



## To Assess the Effectiveness of Sugar Free Chewing Gum with CPP-ACP on *Streptococcus mutans* Count" - A Double Blind Randomized Controlled Study

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### Abstract

**Background:** Dental caries is an important public health problem and its impact on the society and on the individual is significant. There is a need to adopt a more progressive approach towards prevention of dental caries incidence. CPP-ACP has been demonstrated to be a safe and effective tooth-decay preventive agent when used at an optimum dose of 3 - 5 g/day divided in 3 exposures. It is important to determine the effect of using CPP-ACP chewing gums within the recommended concentrations and frequencies long enough to alter the oral micro flora which in turn can retard the proliferation of *Streptococcus mutans* and maintain its effect even beyond interruption among the individuals who are among high caries risk groups.

**Aim of the Study:** To assess the effectiveness of sugar free chewing gum with CPP-ACP on *streptococcus mutans* count.

**Materials and Methods:** Sixty study subjects (first and second year undergraduate dental students) from a dental colleges based on the inclusion and exclusion criteria were assigned into two groups; Group A (Without CPP-ACP) and Group B (With CPP-ACP). The experimental period comprised of baseline data collection, intervention period for 30 days, follow-up and post intervention period for 30 days and assessed for streptococcus mutans quantification at all the time frames.

**Results:** The results showed that the mean age of the study subjects was  $21.12 \pm 1.75$  years, there were significant reductions in salivary levels of *streptococcus mutans* at 30 days after intervention when compared to the baseline and the effect was maintained even beyond interruption. Group B showed greater amounts of streptococcus mutans counts reduction at the end of follow-up phase when compared to Group A.

**Conclusion:** Thus, the use of CPP-ACP chewing gums 2 pellets/2 times/day leads to increased reductions in salivary levels *streptococcus mutans* of even for short periods of 30 days and the effect was maintained even beyond interruption.

**Keywords:** CPP-ACP; Chewing Gum; Saliva; *Streptococcus mutans*

### Introduction

Dental caries and periodontitis are microbiological disease with widespread global occurrence leading to pain, tooth loss, and infection [1]. Tooth enamel consists predominantly of the mineral hydroxyapatite [2]. In the oral cavity, indigenous bacteria are often associated with oral problems [1]. Bacteria which are mainly responsible for initiating caries are *Streptococcus mutans* and Lactobacillus species. These bacteria produce lactic acid in the presence of fermentable carbohydrates. The importance of mutans streptococci in the etiology of dental caries has been recognized since the 1970s [2].

Dairy products including milk, milk concentrates, and cheeses have long been known to exhibit anticaries activity i.e. reduce demineralization and increase remineralization of enamel [1]. The components of milk responsible for this anticariogenic activity have been identified as casein, calcium and phosphate. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a milk derived peptide that has the ability to stabilize ionic calcium and phosphate and limit aggregation to a size that prevents crystal nucleation creating supersaturated solutions which drive remineralization and inhibit demineralization. Numerous in-vitro, in situ and clinical studies have reported that CPP-ACP is efficacious in

remineralizing White Spot Lesions and preventing demineralization [3].

Recently it has been demonstrated that the CPP-ACP complex is able to incorporate fluoride ions thus forming a CPP-amorphous calcium fluoride phosphate (CPP-ACFP) nanocomplex. Thus, confirming that the CPP-ACFP complex has the potential to be a novel delivery vehicle for calcium, phosphate and fluoride ions [1]. Chewing gums have the potential of being an effective vehicle for delivering therapeutic agents because they permit protracted contact of the agent with the teeth with minimal efforts on the part of the patient. CPP-ACP nanocomplexes have been proven to have reduced caries activity.

### Objective of the Study

The main objectives of the research was to assess the levels of *streptococcus mutans* pre and post intervention periods in control and experimental group and to assess the maintenance of salivary level of *streptococcus mutans* post withdrawal of CCP - ACP chewing gum usage. Hence, the present study aimed to assess the effectiveness of sugar free chewing gum with CPP-ACP on *streptococcus mutans* count.

### Methodology

The present study was an interventional randomized controlled double (observer and subject) blinded parallel arm clinical trial conducted to assess the effectiveness of sugar free chewing gum with CPP-ACP on *Streptococcus mutans* count at baseline, 30 days of intervention and 30 days of post intervention among 60 subjects aged 18 - 25 years, from April 2016 to June 2016 selected from a college in Bangalore city, Karnataka, India. Prior to the start of the study, ethical clearance was obtained from the Institutional review board of Bangalore Institute of Dental Sciences, Bangalore. Permissions for conducting the study and clinical examination were obtained from college authorities of Bangalore Institute of Dental sciences

### Sample size estimation

Sample size per group was estimated from power calculation based upon the sample size calculations by computing the values

in the given formula:

$$SD = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

N: population size

n: Sample size

$\sigma$ : Standard deviation

z: Critical value based on Normal distribution at 95% Confidence Interval.

