

Efficacy of Casein Phosphopeptide–Amorphous Calcium Phosphate Containing Propolis on Dental Plaque Development in The Anterior Enamel Tooth Surface of 7–10-Year-Old Children

Peter Andreas¹, Risqa Rina Darwita^{1*}, Faiz Abdurrahman², Revi Aryawedha², Armasastra Bahar¹, Gita Arrifa Sjarkawi¹, Mellisa Adiatman¹, Sri Angky Soekanto³, Muhamad Sahlan⁴

1. Department of Community Dentistry and Prevention, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

2. Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

3. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

4. Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Jakarta, Indonesia.

Abstract

The most common oral health problem is dental caries, caused of dental plaque; thus, materials able to inhibit the development of dental plaque are needed. The study aimed to analyzing the effects of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) containing propolis (10%) compared to CPP-ACP without propolis on dental plaque development in tooth surfaces of 7–10 years old children. This was a quasi-experimental study using a single-group pretest–posttest design. Thirty-two children were stratified into two groups based on propolis inclusion (n = 16 each). The application was performed once a day for four weeks. The average plaque index was obtained by measurement using a modification of the Loe and Silness plaque index method applied between before and after the application on the days 7, 14, and 28. The result indicates that the CPP-ACP propolis group, the plaque index per a general linear model (GLM) repeated measures analysis, the plaque index in the first day was 1.79 decreased significantly 46% (p < 0.05) on the 28th day was 0.97. Meanwhile, In the CPP-ACP without propolis group per the GLM repeated measures analysis, between the first day and the 28th day, the dental plaque score decreased significantly 31% (p < 0.05) from 1.72 in first day to 1.18 by the 28th day. However, there was no significant difference (p > 0.05) in the effect of the application of CPP-ACP with or without propolis. In Conclusion there was no significant difference (p > 0.05) in the effect of the application of CPP-ACP contain propolis (10%) compared to CPP-ACP without propolis in dental plaque development. Thus, the CPP-ACP containing propolis could be an alternative to prevent dental plaque development in enamel surface as a dental caries risk prevention.

Clinical article (J Int Dent Med Res 2020; 13(4): 1542-1547)

Keywords: Casein phosphopeptide–amorphous calcium phosphate, dental plaque, propolis.

Received date: 10 January 2020

Accept date: 15 March 2020

Introduction

Dental plaque is a biofilm or a collection of microorganisms on the tooth surface that plays an important role in the process of dental caries and periodontal disease onset. Some bacteria found in the dental and oral environment such as *Streptococcus mutans* can form colonies on the tooth surfaces and initiate the formation of dental plaque due to the ability to produce extracellular

polysaccharides from sucrose, especially insoluble glucans in water, using *glucosyltransferase* enzyme.¹

The development of dental caries lesions begins when saliva or plaque on the enamel surface reaches a critical value of 5.5 and organic acids from cariogenic bacteria diffuse into the enamel, prompting a demineralization process. Enamel demineralization will initially be visible as white spot lesions. These lesions will continue to develop into cavities or could also experience remineralization.²

In dealing with dental caries problems, alternative methods to inhibit dental caries activity have been explored. In recent years, the adoption of casein phosphopeptide (CPP) as a remineralization material has continued to

*Corresponding author:

Risqa Rina Darwita,
Department of Community Dentistry and Prevention, Faculty of
Dentistry, Universitas Indonesia
Jl. Salemba Raya no.4, Jakarta 10430, Indonesia.
E-mail: risqarina@ui.ac.id

increase. casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) is a product of milk that could help the process of remineralization and prevent dental caries.³ Various studies have begun to develop CPP formulations in order to improve oral and dental health.²

Inhibiting the colonization of *S. mutans* on the tooth surface is believed to be a method to prevent plaque formation and the development of dental caries.¹ Therefore, controlling biofilm or dental plaque is important to good oral health. Plaque control could be done by maintaining dental and oral hygiene, using fluorine-based products, and participating in regular dental visits. In addition, there is strong evidence supporting the benefit of chemical agents and natural ingredients containing antimicrobial activity, such as propolis, in reducing and inhibiting dental plaque formation.^{1,4}

Propolis, in particular, has attracted the attention of many researchers due to its variations in biological activity and therapeutic properties. Propolis is a powerful antibacterial agent. In a previous study, it was found that 4% to 5% of ethanol propolis extract had antimicrobial activity in vitro.² Propolis is nontoxic and contains many pharmacological effects as well as complex chemical compositions. Propolis is capable of reducing the incidence of dental caries in mice.

