing active ingredients in addition to chlorhexidine and triclosan compared with chlorhexidine and triclosan rinses on plaque, gingivitis, supragingival calculus and extrinsic staining

Abstract: *Objectives:* To assess the effectiveness of three different mouthrinses – chlorhexidine, triclosan + sodium fluoride and chlorhexidine + triclosan + sodium fluoride + zinc chloride – on plaque, calculus, gingivitis and stains and to evaluate the occurrence of adverse effects with these three treatments. *Methods:* Forty-eight healthy subjects participated in a double-blind, randomized, parallel experiment and were randomly allocated to any one of the three experimental mouthrinses: group A (0.2% chlorhexidine (CHX) gluconate), group B (0.03% triclosan + 0.025% sodium fluoride (NaF) + 12% ethyl alcohol) or group C (0.2% CHX + 0.3% triclosan + 0.3% NaF + 0.09% Zn chloride ($ZnCl_2$). All the subjects were assessed for gingivitis, plaque, supragingival calculus and extrinsic stains at baseline and at the end of the 21-day experimental period. *Results:* There was a significant difference ($P = 0.046$) in the effectiveness for the prevention of gingivitis and plaque, with subjects of group A and group C presenting least and highest gingival and plaque scores, respectively. Significant differences ($P = 0.03$) were observed for the accumulation of supragingival calculus where the deposition of calculus in group A was nearly double that of the group B, and group B was most effective in the prevention of supragingival calculus. Highest deposition of extrinsic stains was in the group A followed by group C and group B. There was no significant difference between the three treatments for adverse events' occurrence. *Conclusions:* CHX mouthrinse was most effective in controlling plaque and gingivitis but caused greatest deposition of extrinsic stains. Supragingival calculus deposition was least in triclosan + NaF group followed by CHX + triclosan + NaF + $ZnCl_2$ and CHX. More than half of the subjects reported adverse events during the experimental phase.

Key words: calculus; gingivitis; plaque control

Introduction

Dental plaque is the soft deposit that forms the biofilm adhering to the tooth surface or other hard surfaces in the oral cavity, including removable and fixed restorations (1), which provides a surface for accumulation of calculus and is a key factor in the aetiology of gingivitis.

A long-term plaque-free dentition seems to be an unrealistic goal. Nevertheless, to overcome deficiencies in mechanical tooth cleaning, the use of antiseptic mouthrinses could have clear benefits. They represent adjunct to mechanical hygiene measures to facilitate the control of supragingival plaque and gingivitis (2, 3).

Presently, many mouthrinses are available for this purpose, and chlorhexidine has been proved as the most effective chemical agent in plaque control (4).

Chlorhexidine is an antimicrobial agent. It is a biguanide that has shown the highest inhibitory effect on plaque formation and gingivitis (5). However, its long-term daily use is not recommended because it has been associated with a number of local side effects such as brownish discoloration of the teeth, restorative materials and the dorsum of the tongue (6) in addition to interference with taste (7).

Another chemical that has received much attention is triclosan as it does not have any formulation difficulties or local side effects of cationic antiseptics, such as chlorhexidine (8).

Triclosan, a trichloro-2′-hydroxydiphenyl ether, is a nonionic antiseptic of moderate substantivity (9) that reduces plaque accumulation to a much lesser extent than chlorhexidine.

However, the extent of the plaque inhibitory effect of triclosan is dependent upon the presence of copolymers or ionic agents in the formulation to increase its oral retention (10). The effects of triclosan on gingivitis levels are attributable to its anti-inflammatory and plaque inhibitory effect (10–12).

It was observed that zinc + triclosan inhibited plaque accumulation significantly more than either zinc or triclosan alone (13,14).

Chlorhexidine, and zinc in combination with triclosan, are used as antiplaque agents in the prevention of gingivitis. The activity of these compounds against bacterial cells has been proposed to be due to their interference with sugar transport and reduction in glycolysis (15).

However, no experimental gingivitis study could be traced where the effectiveness of a formulation containing CHX + triclosan + zinc salt has been tested in comparison with chlorhexidine or triclosan alone.

Thus, the present study aimed to assess the effectiveness of three different mouthrinses – chlorhexidine, triclosan + sodium fluoride and chlorhexidine + triclosan + sodium fluoride + zinc chloride – on plaque, calculus, gingivitis and stains and to evaluate the occurrence of adverse effects with these three treatments.