This gave the sample size to be 30 per group, which was randomly chosen, from the study population with a significance level of 0.05.

1<sup>st</sup> and 2<sup>nd</sup> year dental students of the institution, study participants who are willing to participate and gave consent, no report of use of antibiotics 3 months prior to the beginning of the study and absence of active dental caries and periodontitis were included in the study. Study participants having systemic diseases and conditions who are under medications, undergoing orthodontic treatment, phenylketonurics, history of gastrointestinal problems and habitual chewing gum users were excluded from the study. Written informed consent was obtained from all the subjects after explaining the nature of the study.

Before commencing the study, information on CPP-ACP chewing gums availability in Indian market was collected. It was found that there were no CPP-ACP chewing gums being marketed in India. Hence the information on its availability was checked in other countries, availability of CPP-ACP chewing gums was confirmed in the United States of America, information on various brands of commercially available CPP-ACP chewing gums were collected. Among the various brands available in US market Trident white Wintergreen and Trident white Peppermint (TRIDENT™, Cadbury, Adams, USA) chewing gums was selected and imported for the study, which was available as pellets containing 1.06g of CPP-ACP in each Trident white Wintergreen chewing gums. The composition of Trident white Peppermint chewing gum and Trident white Wintergreen were alike without the CPP-ACP ingredient which was only

present in the Trident white Wintergreen chewing gum. Training and calibration of the investigator was done under the guidance of an expert examiner.

A pilot study was conducted before the main study to check the adverse effects and toxicity of the chewing gums. As per the above mentioned inclusion and exclusion criteria of the study, the subjects were screened until a final study sample of sixty subjects were obtained and randomly allocated into two groups using lottery method with 30 subjects in each group. Selection bias was addressed by allocation concealment. The assignment of subjects to the groups was performed by a person who was not involved in the examination. Group A (n = 30) - chewed the Trident White peppermint (without CPP-ACP) (1 pellet = 1.06g), 2 pellet/2 times a day for 5 minutes each time, between meals for a period of 30 days and Group B (n = 30) - chewed the Trident White wintergreen (with CPP-ACP) (1 pellet = 1.06g), 2 pellet/2 times a day for 5 minutes each time, between meals for a period of 30 days.

Everyday subjects were instructed to chew the chewing gums for 5 minutes each time after taking food. To avoid performance bias both the examiner and study participants were blinded and was unaware of the chewing gums, which were allotted to the subjects of that particular group. After the distribution of the chewing gums the study participants were monitored by the examiner to ensure that they were using the assigned products and following the instructions. During holidays SMS (Short message service) were sent and calls were made to study participants to ensure chewing gum consumption.

The study was carried out in 3 phases: During the first phase of the study, the examiner carried out oral examination of the subjects during college working hours for the selection of the study subjects satisfying the inclusion and exclusion criteria where the subjects without any active carious lesions and periodontitis were selected for the study. The subjects were asked to refrain from intake of chewing gums and products containing CPP-ACP during the course of the study.

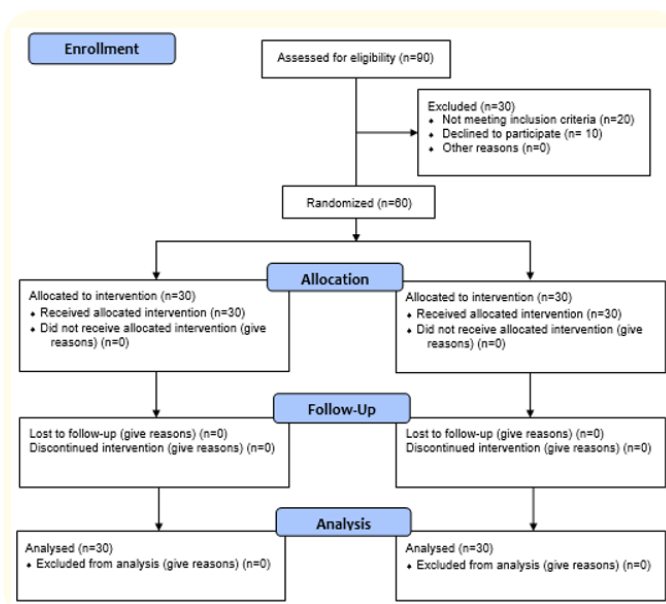
In the second/intervention phase baseline saliva sample collection was done and the intervention was given to both the groups.