As research that discusses CPP-ACP in combination with 10% propolis is limited, this study sought to analyze the application of CPP-ACP containing propolis 10% as an alternative material that could be used to reduce the index of plaque to prevent dental caries.

Materials and methods

This study was a quasi-experimental study using a one-group pretest–posttest design. The study compared the average plaque index of children between before and after CPP-ACP application with or without propolis. The study was conducted at Cipinang Besar Utara Elementary School 09 Morning Cipinang District, East Jakarta from July to September 2018.

Ethical clearance was obtained from the FKG UI Dentistry Research Ethics Committee

(no. 44/ethical approval/FKG UI/VI/2019). The reliability was confirmed using two measurements conducted at two different times among five different subjects. All collecting data were analyzed using the Statistical Package for the Social Sciences software program (IBM Corp., Armonk, NY, USA). The reproducibility result was 0.82, suggesting good reproducibility. In other words, all operators were dentist had performance was reliable and they acted the same in conducting the oral examinations.

The research subjects were children aged 7–10 years, i.e., in the second and third grades of Primary school in East Jakarta. Subjects were divided into two groups that received CPP-ACP containing 10% propolis that was extracted from stingless bees and concocted by the Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia or CPP-ACP as a tooth mousse from GC Corp. Both CPP-ACP with or without propolis was given to subjects every day until the day of the 28th.

The sampling approach was a nonprobability with purposive sampling method consisting of taking samples that were in line with the objectives of this study. The sample size was calculated by Freederer's formula. The minimum sample size for one group based on the formula was 16 children; thus, the total number of children established for both groups was 32 children. Both groups were examined according to dental instrument standards by a dentist, while the dental plaque index used was the Silness–Loe plaque index. After the first dental plaque examination (baseline), group 1 received CPP-ACP containing propolis and group 2 received only CPP-ACP as tooth mousse every morning until the day of the 28th. The dental plaque index was calculated at the day 7th, 14th, and 28th day. The method of making CPP-ACP was taken from the patent of the World Intellectual Property Organization proposed by Eric C. Reynolds (U.S. publication no. 2005/0037948 Calcium Phosphopeptide Complexes) [5].

Results

The application of either CPP-ACP containing propolis or CPP-ACP alone was carried out on upper anterior tooth surfaces and lower anterior tooth surfaces, with the amount of

material required per application being corn kernel-sized and measured by using the tip of the index finger. The application was carried out by two operators who had received training from the principal researcher. The calculation of plaque scores was carried out before (baseline) and after application on Days 7, 14 and 28. Dental plaque scores were obtained using a modification of the Loe and Silness plaque index method as previously stated.

The study population was 46.9% (n = 15 children) male and 53.1% (n = 17 children) female. At the initial examination, the children involved in this study had an average age of 7.6 years with an age range of 7–9 years.

Among the children who received CPP-ACP only (n = 16), the highest average plaque index was recorded at the initial examination before CPP-ACP application, equal to 1.72. Conversely, the lowest average plaque index was recorded after CPP-ACP application on the 28th day, equal to 1.18. The results of the average plaque index of children in this group are shown in figure 1.

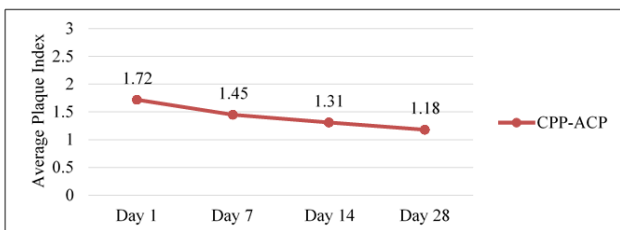


Figure 1. Graph of the average plaque index before and after the application of casein phosphopeptide-amorphous calcium phosphate containing propolis.

The results of the plaque index data normality test for CPP-ACP application indicated $p > 0.05$ at the initial examination and each evaluation, meaning that the data were normally distributed. After the general linear model (GLM) repeated Measures test was conducted, it was found that there were at least two groups of plaque index data with statistically significant differences ($p < 0.05$) after the application of CPP-ACP.

