ORIGINAL RESEARCH

Effect of Tricalcium Phosphate and Calcium Sucrose Phosphate on the Inhibition of *Streptococcus mutans*: An *In Vivo* Study

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Abstract

Aim: To determine the efficacy of tricalcium phosphate (TCP) and calcium sucrose phosphate (CSP) on the inhibition of *Streptococcus mutans (SM)*.

Materials and methods: Thirty healthy children between 13 and 18 years of age were divided into two groups of 15 each; Group I receiving TCP-containing cream and Group II receiving calcium sucrose phosphate-containing cream. On the first day of the study, 30 minutes after breakfast, baseline plaque samples were taken from the buccal surface of first mandibular permanent molar using a sterile wedge which was immediately transferred to sterile container containing 1 mL of saline, and were subjected to microbiological examination. On the following days, both the creams were applied to the respective groups. On the 16th day, plaque samples were collected from the same site, and colony forming units were recorded using agar plate as a culture medium.

Results: The mean of *S. mutans* count before application of TCP-containing paste was 16.27 cfu per mL and before calcium sucrose phosphate-containing paste was 15.33 cfu per mL. The mean after application of TCP-containing paste and calcium sucrose phosphate-containing paste was 3.53 and 9.87 cfu per mL, respectively. And, there was a statistically significant difference found within the groups.

Conclusion: Both TCP and CSP have an inhibitory effect on *S. mutans*.

Clinical significance: This can be an effective preventive tool for children with high caries risk and even for special child. Both TCP and CSP deposit the mineral reservoir in plaque and saliva; it may help resist the future cariogenic challenges.

Keywords: Calcium sucrose phosphate, Dental caries, Mitis Salivarius agar medium, *Streptococcus mutans*, Tricalcium phosphate.

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Introduction

In the present century, great emphasis is placed on caries risk assessment, early diagnosis, and caries control. Thus, the focus has shifted to effective preventive strategies and minimal intervention. Prevention of dental caries and minimal intervention dentistry play a vital role in dental practice.

Dental caries has been defined as a diet- and saliva-modified bacterial disease. Among the various microorganisms present in the biofilm, *S. mutans* is predominant.[1] Various new products have been continuously introduced into the market targeting these organisms. It is very important for both clinicians and community oral health workers to understand the effectiveness of these products. This would enable them to select the most appropriate preventive agent and gain optimum benefit from their preventive strategies.

Calcium phosphate concentrations in plaque, plaque fluid, and saliva play an important role in caries prevention.[2] TCP has been considered as one possible means for enhancing levels of calcium in plaque and saliva.[3] Calcium sucrose phosphate (CSP) has received publicity as a possible cariostatic food additive and also enhanced the level of calcium in plaque and saliva.[4]

Increased calcium and phosphate concentrations in the oral environment can also increase tooth mineral saturation in oral fluids.[5] Therefore, an increased anticaries effect might be anticipated from the release of these ions. Since both TCP and CSP deposit the mineral reservoir in plaque and saliva, it may help resist the future cariogenic challenges. Hence, the purpose of this study was to determine the effect of TCP and CSP on the inhibition of *S. mutans*.

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Materials and Methods

The study was conducted in the Department of Pedodontics, Yenepoya Dental College, Mangaluru. Ethical clearance was obtained, and written consent was taken from the parents of the children.

Thirty healthy children from residential school aged 13–18 years with decayed, missing, and filled teeth (DMFT) >3 were selected.

Children with an age below 13 years and DMFT <3 were excluded from the study.

Initially, all the children were screened, and 30 children were selected based on the inclusion criteria, who were medically healthy and decay-free children with a milk teeth or mixed dentition, who had any history of antibiotic use in the 3–4 weeks preceding the study, and who had no history of fluoride treatment in the 2 weeks preceding the study. Children who refused to participate and those with tooth structure defects were excluded from the study. These children were further randomly divided into two groups of 15 children each.

First group—subjects receiving TCP-containing paste (Clinpro cream).

Second group—subjects receiving calcium sucrose phosphate-containing paste (Toothmin cream) (Fig. 1).

In the first day of the study following 30 minutes after breakfast, baseline plaque samples were taken from all the children. The plaque samples were collected from the buccal surface of first mandibular permanent molar using a sterile wedge (Fig. 2). The samples were immediately shifted to sterile container containing 1 mL of saline each and were transferred within 2 hours to the microbiological laboratory (Fig. 3).

The samples were then vortexed thoroughly before culture. Ten microliter of the sample was pipetted on to Mitis Salivarius Agar and streaked across the plate as per the semiquantitative technique. The plates were then incubated aerobically at 37°C for 48 hours. The colonies were identified by colony morphology, gram stain, and biochemical reactions. The numbers of similar type of colonies were manually counted (Figs 4 and 5). The total number of colonies was calculated by multiplying with conversion factor (i.e., 10^2) and finally expressed as colony forming units per mL of specimen (cfu per mL).

