

Association of Propolis Fluoride with Arrested Dentinal Caries and Dental Plaque Levels

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Abstract

It is estimated that 28.9% of children aged 5–9 have dental problems. However only 35.1% of these receive dental treatment. The study aimed to evaluate the effect topical propolis fluoride application for stopping the activity of dentinal caries, and to analyze the correlation between topical propolis fluoride application and plaque index. Convenience sampling was used to include 183 subjects age 7-9 year old, all student were performed dental examination to get diagnose of dentinal caries surface was measure by index DMF-S, while dental plaque examination was use disclosing solution applied in all tooth surfaces, All tooth surfaces had active dentinal caries were smeared with propolis fluoride 10%. The numbers of active dentinal caries and the plaque index were examined at baseline and follow-up evaluation after 1 month. In conclusion, there was a significant difference between the activity of dentinal caries before and after application of propolis fluoride, and there was also a significant but weak negative correlation ($p=0.015$ and $r=-0.177$) between the plaque index and the percentage of arrested dentinal caries. It showed that propolis fluoride could stop/arrest dentinal caries activity, while the lower plaque index could affect to the process of dentinal caries activity is getting slower.

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Introduction

Dental disease is common in Indonesia, with the prevalence of dental caries increasing from 43.4% in 2007 to 53.2% in 2013.^{1,2} The prevalence of active dental caries in Banten province has been reported to be 37.3%, while 61.2% of the population has a history of caries.³ According to the World Health Organization (WHO), dental caries are a major problem in industrial countries, affecting 60%–90% of school-age children and adults.⁴ The WHO has reported that 9 of 10 children aged 3–6 y in Indonesia had dental caries, while in 2016, Maharani et al. reported that 94% of children aged 6–7 y in the country had tooth decay.⁵

In 2013, the Ministry of Health of Indonesia stated that 25.9% of Indonesians had oral health problems in the preceding 12 months,

but that only 31.1% of those accessed treatment.¹ Moreover, 28.9% of children aged 5–9 y were reported to have oral health problems, among whom only 35.1% received treated.¹ Dental caries may not be treated for a variety of reasons, including financial and physical access barriers, effects on general health, inadequate social welfare, and poor child education.⁶ Untreated dental caries are becoming a pandemic.⁷ However, caries may become concerning when, if left untreated, they extend to the tooth pulp, cause an inflammatory process among the local blood vessels and nerves, leading to symptomatic dental necrosis.²

Dental caries represent a destructive process in which there is a loss of mineral ion from enamel, which in turn, is caused by the actions of pathogenic microorganisms in the presence of carbohydrate substrate.¹ Efforts to reduce the prevalence of caries has been undertaken in Australia, Japan, and Brazil with the use of antimicrobial fluoride varnishes, such as silver diamine fluoride (SDF).⁸ Studies have shown that SDF is effective at arresting the activity of dentinal caries in primary teeth.⁹ Indeed, SDF can effectively decrease demineralization caused by caries and can inhibit cariogenic dental biofilm growth.¹⁰ However, it

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can also cause a metallic taste and can alter the color of enamel, neither of which is desirable.⁹

A natural material, propolis, has become an alternative option to SDF for use in preventing the activity of dental caries. Propolis is a complex structure that has antiseptic, antibiotic, antibacterial, antifungal, and antiviral properties.¹² Specifically, streptococci are sensitive to propolis, which inhibits bacterial cell division by inhibiting protein synthesis and by destroying the bacteria cell membrane, causing lysis. Its flavonoid content also contributes to its antibacterial properties. Propolis has proven efficacy in decreasing gingival inflammation, mouth ulcers, and other oral problems.¹¹ Moreover, it can prevent dental plaque formation by inactivating the glucosyl transferase enzyme produced by *Streptococcus mutans* to catalyze glucan from sucrose.^{11,13} Glucan plays a role in the adhesion and accumulation of streptococci on dental surfaces, and thus, in forming dental plaque.¹³ The hesperidin content of propolis can also inhibit dentin degradation in situ.^{11,14} In dentistry, propolis has been used to heal surgical, skin, and socket wounds, to resolve dentin hypersensitivity, and for direct and indirect pulp capping.¹¹ A laboratory study in 2016 showed that the potential of propolis fluoride to inhibit *S. mutans* biofilms was comparable to that of 38% SDF.¹⁵

