Effectiveness of Casein Phosphopeptide-Amorphous Calcium Phosphate and Xylitol Chewing Gums on Salivary pH, Buffer Capacity, and Streptococcus mutans Levels: An Interventional Study

Abstract
Aim: The aim of the study is to compare the anticariogenic effectiveness of Casein phosphopeptide-Amorphous Calcium phosphate (CPP-ACP) and xylitol chewing gums based on salivary pH, buffer capacity, and Streptococcus mutans levels. Materials and Methods: A group of twenty individuals in the age group of 18-25 years were randomly divided into two Groups A and B. Test arm A received xylitol gums and test arm B received CPP-ACP gums and they were instructed to use the gums thrice daily for 2 weeks. Unstimulated salivary samples were collected before they began the use of the gums for baseline values, 24 h after beginning the usage of chewing gums and at the end of 14 days. The samples were analyzed for pH, buffer capacity, and S. mutans levels. Results: A statistically significant reduction of salivary S. mutans levels, improvement in salivary pH, and buffer capacity were displayed in both groups 24 h and 14 days after the intervention when compared with baseline. Group B showed more statistically significant improvement in pH than group A after 24 h (P = 0.028) and at the end of 2 weeks (P = 0.041). Conclusion: CPP-ACP has better ability than xylitol in improving the pH of saliva. Both CPP-ACP and xylitol gums individually have remarkable ability in bringing down S. mutans levels while simultaneously improving the pH and buffer of saliva.

Keywords: Anticariogenic efficacy, casein phosphopeptide - amorphous calcium phosphate, salivary buffer capacity, salivary pH, Streptococcus mutans, xylitol

Introduction
Dental caries is an irreversible yet preventable odontogenic infection of the calcified structures of the tooth. The genesis of a Dental carious lesion is determined by the interplay of multiple factors. This interplay is best explained by the Keyses’ triad Venn diagram proposed in 1960s which includes tooth, diet, and dental plaque. However, the occurrence of a frank cavitation is attributable to the destructive factors that cause demineralization process to overpower remineralization. The beam balance of caries has the protective factors and the destructive factors on the two pans. The battle that ensues between the destructive and protective factors governs the demineralization-remineralization cycle.

Optimal salivary pH, buffering ability, reduced salivary Streptococcus mutans counts, noncariogenic sugar substrate in the diet, etc., contribute to the protective factors that enhance remineralization process.

Two principal mechanisms that destroy the equipoise of the oral environment include high sugar intake and low salivary pH caused by the microbial breakdown of the sugar substrate. S. mutans is considered as the predominant human type S. mutans that is formidably associated with dental caries. Increased S. mutans levels in saliva favor demineralization by producing acids from sugar substrates. In addition to it, the organisms use the sugars to produce glucans that amplifies their ability to attach to the tooth structure. The fermentable sugar substrate also serves as a reserve source of energy for the S. mutans. Thus, a pragmatic approach would be to orient the preventive strategies toward producing a reduction in S. mutans counts and also enhance the salivary pH and buffer capacity.

Xylitol is a nonfermentable sugar alcohol that has the potential to reduce S. mutans levels by disrupting the organism’s energy cycle by a suicidal mechanism involving...
Casein phosphopeptide and amorphous calcium phosphate (CPP-ACP) is a successful sugar-free anticariogenic compound sequestrated from milk protein casein complexed with calcium phosphate. The Ser(P)-Ser(P)-Ser(P)-Glu-Glu sequence of CPP-ACP compound is responsible for the exceptional stability of calcium phosphate ions thereby encouraging the remineralization process. This compound is also known to ameliorate the salivary pH and buffering capacity. Furthermore, CPP-ACP destroys the plaque bacteria bridging by competing for the calcium that is necessary for the bond.

Multiple studies have been done to assess the anticariogenic efficacy of chewing gums such as xylitol, sorbitol, and mastic gums in the past. Literature search reveals more studies on comparison between xylitol and sorbitol for anticariogenic efficacy. However, only one study by Emamieh et al. has compared Xylitol with CPP-ACP gums for its ability to reduce S. mutans levels in saliva. Reducing the levels of the causative organism while simultaneously improving the salivary defense will prove to be more effectual in combating the disease. Thus, the interventional study was designed to compare the two gums not only in terms of S. mutans reduction ability but also in the lines of salivary pH and buffer capacity which are vital for fortification against dental caries. The aim of the present study is to compare the anticariogenic effectiveness of CPP-ACP and xylitol chewing gums based on salivary pH, buffer capacity, and S. mutans levels.