On the second day, following 30 minutes after breakfast, children belonging to Group I were given Clinpro cream and children belonging to Group II were given Toothmin cream. The paste was dispensed onto the index finger of every child, and they were asked to spread it on all the surfaces of their teeth. Following application, the children was asked not to eat or drink for half an hour. For 15 consecutive days, the pastes were dispensed at the same time in the morning, and the similar procedure of application was followed.

Fig. 1: Toothmin cream and Clinpro cream used in the present study

Fig. 3: The samples transferred to sterile container containing 1 mL of saline

Fig. 2: Plaque samples collected from the buccal surface using a sterile wedge

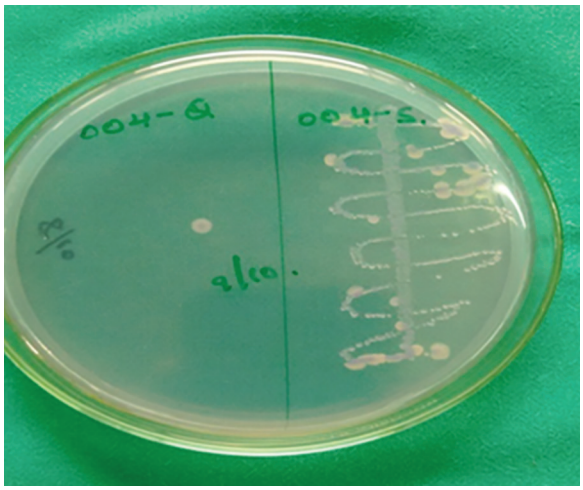

Fig. 4: Mitis Salivarius agar containing *S. mutans* colonies of CSP group

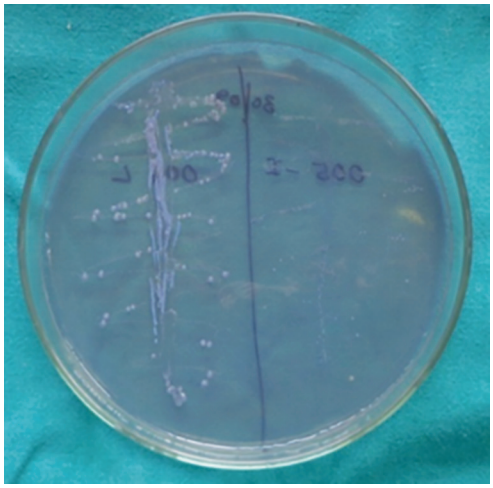

Fig. 5: Mitis Salivarius Agar containing *S. mutans* colonies of TCP group

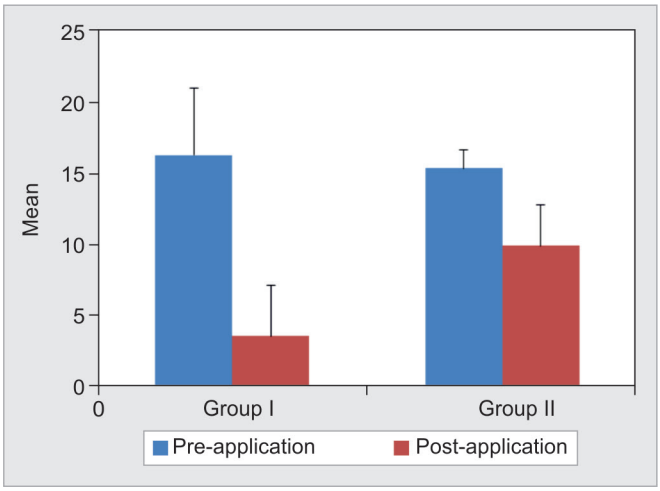

Fig. 6: Comparison of pre- and post-application *S. mutans* count within the groups

Table 1: Evaluation of *S. mutans* count before application of experimental pastes

Pre-application	*N*	*Minimum*	*Maximum*	*Mean (cfu/mL)*	*Std. deviation*	*t value*	*p value*
Group I	15	10	26	16.27	4.74	0.607	0.549
Group II	15	11	22	15.33	3.60		NS

NS, not significant

Table 2: Evaluation of the pre- and post-*S. mutans* count within the groups

Groups	*N*	*Min*	*Max*	*Mean (cfu/mL)*	*SD*	*Mean difference (cfu/mL)*	*Change (%)*	*t value*	*p value*
Group I Pre-application	15	10	26	16.27	4.74	12.733	78.28	9.907	0.000
Group I Post-application	15	2	6	3.53	1.30				<0.001, HS
Group II Pre-application	15	11	22	15.33	3.60	5.467	35.65	7.632	
Group II Post-application	15	2	15	9.87	2.92				0.000 <0.001, HS

HS, highly significant

Table 3: Evaluation of the efficacy of experimental pastes between the groups

Groups	*N*	*Mean difference (cfu/mL)*	*SD difference*	*Change (%)*	*t value*	*p value*
Group I	15	12.733	4.978	78.28		0.000
Group II	15	5.467	2.774	35.65	4.939	<0.001, HS

HS, highly significant

On the 16th day following 30 minutes after breakfast, plaque samples were collected from the same site. As previously done, all samples were transferred to the microbiological laboratory for inoculation into the agar medium. After this incubation, the colony-forming units of *S. mutans* were counted and recorded.