Research into the use of propolis for the treatment of dental caries is still limited. Therefore, this study was to evaluate the effects of topically applied propolis fluoride on dental caries activity, which is important for potential application of this pharmaceutical in clinical practice.

Materials and methods

This was a non-random experimental study, the respondent is the primary school student age 6-9 year old who were live in South Tangerang city. The Convenience sampling was used to identify first- and second-grade primary school children from SDN 01 Rawabuntu, South Tangerang. The study was approved by the research ethics committee of the Faculty of Dentistry of the University of Indonesia (number 66/Ethical Approval/FKGUI/VIII/2016) and conducted on September-November 2017).

All teeth were examined with dental instruments standard to obtain dental caries,

missing teeth because of dental caries and dental filling, to measure the severity of the the condition of dental caries disease using the def-s (decayed, extracted, filled - surface) index, def-s index is the index which use to count the total number of dental caries which found in tooth surface, while plaque index is instrument that used to assessing the number of dental plaque which sign red color by disclosing solution in tooth surface, total red color that found in tooth surfaces were count then divide to 28 tooth. The collected data were recorded on an odontogram record. Thereafter, propolis fluoride 10% was applied by a trained operator to the surfaces of dental caries, using a Microbrush[®]. Subjects were instructed not to eat or drink for 30–60 minutes and were given advice about maintaining their oral health. The dental plaque index and number of active dental caries were then re-evaluated after a month. Dental caries were evaluated with the aid of an explorer along the tooth surface on which the propolis fluoride had been applied. Arrested caries were characterized as having hard surfaces inside of dental cavity that were difficult to penetrate under mild pressure, while active caries were characterized as having soft surfaces that could be penetrated with light pressure.

The data were analyzed using the statistic computer software, and differences between the first examination and first propolis fluoride application as a baseline data was compared to second examination as an one month evaluation examinations were analyzed using Wilcoxon tests. The correlation of the plaque index with propolis fluoride success was analyzed using Spearman tests.

Results

The study included 183 children, and their age and sex are shown in Table 1. The prevalence rates of dental and dental caries were 93.6% and 90.6%, respectively.

As shown in Table 2, examination revealed that, on average, each child had a history of 16.28 caries, with the decay component (d-s) having the highest sub-score. Examination revealed that no child had received a restoration filling, so this was ignored. The total number of surfaces affected by active dental caries was 1144.

Table 3 shows that there was significant difference ($p = 0.001$) in the median number of active dentinal caries at baseline (4 surfaces/child) and after application of propolis fluoride for 1 month (1 surface/child). There was also a significant difference between the number of active dentinal caries at baseline (1144 surfaces), then after propolis fluoride application for one month shows that 811 surfaces (70.89%) were arrested, while 333 surfaces (29.11%) were active dentinal caries.

Gender	Male, n (%)	96 (52.5%)
	Female, n (%)	87 (47.5%)
Age (y)	Overall, (Mean \pm SD; range)	6.6 \pm 0.681; 5–8

Table 1. Participant Characteristics (n = 183).

Variable	N	Mean \pm SD	Median	Min–Max	Sum
d-s	183	9.35 \pm 6.34	8	1–35	1712
e-s	183	6.92 \pm 9.32	5	0–59	1268
def-s	183	16.28 \pm 11.36	14	1–64	2980

Table 2. History of Primary Dental Caries Surfaces in The Included Children.