Materials and methods

Subjects

This study was approved by Ethical Committee of Darshan Dental College and Hospital, and written informed consent was obtained from each volunteer. Target population comprised dental undergraduate students at Darshan Dental College and Hospital, Udaipur, India. All the undergraduate students were invited to participate in the study, and 124 subjects voluntarily offered their willingness to participate. However, only 64 individuals met the selection criteria, which constituted full set of sound dentition, periodontal pockets <4 mm and no orthodontic or removable dental appliances. Consequently, 48 healthy subjects (26 men and 22 women) with a mean age of 21.4 ± 2.13 years were included after excluding 16 subjects. The following exclusion criteria were applied: periodontal surgery during the previous 3 months; treatment during previous 1 month with the mouthrinses under study or with any other medication that might affect the periodontal condition; history of allergy or hypersensitivity to any of the ingredients of mouthrinses; systemic diseases, particularly chronic ones, which might interfere with the pathology under study (gingivitis).

A sample size of 48 subjects with 16 in each group achieves a power of 0.81 using the F-test with a target significance level of 0.050 and an actual significance level of 0.045. All the eligible subjects were given information about the agents and purpose of the study.

Procedure

This was a double-blind, randomized, three-group parallel study with random allocation of subjects to any one of the three experimental mouthrinses. The experimental phase preceded by a pre-experimental phase of 14 days. During this phase, all the subjects were instructed to maintain excellent oral hygiene by brushing their teeth for a minimum of three minutes twice daily so as to assure the minimal presence of plaque and gingivitis before the start of the experimental phase.

The experimental phase was initiated with a thorough supragingival dental prophylaxis to remove stains, calculus and plaque.

All the subjects were assessed for plaque, gingivitis, calculus and stains at baseline and at the end of the 21-day experimental period. During the experimental phase, subjects were treated solely with one of the three mouthrinses under study.

Each subject rinsed their mouth with 10 ml of the mouthrinse assigned to them, twice daily for one minute after breakfast and dinner. They were also instructed to swish it properly around the mouth and avoid its ingestion. They also had to avoid rinsing with water afterwards and had to keep away from eating or drinking within 30 min after using the mouthrinse.

A CONSORT-type diagram explaining the design of this study is presented in Fig. 1.

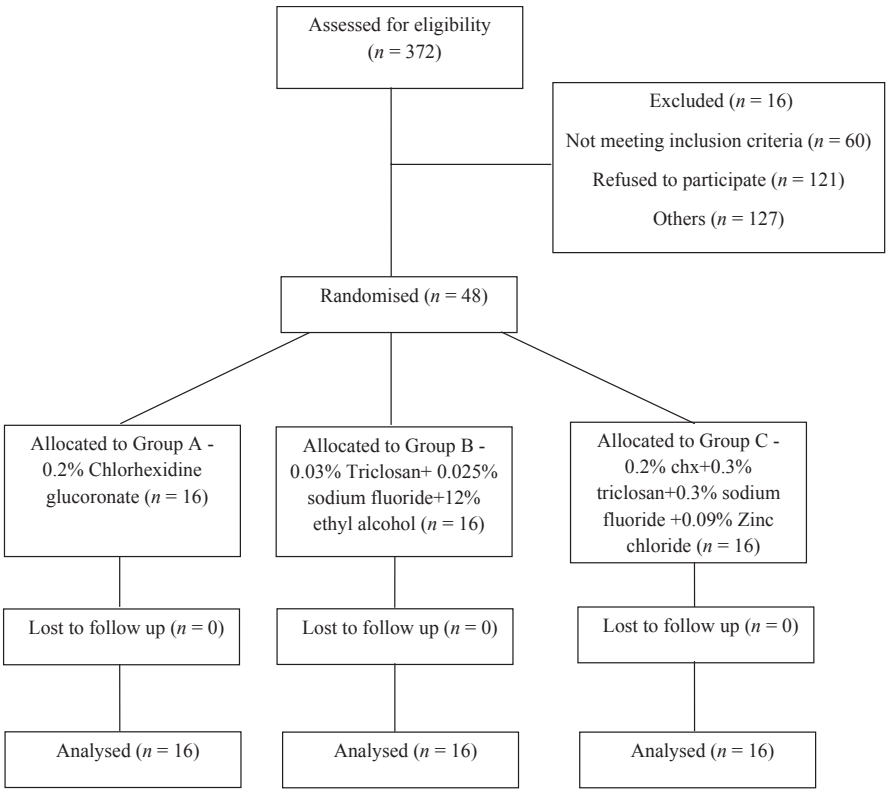

Fig. 1. CONSORT diagram depicting the study design.