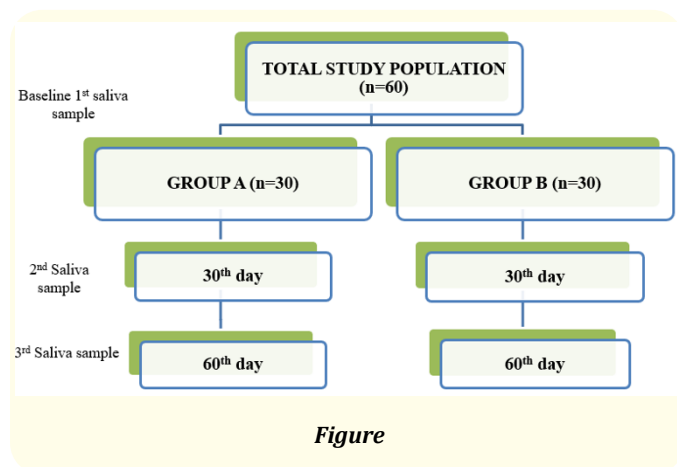
Subjects were divided into Group A (Without CPP-ACP) and Group B (With CPP-ACP). The baseline saliva sample collection was done to assess the mutans streptococcus and the chewing gums were packed in identical plastic covers (1 pack = 2 pellets), blinded and coded were distributed. Before the start of the intervention a demonstration about the chewing gums and its disposal was given to the subjects.

During the third and final phase, first follow-up was done. The entire procedure of collecting the saliva samples, microbial analysis for mutans streptococci quantification were repeated again after 30 and 60 days of post intervention and checked for the differences.

### Saliva sample collection

All the study subjects undergoing investigations were given clear instructions to refrain from eating for one hour before collection of saliva and all necessary things required for saliva collection and analysis were assembled before the sample collection. The subjects were asked to chew on paraffin wax blocks for five minutes and the accumulated saliva was collected continuously into a coded and sterilized disposable plastic cups by asking the subjects to spit into these measuring cups. Immediately following this, 1 ml of saliva was taken in disposable syringes and transferred to test tubes containing 4 ml thioglycolate solution, which was used as a transport media.





Figure

**Statistical analysis**

The data was processed and analyzed using the SPSS software version 22. Unpaired ‘t’ test was done for the inter group comparisons of the continuous variables such as logarithm values of the *mutans streptococci* counts at baseline and all the time intervals along with Repeated measures ANOVA was used for the analysis of the Continuous variables such as logarithm values of the *mutans streptococci* counts for the intra group comparisons at baseline and all the time intervals.

**Results**

A total of 60 participants (8 males and 52 females) were included in the study. They were randomly divided into two groups, such that each group contained 30 subjects. The mean age of the participants was 21.12 years (standard deviation of 1.75) with minimum age of 18 years and maximum age of participants were 25 years as shown in table 1.

Study groups	Number of Participants		Total Participants N (%)
	Male n (%)	Female n (%)	
Group A	4 (6.6%)	26 (43.3%)	30 (50%)
Group B	4 (6.6%)	26 (43.3%)	30 (50%)
Total	8 (13.3%)	52 (86.7%)	60 (100.0%)

Table 1: Distribution of study participants according to their gender in both the groups.

Table 2 shows intergroup comparison of *Streptococcus mutans* count in Saliva (Expressed as 10<sup>4</sup> CFU/ml) between Group A and Group B at baseline were 442.62 ± 22.79 and 486.73 ± 18.36 and the difference was not statistically significant (p = 0.32). At 30<sup>th</sup> day and 60<sup>th</sup> day the counts were 426.89 ± 22.33 and 306.75 ± 13.33, 429.71 ± 22.38 and 381.30 ± 14.86 respectively. There was statistically significant difference between the counts between the 2 groups (p < 0.01\*).

Groups	Time interval	Number	Mean ± std deviation	Mean difference	t value	p value
Group A	Baseline	30	442.62 ± 22.79	44108.76	0.82	0.32
Group B			486.73 ± 18.36			
Group A	30 <sup>th</sup> day	30	426.89 ± 22.33	120145.633	2.53	0.01*
Group B			306.75 ± 13.33			
Group A	60 <sup>th</sup> day	30	429.71 ± 22.38	48410.60	0.98	0.01*
Group B			381.30 ± 14.86			

Table 2: Comparison of *Streptococcus mutans* counts among study participants between Group A and Group B at baseline, 30<sup>th</sup> day and 60<sup>th</sup> day (Expressed as 10<sup>4</sup> CFU/ml).