Assessment	N (children)	N (surface units)	%	p-value (Wilcoxon test)
Baseline	183	1144	100	
Arrested surfaces	183	811	70.89	0.001
Active dentinal caries surfaces	183	333	29.11	

Table 3. Comparison of active dentinal caries and arrested dentinal caries from baseline to 1 month propolis fluoride application. * $p < 0.05$

Table 4 shows that there was a significant difference ($p=0.006$) between the median plaque index at baseline and after a month propolis fluoride intervention.

Table 5 shows the correlation between the plaque index and propolis fluoride retention in dentinal caries surfaces, the meaning is the retention of propolis fluoride effect to inhibit process dental caries activity. There was a significant negative correlation (inverse relation), the meaning is that the higher plaque index will accelerate the occurrence of dental caries activity. The correlation coefficient was -0.177 , can be interpreted that the increasing in the

plaque index of 1 unit was followed by the lower in the percentage (17.7%) of arrested dentinal caries reduction, although that relationship was significant ($p=0.015$).

Variable	N	Mean \pm SD	Median	Min–Max	Sum	p-value (Wilcoxon test)
Baseline	105	1.75 \pm 0.74	1.8	0.17–3	681	0.006
1 Month	105	1.60 \pm 0.62	1.6	0.33–3	204	

Table 4. Change in the Plaque Index from Baseline to 1 Month.

Variable	Correlation Coefficient	p-value (Spearman test)
Plaque index	-0.177	0.015

Table 5. Correlation of the Plaque Index with The Percentage of Arrested Dentinal Caries.

Discussion

The prevalence of dentinal caries exceeded 90% in this study of children aged 5–8 y. This age range reflects the period during which the first permanent teeth (molars or mandibular incisors) erupt.¹⁸ However, the child's oral cavity is still dominated by the deciduous teeth, which will be completely replaced by age 12, the topical use of fluoride preparations such as sodium fluoride topical application, pit fissure sealant use dental material release fluoride ion, silver diamine fluoride, etc.^{4,5,7,9,10}

There was a significant decrease 29.11% in Table 3 ($p < 0.05$) the median numbers surfaces of active dentinal caries at baseline compared to the median numbers surfaces after 1 month of single topical application of propolis fluoride use. This result was supported by the laboratory study, it was shown that the minimum concentrations content of propolis fluoride on *S. mutans* bacteria was achieved at propolis extract concentrations of 10% and fluoride concentrations of 2.14%, and that there was no colony regrowth at these levels.^{15,16} In the present study the 70% of dentinal caries was significantly stop, probably due to the combined activity of propolis extract (10%) and fluoride (2.14%).¹⁶

Several mechanisms of action have been proposed for the anticariogenic properties of propolis. These include its antimicrobial activity against cariogenic bacteria and its ability to inhibit the activity of the glucosyltransferase

enzyme, which plays a role in the attachment of *S. mutans* and other bacteria on tooth surfaces before dental plaque formation.¹² Propolis also contains flavonoids that have antibacterial activity against *S. mutans*. Flavonoids can inhibit the division of bacterial cells by destroying the cytoplasm and bacterial cell membranes, causing lysis, as well as by inhibiting protein synthesis.^{14,16-17} In addition, *in vitro* studies using electron microscopy have proven that, when applied to dentin, propolis can significantly reduce dentin permeability by plugging tubular dentin.¹²

Fluoride also confers protection against caries in several ways.^{4,5} It plays a role in slowing the progression of carious lesions by inhibiting demineralization, by increasing enamel resistance to acids, and by accelerating remineralization when it reacts with hydroxyapatite to form fluorapatite.^{4,5} The main anticariogenic effect of fluoride occurs topically on erupted teeth.^{4,5} The high levels of fluoride achieved with the use of topical gels or varnishes produce a temporary layer of material (i.e., calcium fluoride) on the enamel surface. Fluoride concentrated in plaque and saliva can inhibit the demineralization of hard tooth tissue,^{4,5,10} and along with calcium and phosphate, form a more acid-resistant crystalline structure.¹⁰ When the pH falls in response to the presence of dietary acid, fluoride is released and triggers remineralization.^{10,21,22} Fluoride has also been shown to inhibit the process by which cariogenic bacteria metabolize carbohydrates to produce acids.