The pairwise comparison test showed that there were no statistically significant differences ($p > 0.05$) between the mean plaque index before application and after application on Days 7, 14,

and 28; between the Days 7 and 28; and between Days 14 and 28. However, there was a statistically significant difference ($p < 0.05$) between Days 7 and 14.

The subjects' plaque index values were examined using the modified Loe and Silness method. Figure 2 presents the average results of the plaque index in children who received CPP-ACP containing propolis (n = 16). The highest average was seen at the initial examination before the application of CPP-ACP containing propolis, which was equal to 1.79. The lowest average plaque index was seen after the application on Day 28, which was equal to 0.97.

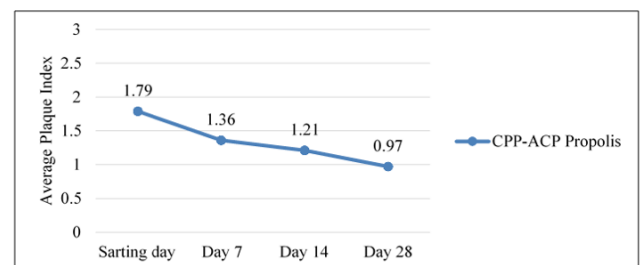


Figure 2. Average plaque index before and after application of casein phosphopeptide-amorphous calcium phosphate containing propolis.

The results of the plaque index data normality test in the application group of CPP-ACP containing propolis indicated $p > 0.05$ at the initial examination and each evaluation thereafter, which means that the data were normally distributed. Furthermore, hypothesis testing was conducted using a parametric test—namely, the GLM repeated measures approach—to determine the differences in plaque index between before and after CPP-ACP propolis application.

Table 1 describes that there was a significant decrease in plaque index after application with CPP-ACP containing propolis ($p < 0.05$).

The pairwise comparison test was used to elucidate which groups of plaque index data demonstrated a significant difference, the results of which are shown in Table 2. It was found that there were statistically significant differences ($p < 0.05$) before application and after CPP-ACP propolis on Days 7, 14, and 28; between Days 7 and 14; between Days 14 and 28; and between Days 14 and 28.

Plaque Index	Mean (SD)	p-value
Baseline	1.79 (0.59)	0.01
Day 7	1.36 (0.48)	
Day 14	1.21 (0.53)	
Day 28	0.97 (0.51)	

Table 1. GLM Repeated measures test results in the CPP-ACP propolis application group.

Examination		Mean Difference	p-value
Baseline	Day 7	0.43	0.00
	Day 14	0.59	0.00
	Day 28	0.82	0.00
Day 7	Baseline	-0.43	0.00
	Day 14	0.15	0.01
	Day 28	0.39	0.00
Day 14	Baseline	-0.59	0.00
	Day 7	-0.15	0.01
	Day 28	0.23	0.00
Day 28	Baseline	-0.82	0.00
	Day 7	-0.39	0.00
	Day 14	-0.24	0.00

Table 2. Pairwise comparison results between before and after the application of CPP-ACP containing propolis.

The comparison of mean plaque index in the CPP-ACP group containing propolis and the CPP-ACP-only group after 28 days of application is shown in figure 3.

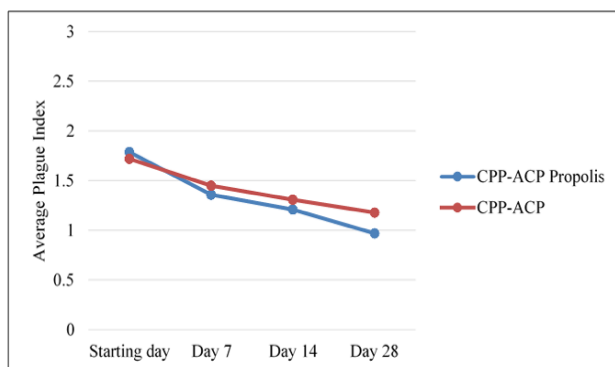


Figure 3. Comparison of the average plaque index.

The results of an independent t-test, seen in Table 3, indicated that there were no

statistically significant differences ($p > 0.05$) in the average plaque index between before and after application on Days 7, 14, and 28. Both groups experienced a decrease in the average plaque index after application. In the CPP-ACP application group containing propolis, the average plaque index at the start of the examination was 1.79, decreasing to 0.97 on Day 28. In the CPP-ACP application group, the average plaque index at the beginning of the examination was 1.72, decreasing to 1.18 on Day 28.