*Streptococcus mutans* count in saliva in Group B study participants at Baseline, 30<sup>th</sup> day and 60<sup>th</sup> day (Expressed as 10<sup>4</sup> CFU/ml). The mean *S. mutans* count in saliva (Expressed as 10<sup>4</sup> CFU/ml) in Group B with CPP-ACP at Baseline, 30<sup>th</sup> day and 60<sup>th</sup> day were 486.73 ± 18.36, 306.75 ± 13.33, 381.30 ± 14.86 respectively. There was statistically significant difference between the counts across the group (p < 0.00\*) as depicted in figure 1.

Table 3 shows percentage reduction in mean *Streptococcus mutans* counts at 30<sup>th</sup> day and 60<sup>th</sup> day between Group A and Group B. At the end of 30<sup>th</sup> day, Group B had shown the maximum percentage reduction of 36.97% when compared with Group A which had

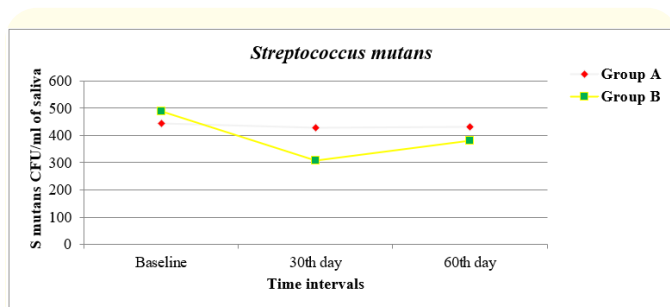


Figure 1

Study groups	<i>S mutans</i>	
	30 <sup>th</sup> day	60 <sup>th</sup> day
Group A	3.55%	0.66%
Group B	36.97%	24.30%

Table 3: Percentage reduction in mean *Streptococcus mutans* counts at 30<sup>th</sup> day and 60<sup>th</sup> day between Group A and Group B.

\*Group A without CPP-ACP, Group B with CPP – ACP.

shown 3.55% reduction rates. At the end of 60<sup>th</sup> day Group B had shown the maximum percentage reduction of 24.30% when compared with Group A which had shown 0.66% reduction rates.

There was an increase in the mean percentage reduction of *S. mutans* counts in Group B at 30<sup>th</sup> day when compared with Group A at 30<sup>th</sup> day, but there was decrease in *S. mutans* counts mean percentage reduction in Group B at 60<sup>th</sup> day when compared at 30<sup>th</sup> day reduction count.

### Discussion

According to the World Health Organization (WHO) in 2003, caries is the major public health problems in the majority of industrialized and developing countries. It affects 60 to 90% of children of school-going age, and the large majority of adults [4].

### Oral hygiene practices

The oral hygiene practices of the study participants were recorded at the beginning of the study and were subjected to statistical tests. The results obtained showed uniformity between the study subjects and no marked variation was observed between the two groups.

### CPP-ACP chewing gums

Many studies report a reduction in levels of salivary *S. mutans* with the prolonged use of CPP-ACP chewing gum, indicating that CPP- ACP may decrease the ability of bacteria to multiply in its presence [5]. CPP-ACP chewing gum has shown to be effective as a caries preventive agent. CPP-ACP currently is available in many forms such as; chewing gums, mints, chewable tablets, lozenges, tooth-pastes, mouthwashes, cough mixtures and nutraceutical products. CPP-ACP are available in a wide range of commercial products, although there are no direct head-to-head comparisons available, the current concept seem to favor chewing- gums because of its superior saliva-stimulating capacity [6].

### Relation of dose and frequency of CPP-ACP chewing gum chosen

The American Academy of Pediatric Dentistry (AAPD) recognizes the benefits of caries preventive strategies involving sugar substitutes on the oral health of infants, children, adolescents and persons with special health care needs with the use of CPP-ACP based products in caries prevention [7].

There is a conflicting evidence in the literature from CPP-ACP toothpaste studies suggesting the lower doses and less frequent exposures might be effective, but the synergistic effects of CPP-ACP and fluoride cannot be ruled out [7]. But a substantial body of literature suggests that a minimum of 3 to 6 grams and 2 exposures per day of CPP-ACP, from chewing gum are needed for a clinical effect to be achieved. Hence keeping this in mind the dose and frequency of CPP-ACP chewing gum consumption was decided in the present study to be 3.02g (2 pellets/2times/day) of CPP-ACP chewing gums.