Materials and Methods

The study was designed as a triple-blinded randomized interventional study to compare the anticariogenic effectiveness of CPP-ACP and xylitol chewing gums by assessing pH and buffer capacity of saliva and salivary S. mutans levels. After obtaining the approval from the Ethics Committee of the institutional review board of Sri Venkateswara Dental College and Hospital, twenty healthy individuals in the age group 18–25 years with DMFS score <3 were enrolled for the study. Written informed consent was obtained from all the subjects after elaborating the purpose of the study. Individuals who were regular users of CPP-ACP or xylitol gums, individuals with systemic conditions, individuals who were on antibiotics in the last 2 weeks, individuals who were allergic to these gums were excluded from the study. The study was registered with the Clinical Trial Registry of India (No. REF/2017/03/013842).

The study was devised with 2 parallel arms (test arm A and test arm B) of ten subjects each. Subjects were randomly allocated to test arms A and B by lottery method. Subjects in test arm A received xylitol chewing gums (Orbit White, Wrigley India Pvt. Ltd., Bengaluru, Karnataka, India) and subjects in test arm B received CPP-ACP chewing gums (Recaldent, Nihon Kraft foods limited, Tokyo, Japan). However, the subjects were unaware of the type of chewing gum received by each test arm during the trial period. All the above allocations were done by a single operator. To facilitate blinded, chewing gums of same color, shape, and size were used. Furthermore, the chewing gums were removed from their original boxes and were repacked into uniform packets. The individuals were instructed to follow uniform oral hygiene measures throughout the study. The subjects were directed to use the gums thrice daily 15 min after each meal, i.e., breakfast, lunch, and dinner for 5 min. They were asked to follow this for 2 weeks. The subjects were regularly monitored during the trial period to prevent confounding errors due to intake of antibiotics or other factors. They were asked to report if any side effects occurred, and it was decided that those will be excluded from the study. The salivary samples from the subjects were collected in a 5 ml sterile container before they began the use of the gums for baseline values, 24 h after beginning the usage of chewing gums and at the end of 14 days by an investigator who was also blinded.

The pH of the unstimulated saliva was recorded using a pH meter (Digital pH meter, MIFA Systems Private Limited, Ahmedabad, Gujarat, India). 0.1 ml of the sample was taken, diluted, and vortex mixed. 0.1 ml of this diluted sample was then taken to inoculate onto the Mitis Salivarius agar base (HIMEDIA, Mumbai, Maharashtra, India). The petri plates were then incubated at 37°C with 3% CO₂ for 48 h. The organisms were identified based on colony morphology. The colony forming units were counted manually. A handheld pH meter was used to assess the buffering capacity. The pH sensitive electrode was first calibrated for pH 4.0 and 7.0 using standard pH pellets. Two hundred and fifty microliters of lactic acid (pH 3, 1.5 mM) were then titrated into the test sample and mixed. The pH value of the titrated sample was noted by using the handheld pH meter with digital reading display. The results were ranked as high (>5.8), medium (>4.8 or <5.7), or low (pH <4.7).

The type of chewing gum given to each of the test arms was not revealed to the statistician either. The data obtained for baseline, 24 h, and 14 days were recorded using Microsoft Office Excel 2007 and subjected to statistical analysis using SPSS software version 20 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).
One sample Kolmogorov–Smirnov test was used to assess the normal distribution of the variables. The variables were normally distributed for S. mutans count and salivary pH, and hence independent sample t-test was used for intergroup comparisons and paired t-test was employed for within group comparisons. Since the buffer capacity data were ordinal in nature, Mann–Whitney U-test, and Wilcoxon-signed rank test were applied for intergroup and within group comparisons, respectively. The level of statistical significance was set at 5% ($P < 0.05$).

**Results**

All subjects completed the trial with good compliance, and no adverse effects were reported by any of the subjects enrolled. Figure 1 provides information on the subjects excluded during the screening process. Table 1 shows the demographic details and mean age of the subjects enrolled in test arm A and B. There was no statistically significant difference in the distribution of the subjects.