In research by Koo et al., *in vitro* evaluation of the effects of propolis in combination with sodium fluoride in mice revealed that apigenin and *tt*-farnesol significantly reduced the virulence of *S. mutans*, enhancing the cariostatic effects of fluoride.^{14,22} According to laboratory research, in 2016, the potential of propolis fluoride to inhibit the formation of *S. mutans* biofilms was comparable to that of 38% SDF.¹⁶ The percentage efficacy of 38% SDF in Chinese research was reported that the activity of dentinal caries to be 70%–83% was good, depending on how SDF was applied in tooth.²³⁻²⁶ However, some studies suggested that one application only caused 50% of carious surfaces to have arrested progression at 6 months, with cases returning to active states by 24 months.^{8,9,24,27} In addition, it was stated that

SDF application was only 38% effective in stopping carious lesions, with its effectiveness decreasing over time.^{8,9} Reapplication may therefore be necessary to maintain an arrested state.²⁴ Application frequency after baseline has been recommended at 3 months and then every 6 months for 2 y.²⁴

The present results in Table 4 showed a significant decrease of median plaque index at 1 month after intervention with propolis fluoride from baseline plaque index. It can therefore be concluded that propolis fluoride was less effective at lowering plaque indexes than it was at arresting the progression of dentinal caries. However, this result is in line with the research conducted by Koo et al., who found that propolis can inhibit the activity of glucosyl transferase produced by *S. mutans*, which normally catalyzes the formation of glucan from sucrose. Thus, the availability of glucan is reduced, inhibiting the ability of streptococci to adhere to and accumulate on tooth surfaces.²⁶ The study also indicated that the apigenin content of one of the flavonoids in propolis may affect the potency of dental plaque associated with caries by reducing the synthesis of extracellular glucan.²⁶

According to description of Shah et al., topically applied fluoride can lead to fewer plaque accumulations, and ultimately, fewer dental caries.²⁷ It was also suggested that the preventive effect on the plaque index was associated with improved regularity of dental hygiene and not with topical fluoride application.²⁷ In the present study, there was a significant correlation between the plaque index and the percentage of arrested dental caries. The negative correlation coefficient meant that the higher the plaque index the lower on the percentage of arrested caries, and vice versa. This result was similar to that reported by Crystal et al. with the use of SDF, suggesting that individuals with high plaque indexes exhibit lower rates of arrest.²⁵ This may be attributable to the acid ions constantly produced in plaque from processed carbohydrates, effectively causing the remineralization process to lose its effectiveness in neutralizing demineralization.^{18,26,28,29}

Research suggests that children who frequently consume sugar-containing beverages, and who keep those drinks in their mouths for longer periods, are at increased risk of dental caries.²⁸⁻³⁰ One of the most effective ways of removing plaque is by brushing teeth. Based on

a study by Perinetti et al., there was a significant association between the habit of tooth brushing and the frequency of dental caries in children.²⁹ Other studies have suggested that there was a significant difference in the occurrence of caries in tooth enamel between teeth brushed twice daily with fluoride-containing toothpaste and teeth brushed daily.^{28,30} By addressing risk factors for caries, such as plaque, we may increase the rates of successful treatment.^{28,30}

Conclusions

In conclusion, the active dentinal caries on tooth surfaces were significantly stop from baseline to 1 month after single treatment with topical propolis fluoride. There was also a significantly relationship between the number of plaque index and the inhibition of dentinal caries activity by propolis fluoride. The research result can be recommended for use in preventing the severity of dental caries on the surface of dentin and enamel in the community pre-school and school children by dentist.

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Declaration of Interest

The authors report no conflict of interest.

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