	Mean			
	Baseline	Day 7	Day 14	Day 28
CPP-ACP only (n = 16)	1.72 (0.69)	1.45 (0.39)	1.31 (0.42)	1.18 (0.53)
CPP-ACP with propolis (n = 16)	1.79 (0.59)	1.36 (0.48)	1.21 (0.53)	0.97 (0.51)
p-value	0.73	0.58	0.54	0.26

Table 3. Results of independent analysis of t-test regarding the average comparison of plaque indexes.

In addition, to analyze the difference in average plaque index decline taken from both groups, a comparison of the difference in the initial plaque index between before and after application on Day 28 was implemented.

The results of the normality test indicated $p > 0.05$, which means that the data were normally distributed. The average decreases in plaque index with the application of CPP-ACP only was 0.53, and CPP-ACP with propolis was 0.82, respectively. Table 4. indicates that there were no statistically significant differences ($p > 0.05$) in decreasing the mean of plaque index between CPP-ACP only group compared to CPP-ACP propolis group.

Discussion

The results of this study indicated that a decrease in the average of dental plaque index occurred between before and after CPP-ACP application. After conducting statistical analysis using the GLM repeated measures test, which was followed by the pairwise comparison test, it was found that there was no significant difference between the highest mean before the application and at the point after the application that had the lowest average plaque index. However, there

was a decrease in the average plaque index between at the time before the application and after the application, from 1.72 to 1.18 over a period of four weeks. This could have occurred due to the involvement of casein (in the form of CPP) in CPP-ACP with the ability to influence the process of forming dental plaque.⁶

Dental plaque forms due to the bond of bacteria and salivary pellicles. In the process of dental plaque formation, which occurs during the initial colonization of bacteria, bacterial cells will attach to the salivary pellicles that line the tooth surfaces through bacterial receptors on the salivary pellicles. More specifically, the bacteria can attach to the tooth surface in this manner because of the bond between the protein matrix of the extracellular polysaccharides and the bacterial receptor on the pellicles. CPP-ACP, through its casein content in the form of CPP, has the ability to inhibit bacterial metabolism. CPP could break the bonding structure between the bacterial polysaccharide protein matrix and bacterial receptors on the pellicle. CPP is able to break this bond and release the attachment of bacteria from the tooth surface. The CPP will then bind to the salivary pellicle receptors, resulting in a bond between CPP and the salivary pellicles. This is how CPP inhibits the presence of dental plaque-forming bacteria.⁶

The results also indicates a decrease in the mean plaque index existed between before and after the application of CPP-ACP with propolis. Based on the results of statistical analysis using a GLM repeated measures test followed by a pairwise comparison test, there was a statistically significant difference between the highest average plaque indices between before the application and after the application. The lowest average plaque index was shown at the time of the third evaluation—namely, after the application of CPP-ACP with propolis for four weeks. A decrease in plaque score could occur due to the antimicrobial activity of propolis against most microorganisms inside the mouth.^{4,7}

The formation of dental plaque is a biological process associated with the attachment and proliferation of oral bacteria to the pellicle protein on tooth surfaces. The main bacteria that participate in the formation of dental biofilms include *S. mutans*, *S. mythic*, *S. sanguis*, *S. sobrinus*, and *Lactobacillus casei* [8]. These

bacteria produce an extracellular enzyme called glucosyltransferase (GTF), which can convert sucrose into extracellular polysaccharides called glucans.⁹ Glucans play roles in the early stages of colonization and accumulation of cariogenic microorganisms as well as contribute to biofilm formation. Glucose formation in dental plaques mediates the binding of *S. mutans* and *S. sanguis* to each other and to other bacteria.¹⁰ Therefore, inhibiting the activity of GTF is important in preventing dental plaque formation.