The effect was declining with lower doses while increasing the daily dose showed no additional benefit [8]. From recent studies it has become evident that a dose-response relationship exists concerning the impact of CPP-ACP on oral ecology and also that the frequency of administration could affect the outcome [6].

### Effect of CPP-ACP chewing gums after meals

In recent years, a great deal of interest has centered on the use of chewing gums after meals as a means of stimulating salivary flow to prevent the formation of dental caries. The increase in salivary



flow enhances the buffering capacity of saliva, which effectively neutralizes the drop in plaque pH that occurs after eating which leads to a reduction in caries rates as it enhances remineralization and prevents demineralization. Hence we also in the present study have considered administering the CPP-ACP chewing gums after meals to enhance its effect [9].

### Relation between CFU of mutans streptococci and CPP-ACP chewing gum

The results in the present study revealed that there was a statistically significant difference in the salivary mutans streptococci counts ( $10^4$  CFU/ml) when the study subjects among Group A and Group B were compared at baseline, 30 days and 60 days post intervention time intervals. Similar findings were noted by Rose R K in 2000, Cross K J, Huq N L, Reynolds E C in 2007, Hanning M, Hanning C in 2010 and Maria C., *et al.* in 2014 where comparison of levels of *Streptococcus mutans* in saliva in baseline conditions and after chewing CPP-ACP chewing gum or without CPP-ACP showed statistically significant differences [2,10-13]. Studies such as those carried out by Subramanian P in 2009, Arzu Aykut Yetkiner., *et al.* in 2014, Vasisht R., *et al.* in 2013 and Shila Emamieh., *et al.* in 2015 have shown that chewing a CPP-ACP chewing gum reduces the levels of *S. mutans* in saliva [13-15]. Whereas in a laboratory study conducted by Erdem., *et al.* in 2011 reported that there were changes in *Streptococcus mutans* after the use of CPP-ACP but it was not statistically significant after 18 weeks [16].

### Taste and acceptance of CPP-ACP

None of the study subjects in this study had reported of any side effects and issues about the taste of the CPP-ACP chewing gums consumed by them as the CPP-ACP chewing gums used were wintergreen and peppermint flavored and it was well accepted by the study subjects who participated in the present study.

According to a recent review of randomized field trials and observational studies, it was concluded that the evidence is strong enough to support the regular use of CPP-ACP chewing gums as a way to prevent caries and it can be promoted as a public health measure [5,8]. Furthermore, health-economic evaluations of preventive programs with chewing-gums are needed. Hence this study can mark a beginning to assess the short-term therapy and maintenance of its effects even beyond interruption so that it can gain attention towards being used as a strategic public health measure.

### Recommendations

Patients at caries high risk could be recommended to use CPP-ACP products as a complement to the daily exposure to fluoride, at least 3g of CPP-ACP/day and at recommended daily intake to be fractioned at least 2 times over the day and the chewing period should not be shorter than 5 minutes. The taste and acceptance of these CPP-ACP chewing gums need to be further studied among larger group of population. Extensive research is needed to test the cost effectiveness of CPP-ACP chewing gums by conducting more and more field trials. Recent studies indicate that preventive programs should include as many complementary strategies as possible especially when directed towards caries-active patients. Hence CPP-ACP should be considered as an adjunct to other measures such as fluoride exposure, fissure sealants and dental health education in individuals assessed as being at high risk for future caries development. There is a huge potential for the use of CPP-ACP chewing gums in improving oral health but more long term-clinical trials and preventive programs are needed before any concrete statements regarding CPP-ACP as a public health measure.

### Limitations of the Study

The present study points towards the positive effects of the CPP-ACP chewing gums on the oral health. But the study also show that certain limitations like semi-quantitative nature of the microbial estimations and the sample size taken in the present study was 60. Future studies should include bigger sample size to overcome this limitation. Hence, these results should be interpreted with caution. Follow up in this study was for 30 days. Hence, long follow-up period should be considered in order to be able to generalize the benefit demonstrated by CPP-ACP.

### Conclusion

This research marks a new beginning to the evaluation of the effectiveness of CPP-ACP chewing gums on oral micro flora which attribute to the caries preventive action. There were significant reductions in salivary levels of *Streptococcus mutans* at 30 days after intervention when compared to the baseline and the effect was maintained even beyond interruption. Group B showed greater amounts of *Streptococcus mutans* counts reduction at the end of follow-up phase when compared to Group A. This study supports the action of CPP-ACP chewing gums at the dose and frequency used in our study in reduction of *Streptococcus mutans* and this strategy can be used as a targeted caries-preventive measure after

the evaluation of its cost-effectiveness through various preventive programs.

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### Conflict of Interest

None.

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