Table 2 shows within group comparisons of CPP-ACP and xylitol gums at different time intervals. After the 2 week intervention period, there was an improvement in salivary pH and buffer capacity compared to baseline and 24 h in both xylitol and CPP-ACP groups which was statistically significant. S. mutans levels decreased in both groups during all the three-time intervals (i.e., baseline to 24 h, 24 h to 14 days, and baseline to 14 days), and the difference was found to be statistically significant.

Table 3 shows the intergroup comparisons between xylitol and CPP-ACP at different time intervals. The difference in the baseline values of salivary pH, buffer, and S. mutans levels between the two groups was not statistically significant. The Mann–Whitney U-test revealed that there was no significant difference between the two gums in improving the buffering capacity after 14 days. The results of the independent sample t-test revealed that the difference between xylitol and CPP-ACP was not statistically significant in reducing the salivary S. mutans levels after 14 days. However, the same test performed for pH comparisons showed that CPP-ACP had better ability to increase the salivary pH than xylitol immediately after 24 h ($P = 0.028$) and at the end of 14 days ($P = 0.041$), and this was found to be statistically significant.

**Discussion**

Caries prevention has always been an area with immense scope for research. There has always been a constant search for identifying preventive measures for caries. Enhancing the inherent defense mechanism of saliva and diminishing the microbial levels seems to be a rational approach. The protective mechanisms of saliva can be divided into two categories – physical and chemical. The physical defense actions of saliva include flushing and displacement of microbes from their niche. Salivary pH and buffer are two natural endogenous chemical protective mechanisms that rein the caries balance from swaying toward
Table 3: Between the group comparison of Streptococcus mutans count, pH, and buffer capacity of the two test arms at different time intervals

| Time intervals | Group       | n  | Median Streptococcus mutans count | Inter-quartile range | P     | pH mean | P       | Buffer capacity (mean rank) | P     |
|---------------|-------------|----|---------------------------------|----------------------|-------|---------|---------|----------------------------|-------|
| Baseline      | Group A     | 10 | $6.96 \times 10^5$             | $6.6475 \times 10^5$ | 0.749 | 6.0180  | 0.064   | 11                         | 0.615 |
|               | Group B     | 10 | $7.045 \times 10^5$            | $8.461 \times 10^5$  |       | 6.2320  |         | 10                         |       |
| 24 h          | Group A     | 10 | $5.83 \times 10^5$             | $6.92 \times 10^5$   | 0.202 | 6.1320  | 0.028*  | 10.7                      | 0.861 |
|               | Group B     | 10 | $3.43 \times 10^5$             | $6.6275 \times 10^5$ |       | 6.5510  |         | 10.3                      |       |
| 14 days       | Group A     | 10 | $1.62 \times 10^5$             | $1.933 \times 10^5$  | 0.355 | 6.7760  | 0.041*  | 10                         | 0.648 |
|               | Group B     | 10 | $6.945 \times 10^4$            | $1.42 \times 10^5$   |       | 7.0550  |         | 11                         |       |

*Significant at 5% interval

Xylitol and CPP-ACP are two compounds which are known to reduce the S. mutans levels and also improve the pH and buffer capacity of saliva. This has been proven by various studies in the literature. [9-14,17,20,23] CPP-ACP has been used in various studies to assess its anticariogenic potential in the form of tooth mousse (toothpaste form). Delivering CPP-ACP in the form of chewing gums has its own advantages. The effectiveness of CPP-ACP in the form of chewing gums has been evaluated in this study. Moreover, exploration of literature reveals there is a scarcity in comparative evaluations of CPP-ACP chewing gums. Hence, in this current study, CPP-ACP chewing gums are compared with xylitol gums under three criteria, which are critical for protection against dental caries. Emamieh et al. [35] have carried out this comparative study in lines of S. mutans reduction ability and concluded that CPP-ACP have better efficacy than xylitol, but the results of this study revealed that there is no difference between xylitol and CPP-ACP gums in diminishing the S. mutans levels. However, in addition to this, maintenance of the pH and buffer capacity was assessed in the current study as these are critical regulators in the process of cariogenesis. Both the gums showed equal effectiveness in maintaining the buffer capacity while CPP-ACP was better than xylitol in maintaining the pH.