Experimental products

All subjects who fulfilled the selection criteria were randomized to rinse with one of the three experimental mouthrinses:

Group A: Hexidine® (ICPA, Ankleshwar, India) 0.2% CHX gluconate;
Group B: Colgate Plax (Colgate, Daman, India), 0.03% triclosan, 0.025% NaF, 12% ethyl alcohol; and
Group C: Guard-OR mouthwash (Group Pharmaceutical Ltd, Mumbai, India) 0.2% CHX, 0.3% triclosan, 0.3% NaF, 0.09% $ZnCl_2$.

To minimize any interference with the assessment of response to the mouthrinse, all subjects were carefully and adequately educated how to maintain their oral hygiene optimally during the pre-experimental period (14 days). They were also carefully instructed that during the experimental period (21 days), they are strictly not allowed to use any other oral hygiene products such as toothpaste, mouthrinses other than the mouthrinse they are provided with. Subjects using <70% of the planned amount of mouthrinse were considered as non-compliant; however, none of the subjects were non-compliant.

Clinical evaluation

As this study was a double-blinded trial, all the subjects and examiner were not aware of the intervention to which the subjects belonged. Gingivitis, plaque, supragingival calculus and extrinsic stain levels were assessed at baseline and after 21 days by a single examiner.

Loe–Silness index (16) was used for the assessment of gingivitis on the mesial, distal, facial and lingual surfaces of the six Ramfjord teeth (17), while the whole set of dentition except the third molars were assessed for plaque on buccal and lingual surfaces after staining with the disclosing agent using Turesky modified Quigley–Hein index for plaque (18).

Calculus assessment was made by Volpe–Manhold index for calculus. The amount of supragingival calculus was measured on the six lower incisors in millimetres to the nearest 0.5 mm (19).

Extrinsic dental stains were estimated using the stain index suggested by Macpherson *et al*., (20). It was done by assigning separate scores to the mesial and distal sites of each tooth, in addition to the standard gingival area and tooth body. For each site, stain intensity and extent were scored. The final score for each individual was the sum of (intensity × area) scores at each site.

At the end of the experimental phase, all the subjects were individually asked about their experience. They were also systematically enquired if they faced some discomfort or experienced any adverse events.

None of the subjects violated the protocol, and hence, intention-to-treat analysis was not used.

All measurements were taken by single calibrated and experienced examiner (SK). To investigate examiner repeatability with respect to clinical parameters, 15 subjects were selected and re-evaluated at baseline and after experimental phase. Reliability was assessed by means of the intraclass correlation coefficient (R). The intraclass correlation coefficient for

gingival index was 0.98, while for plaque index, it was 0.96. The corresponding figures for calculus and stain indices were 0.96 and 0.89, respectively.

Statistical analysis

Descriptive measures were assessed and presented as means and standard deviations. The Kruskal–Wallis test was executed to assess the statistical difference in the measures of clinical parameters between the treatment groups at baseline and after 21 days. Friedman's repeated-measures test was used to compare the evolution of the treatments. Chi-squared test was used to compare the proportion of individuals reporting various adverse events in different treatment groups.

Results

Forty-eight subjects (26 men and 22 women) with a mean age of 21.4 ± 2.13 years were included in the study. All the subjects completed pre-experimental and experimental phases of the study and were homogenous at baseline for all the four indices.

There was no statistical difference for gingival index at baseline and after 21 days between the interventions. However, there was a significant difference ($P = 0.046$) in the evolution of gingivitis, with subjects of group A and group C presenting least and highest gingival scores, respectively (Table 1).

There was a significant difference in plaque scores, with group C presenting the worst plaque scores in comparison with other groups after the experimental period. Group C was least effective in preventing plaque formation, with plaque index increasing by 2.12 ± 0.03 from baseline, while it increased only by 1.46 ± 0.01 in the CHX group.

Triclosan + NaF exerted greater effectiveness on preventing supragingival calculus, followed by CHX + triclosan + NaF + $ZnCl_2$ and CHX. Significant differences ($P = 0.03$) were observed for the accumulation of supragingival calculus where the deposition of calculus in group A was nearly double that of the group B.