In propolis, there are more than 300 components, most of which are composed of phenolic compounds such as flavonoids, aromatic compounds, terpenes, and essential oils. Flavonoids and derivatives of cinnamic acid are considered to be the main active biological components.¹¹ Most of the biological activities of propolis come from the flavonoid content. The high flavonoid content in propolis has an antibacterial role, especially inhibiting the growth of bacteria in the oral cavity.⁹

Flavonoids in propolis contain apigenin and tt-farnesol, which could inhibit the process of plaque formation. The mechanism of apigenin in inhibiting plaque formation acts by inhibiting the activity of the GTF enzyme in *S. mutans* which then inhibits the formation of extracellular polysaccharides by bacteria. Meanwhile, tt-farnesol has a high antibacterial ability to inhibit the growth and metabolism of *S. mutans* by disrupting the formation of bacterial membranes.⁹

In contrast with CPP-ACP, in preventing plaque formation, propolis plays a role by inhibiting the activity of GTF,¹⁰ while CPP-ACP, through its casein in the form of CPP, breaks the bonding structure between bacterial polysaccharide matrix proteins and bacterial receptors in the pellicle, resulting in a bond between CPP and salivary pellicles.¹² Based on the results of this study, a combination of CPP-ACP and propolis could prevent plaque formation, which is indicated by a decrease in the plaque score after application for four weeks.

Both groups in this study experienced a decrease in the average plaque index after four weeks of application. In the CPP-ACP with propolis application group, the average plaque index at the beginning of the examination was 1.79, which then dropped 46% to 0.97 by the

third evaluation in the day of 28th. Whereas, in the CPP-ACP–only application group, the average plaque index at the beginning of the examination was 1.72, dropping 31% to 1.18 at the third evaluation in the day of 28th. Based on the results of this study, there were no statistically significant differences in the mean plaque index between before and after application on Days 7, 14, and 28.

The decrease in plaque score in the CPP-ACP with propolis application group showed a higher rate than that in the CPP-ACP–only group, with averages of 0.82 and 0.53, respectively. This might have occurred because of the antibacterial properties contained in the flavonoid and tt-farnesol found in propolis. However, the difference in the decrease in the average plaque index also did not show a statistically significant difference.

Conclusions

In Conclusion there was no significant difference ($p > 0.05$) in the effect of the application of CPP-ACP contain propolis (10%) compared to CPP-ACP without propolis in dental plaque development. Thus, the CPP-ACP containing propolis could be an alternative to prevent dental plaque development in enamel surface as dental caries risk prevention.

CPP-ACP with propolis might be used as an alternative ingredient able to reduce the index of plaque to prevent the occurrence of dental caries.

Acknowledgements

The study received an international publication academy grant from Universitas Indonesia.

Declaration of Interest

The authors report no conflict of interest.

References

1. Sanghani NN, Shivaprasad BM, Savita S. Health from the hive: Propolis as adjuvant in the treatment of chronic periodontitis - A clinicomicrobiologic study. *J Clin Diagnostic Res.* 2014;8(9):ZC41-ZC44.
2. Santiago KB, Piana GM, Conti BJ, et al. Microbiological control and antibacterial action and antibacterial action of propolis-containing mouthwash and control of dental plaque in humans. *Nat Prod Res.* 2018;32(12):1441-1445.
3. Listyasari NA, Santoso O. Inhibition of the dental plaque formation by toothpaste containing propolis. *Dent J.* 2012;45(4):208-211.
4. Hajishengallis GN, Apostolopoulos AX. [Glucosyltransferase (GTF) and immunization against dental caries in humans]. *Odontostomatol Proodos.* 1989;43(4):315-321.
5. Reynolds EC. Calcium phosphopeptide complexes. Google Patents. August 24, 2004.
6. Jafer M, Patil S, Hosmani J, et al. Chemical plaque control strategies in the prevention of biofilm-associated oral diseases. *J Contemp Dent Pract.* 2016;17(4):337-343.
7. Nordstrom M. Oral Hygiene Indices. Malmö University. 2018.
8. Newman M, Takei H, Klokkevold P, Carranza F. Carranza's clinical periodontology. 12th ed. St. Louis, Missouri: Elsevier Saunders; 2015:904.
9. Marsh PD. Microbiology of dental plaque biofilms and their role in oral health and caries. *Dent Clin North Am.* 2010;54(3):441-454.
10. Libério SA, Pereira ALA, Araújo MJAM, et al. The potential use of propolis as a cariostatic agent and its actions on mutans group streptococci. *J Ethnopharmacol.* 2009;125(1):1-9.
11. Krell R. Value-added products from beekeeping. Vol. 2, Food and Agriculture Organization of the United Nations: Rome; 1996:136-162.
12. Andriani M, Titien I, Rantinah SB. The effect of application of topical casein phosphopeptide amorphous calcium phosphate (CPP-ACP) against alpha Streptococcus growth and accumulation of dental plaque. *J Ked Gi.* 2013;4(4):267-273.