Statistical Analysis

The present study data were analyzed using the Statistical Package for Social Sciences, version 20 (SPSS Inc., Chicago, Illinois, USA). Values were analyzed statistically by Student's paired and unpaired *t*-tests. And, a *p* value of <0.05 was considered statistically significant.

Results

The results showed that the mean of *S. mutans* count before application of TCP-containing paste was 16.27 cfu per mL and before calcium sucrose phosphate-containing paste was 15.33 cfu per mL. The mean after application of TCP-containing paste and calcium sucrose phosphate-containing paste was 3.53 and 9.87 cfu per mL, respectively. And, there was a statistically significant difference found within the groups (Fig. 6 and Tables 1 and 2).

There was a mean of 78.28% reduction in the *S. mutans* count after application of TCP-containing paste for 15 days, and the unpaired *t*-test value was 4.9 that was highly significant. The result of calcium sucrose phosphate-containing paste showed that there was a mean of 35.65% reduction in the *S. mutans* count after the application for 15 days (Table 3).

Discussion

Changes within the chemical composition of dental plaque, following exposure of sucrose, reflect a combination of reticulated

factors and processes that embrace diffusion, microorganism metabolism, the mineral ions release, buffering, and the influence of saliva.[6] Clearly, such changes can result in the establishment of the necessary conditions for caries occurrence. *S. mutans*, although naturally present among the human oral microbiota, is the microbial species most strongly associated with carious lesions. The growth and metabolism of *S. mutans* changes the environmental conditions of the oral flora, which enables fastidious organisms to colonize and causes the formation of dental plaque.[7]

In this study, the children from residential school were selected to keep the parameters, like diet and oral hygiene practices, constant. Older children were selected as they would understand and apply the paste regularly. Since the reduction of *S. mutans* count would be beneficial for a high caries risk, children with DMFT score >3 were selected. Among both creams, the TCP-containing paste significantly reduces the *S. mutans* when compared with the other paste. The possible mechanism responsible for reducing *S. mutans* by TCP-containing cream could be due to the fluoride content, whereas in calcium sucrose phosphate-containing cream, it could be due to the sorbitol content. It is possible that the surface properties of enamel are altered by the presence of both the creams. If the consumption of sugar substitution is more, it can transfer the caries activity toward the preservation of tooth structure and perhaps remineralization, and it also can help to prevent caries but not in the curative sense of an agent, such as fluoride.[8]

There is no scientific literature on TCP's antimicrobial efficacy. TCP, on the contrary, has been extensively researched as a potential bone substitute due to its biodegradable and high-osteoconductive properties.[9] Study conducted by Vashisht et al.[10] evaluated the reduction of *S. mutans* by casein phosphopeptide-amorphous calcium phosphate (CPP/ACP) containing remineralizing paste which had similar results. Other study by Emine et al.[11] evaluated the effect of remineralizing agent on *S. mutans* biofilm adhesion which didn't have significant effect. In a study where a systematic review was done by Raphael and Blinkhorn,[12] it was stated that lacked sufficient evidence to support the CPP/ACP containing remineralizing paste had a major role in preventing dental caries. Samuel et al.[13] demonstrated that the use of CPP/ACP with fluoride, calcium sodium phosphosilicate, and TCP twice daily resulted in a significant reduction in *S. mutans* levels in saliva. When used in oral care products, inorganic compounds based on calcium and/or phosphate (e.g., sodium bicarbonate, hydroxyapatite, calcium carbonate) provide several advantages. Calcium release can inhibit demineralization, and hydroxide and phosphate ion release may aid in acid buffering.[14] A study done by Fadl et al.[15] in preschool children found that CPP/ACP reduced *S. mutans* in the plaque after 2 weeks of daily application when compared to the baseline. The study's short follow-up period of 16 days may be considered a major limitation.

The remineralizing effect of TCP and CSP is well documented. This clinical trial emphasizes the anticariogenic activity of both the creams as well as its role in minimal intervention dentistry. Thus, the present clinical study demonstrates that short-term remineralizing agents resulted in a significant reduction in the *S. mutans* bacterial count. Prolonged administration of remineralizing pastes may play a preventive role in the development of caries. Long-term clinical trials with a larger sample size on the effects remineralizing pastes on *S. mutans* bacterial count inhibition would be commendable.

Conclusion

Both the creams reduced the count of *S. mutans,* which suggests that these creams can be used as a potent agent in inhibiting the growth of this organism. Before drawing a conclusion, a long-term study is required so that these creams can be useful for children with high caries prevalence and even special child, which would in turn open a new phase of preventive dentistry.

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