Table 1 reveals that highest deposition of extrinsic stains was in the group A followed by group C and group B. Extrinsic stain score after the experimental phase in CHX group was 0.89, which was significantly greater than 0.70 and 0.78 observed in Triclosan + NaF and CHX + triclosan + NaF + $ZnCl_2$ groups, respectively.

At the end of the experimental phase, 30 (62.5%) of 48 subjects reported some adverse events with the mouthrinses during the experimental phase (Table 2). None of the subjects reported more than one adverse effect or any other findings. These adverse effects were not severe, and the treatment withdrawal was not required. There was no significant difference between the three treatments for the occurrence of adverse events. Majority of the volunteers who reported adverse events belonged to the triclosan + NaF group and complained of oral itching and aphthous ulcers, while oral soreness was complained by subjects of CHX category and dryness of the mouth was reported by subjects belonging to group C.

Discussion

The focus of this study was to detect the mouthrinse that proves to be wholesome in supragingival plaque and calculus inhibition in addition to minimizing gingivitis. For this purpose, we compared three mouthrinses with different formulations that are commercially available in India. Moreover, we also assessed extrinsic dental stains along with the occurrence

Table 1. **Descriptive statistics for indices at baseline and after 21-day period of experimental treatment with each mouthrinse**

	Baseline		After		Difference	
Index	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
Gingivitis*						
Group A	0.33 (0.26)	0.33 (0.04)	0.84 (0.12)	0.85 (0.12)	0.51 (0.12)†	0.55 (0.18)
Group B	0.30 (0.39)	0.31 (0.05)	0.95 (0.20)	0.98 (0.35)	0.61 (0.23)	0.65 (0.41)
Group C	0.29 (0.70)	0.31 (0.11)	0.97 (0.15)	0.99 (0.26)	0.68 (0.14)	0.68 (0.20)
Plaque*						
Group A	0.85 (0.10)	0.82 (0.11)	2.31 (0.10)†	2.28 (0.11)	1.46 (0.01)†	1.34 (0.31)
Group B	0.80 (0.13)	0.77 (0.23)	2.62 (0.33)	2.66 (0.24)	1.81 (0.34)	1.91 (0.23)
Group C	0.89 (0.10)	0.91 (0.18)	3.01 (0.10)	3.02 (0.18)	2.12 (0.03)	2.12 (0.12)
Supragingival calculus*						
Group A	0.23 (0.06)	0.22 (0.10)	1.05 (0.22)†	1.1 (0.3)	0.81 (0.23)†	0.79 (0.39)
Group B	0.27 (0.11)	0.22 (0.15)	0.72 (0.20)	0.70 (0.3)	0.46 (0.20)	0.39 (0.33)
Group C	0.20 (0.07)	0.20 (0.12)	0.84 (0.25)	0.80 (0.3)	0.63 (0.24)	0.61 (0.27)
Extrinsic stains*						
Group A	0.13 (0.04)	0.13 (0.08)	0.89 (0.12)†	0.96 (0.24)	0.75 (0.13)†	0.78 (0.26)
Group B	0.15 (0.02)	0.16 (0.06)	0.70 (0.14)	0.65 (0.30)	0.54 (0.14)	0.49 (0.25)
Group C	0.14 (0.04)	0.14 (0.06)	0.78 (0.16)	0.93 (0.33)	0.64 (0.16)	0.73 (0.34)

SD, standard deviation; IQR, interquartile range.
*Significant differences between the mouthrinses for the evolution of the treatments using Friedman's repeated-measures test.
†Significant differences between the treatments assessed using Kruskal–Wallis test.

Table 2. **Adverse events reported by study subjects during the experimental phase**

Adverse events	CHX (n = 16)	Triclosan + NaF (n = 16)	CHX + triclosan + NaF + $ZnCl_2$ (n = 16)
No	7 (43.75%)	4 (25.00%)	7 (43.75%)
Yes*	9 (56.25%)	12 (75.00%)	9 (56.25%)
Oral itching	5 (31.25%)	8 (50.00%)	5 (31.25%)
Oral soreness	4 (25.00%)	0	0
Aphthous ulcer	0	4 (25.00%)	0
Dryness	0	0	4 (25.00%)

Chi-squared test = 1.95; P = 0.377.
*No subject reported more than one adverse event during the use of each mouthrinse.

of some adverse events such as oral itching, dryness, soreness, aphthous ulcers, if any, during the experimental phase.