Xylitol and CPP-ACP when used in the form of chewing gums have the inherent benefit of improving the salivary flow rate which boosts the physical defense mechanism of saliva. [37] Thus, in this study, xylitol and CPP-ACP in the form of chewing gums were employed. Collecting stimulated saliva might result in alterations in the composition, concentration, and pH. [38,39] Therefore, unstimulated salivary samples were collected for analysis in our study. Lactic acid is considered safer than the hydrochloric acid that is employed in Ericsson method for assessing salivary buffer capacity. A quantitative method was used for assessing the buffer capacity. Kitasako et al. [40] proved that this quantitative test shows a strong positive correlation with the Ericsson method. Hence, lactic acid along with handheld pH meter was used in this study to assess the buffering capacity of saliva. The recommended dosing frequency of the gums is to chew them thrice daily for 5 min within 20 min following each meal. [41] The time frame of 5–20 min immediately after each meal is when the pH rate falls down rapidly. [42,43] Providing the intervention in that time frame helps in counterbalancing the pH drop effectively. This is because the chewing gums improve the salivary flow rate with concurrent rapid rise in pH. In addition, this rise is closely associated with a rise in bicarbonate buffering ability. [44] Hence, the same dosing frequency was followed in the current study.

Xylitol is very well known for its antibacterial effect against S. mutans as supported by various studies in the literature. [9-14] The results of our study are also in conformity with the above statement. The results of the present study revealed that after 2 weeks use of xylitol gums, the pH and buffer capacity of saliva improved which was found to be statistically significant ($P = 0.001$ and $P = 0.004$). This is similar with the study results of Ribelles et al. [45] The reason might be that xylitol is a natural sweetener with 5 carbon atoms that renders it nonfermentable by oral microflora. [39] This property gives the gum superiority over other sugar free gums when it comes to antibacterial effect. [16]

The current study shows that CPP-ACP is effective in purging S. mutans levels in saliva, and this reduction was found to be statistically significant ($P = 0.002$). The above findings mirror the results from the study of Subramanian and Naidu [46] and Vasishth et al. [47] This is probably due to the fact that the casein fractions from milk alter the adhesion of S. mutans on to the tooth surface and also selectively modulate the microbial composition of plaque biofilm. [48-50] The results of our study indicated an improvement in the pH and buffering ability of saliva after using CPP-ACP gums which is in accordance with the study done by Chaitanya et al. [23] CPP-ACP nanocomplexes serve as a reservoir of calcium and phosphate ions which aids in maintaining the pH and buffer of saliva. Thus, CPP-ACP has dual advantage over xylitol that it not only opposes demineralization but also enhances remineralization by direct incorporation of the calcium and phosphate ions. [17]
In this study, CPP-ACP gums have proven to be more effective in improving the pH when compared to xylitol, and this was found to be statistically significant ($P = 0.041$). This is probably due to the fact that a single CPP-ACP gum can contain mineral ions nearly as much as a liter of a typical remineralizing solution or saliva.[23] Thus, the increased availability of ions would offset any fall in pH. Furthermore, the neutral CaHPO$_4$ which is formed by the pairing of ions released from CPP-ACP takes the credit of consuming majority of the acid generated by the cariogenic bacteria.[51,52] Cai et al. proposed that CPP-ACP has the credibility of promoting remineralization even in acidic environments.[53] Although the results of the study emphasize the fact that both CPP-ACP and xylitol have good anticariogenic properties, it should be stressed that long-term trials are required to constantly monitor their effects on a dynamic process such as dental caries.

**Conclusion**

Within the limitations of the present study, it can be concluded that CPP-ACP has better ability than xylitol in improving the pH of saliva and both CPP-ACP and xylitol gums individually have remarkable ability in bringing down *S. mutans* levels while simultaneously improving the pH and buffer of saliva. Thus, it is very evident that usage of these gums over a short period will definitely provide benefit against caries.

With the ever changing scenario of preventive dentistry, studies on comparisons between the anticariogenic effectiveness of two compounds such as xylitol and CPP-ACP can act as a guide and help the clinicians choose between the two and provide the right suggestion to the patients.

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**Conflicts of interest**

There are no conflicts of interest.

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