However, the lack of a true control in the present study makes the conclusion less valid.

The experimental phase of the present study lasted for 21 days, based on the experimental gingivitis model described by Loe *et al.* (21), which stated that 10–21 days was sufficient for the development of gingivitis in the absence of any mechanical oral hygiene practice. Moreover, the pre-experimental phase of 14 days was included so as to assure the minimal presence of plaque and gingivitis before the start of the experimental phase.

Of the numerous types of mouthrinses currently available, there are relatively few that have been shown unequivocally to reduce both plaque and gingivitis. However, no experimental study could be traced where the effectiveness of a formulation containing CHX + triclosan + NaF + $ZnCl_2$ has been tested in comparison with CHX or triclosan + NaF.

It has been shown that a 0.2% chlorhexidine gluconate mouthrinse will prevent the development of experimental gingivitis after the withdrawal of oral hygiene procedures (22).

Excluding chlorhexidine rinses, only the essential oil rinse has been extensively evaluated and subsequently been shown to be of value as an adjunct to mechanical oral procedures (23–26). Triclosan has been used recently in a number of mouthrinses and produces moderate plaque inhibitory effects when used as a mouthrinse in combination with zinc (8, 27, 28).

After the experimental phase, CHX presented highest effectiveness in preventing gingivitis and plaque, while the CHX + triclosan + NaF + $ZnCl_2$ group was least effective.

Till date, CHX has shown the highest inhibitory effect on plaque formation and gingivitis (5). Similarly, triclosan has been found to inhibit the formation of several important mediators of gingival inflammation (12). Although CHX + triclosan + NaF + $ZnCl_2$ mouthrinse contained many ingredients, it failed to show synergistic action in plaque prevention, which might be due to the incompatibility between the various ingredients.

Group C showed the highest plaque accumulation, followed by triclosan + NaF and CHX groups. Chlorhexidine rinses were always significantly more effective than the triclosan rinse in plaque inhibition (29). Triclosan at 0.1% showed limited plaque inhibition, which is <0.01% chlorhexidine (30). However, Ramberg *et al.* (31) in their crossover study compared the effect of 0.06% triclosan, 0.12% chlorhexidine and placebo mouthrinses on de novo plaque formation over 18 days at healthy and inflamed gingival sites of ten volunteers, both active mouthrinses produced significant reductions in plaque formation compared with the control mouthrinse.

Triclosan better prevented supragingival calculus than the comparative mouthrinses. As an efficient plaque inhibitor, CHX was expected to decrease supragingival calculus better than the comparative mouthrinses, but the calculus deposition in CHX group was nearly double that of the triclosan + NaF group. Loe *et al.* in 1976 reported that despite being a potent antiplaque agent, CHX has the drawback of increasing the calculus levels. Moreover, Svatun and Saxton (32) observed that triclosan-assisted plaque control, promoted gingival health and inhibited the calculus formation. However, no synergistic effect of triclosan and zinc chloride was noted on supragingival calculus, and the effectiveness of CHX + triclosan + NaF + $ZnCl_2$ group was lower than that of the triclosan group.

The score for extrinsic stains after experimental phase in group A was 0.89, which was significantly greater than 0.78 in group C, and the lowest score (0.70) was observed in group B, which could be attributed to the staining property of CHX present in group A and group C mouthrinses. The lowest extrinsic stain deposition exhibited by triclosan + NaF group is in accordance with previous studies where mouthrinses containing NaF showed lower dental staining at the end of the experimental phase (33, 34).

There was no significant difference between the experimental groups for adverse effects. Although triclosan has been found to have anti-inflammatory properties (35), majority of the participants who reported adverse events belonged to the triclosan + NaF group. This finding could be probably due to the additional ingredient that was present in the mouthrinse.

Conclusions

CHX mouthrinse was most effective in controlling plaque and gingivitis but caused greatest deposition of extrinsic stains. Supragingival calculus deposition was least in triclosan + NaF group followed by CHX + triclosan + NaF + $ZnCl_2$ and CHX. More than half of the subjects reported adverse events during the experimental phase.

Conflicts of interest

The authors have no conflicts of interest.

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