An Invitro Study of Caries Arresting Effect of Propolis Fluoride and Silver Diamine Fluoride on Dentine Carious Lesions

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Abstract

According to Riskesdas, 25.9% of Indonesians have caries. Most are from the lower economic groups. Limitations of health facilities led to the need for treatments for caries that are easy to apply and affordable. The objective of the study was to compare the antibacterial and remineralization ability of propolis fluoride (Propolis fluoride) and silver diamine fluoride (SDF) on arresting caries of primary teeth. Methods using in the study was propolis fluoride and SDF materials were tested with the total plate count (TPC) method to determine their antibacterial ability. Observations using SEM and EDX were conducted to determine Propolis fluoride and SDF's remineralization ability. As the results, in the TPC method, Propolis fluoride has the ability to significantly decrease the growth of *Streptococcus mutans*. In the SEM method, the negative control group looked more porous than the positive control group. In the Propolis fluoride group, it appears that the demineralization porous areas are covered by a granulated layer of Propolis fluoride. It was concluded that propolis fluoride has major potential to be an alternative to SDF for arresting dentinal caries.

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Introduction

According to the Indonesian Ministry of Health of Health (2013), tooth and oral disease are significant problems among Indonesians. The DMF-T Index among Indonesian reached 4.6, with caries prevalence rising from 23.2% in 2007 to 25.9% in 2013.[1,2] Although primary teeth are replaced by permanent teeth, primary teeth still need to be taken care of. A few functions of primary teeth are supporting phonetic and masticatory function, and also guiding the eruption of permanent teeth.[3] Sadly, according to the Ministry of Health (2013), 28.9% of children aged 5–9 suffer from tooth and oral diseases, and only 35.1% are treated.[1]

There are some alternatives in preventing progression of dental caries, one of which is the application of silver diamine fluoride (SDF) on dental cavities. SDF has been widely used in Australia, Japan, and China.[4] The silver content in SDF has an antibacterial action and thus prevents bacterial growth on the applied surface.[5] The fluoride content of SDF has the ability to form fluoroapatite, which has higher resistance than hydroxyapatite toward acid challanges.[5] Eventhough it has excellent antibacterial ability, the silver content of SDF has some undesired side effects, such as causing black discoloration on the applied surface and causing an unpleasant metallic taste in the mouth.[6]

Some herbal products have been proven to have antibacterial abilities,[7] one of which is propolis.[10] Propolis is one of many herbal products that possesses antibacterial properties.[8] According to research, propolis from Indonesia was proven to be safe for human application. The mechanism of propolis as an antibacterial agent is by interfering with bacterial cell membranes and cytoplasms, and suppression of DNA

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synthesis.[8-10]

This research aims to compare the ability of propolis in conjunction with fluoride with commonly used SDF as a caries arresting agent. Propolis fluoride can be an alternative to SDF that does not possess SDF's previously stated undesired side effects. Until now, there has been little to no research on propolis fluoride, let alone their cariostatic ability. Therefore, further research about propolis fluoride is needed.

Methods

This research was divided into two studies: an vitro study to compare the antibacterial and remineralization properties of propolis fluoride and SDF, and an in vivo study to compare the ability of propolis fluoride and SDF on arresting dental caries on primary teeth.

A comparison of antibacterial properties was done using the total plate count (TPC) method. Solid and liquid TYS20B agar media was made as the place for bacteria to grow. After the medium was successfully made, 0.75mL of liquid media was injected into 21 different tubes. Seven were injected with 0.75mL of Propolis fluoride, seven were injected with 0.75mL of SDF, and seven had nothing added. To each tube, one colony of *Streptoccocus mutans* was added. Then, those tubes were incubated for 2d. After 2d, mixtures from each tube were inoculated in 21 different plates containing solid agar media. After 2d of incubation, the number of bacteria colonies from each plate was counted using a colony counter. To minimise contamination, every procedure was done inside a class IIA biosafety cabinet.

Comparison of remineralization properties was done qualitatively using scanning electron microscopy (SEM) and quantitatively using electron dispersive X-ray (EDX). Four first maxillary premolar teeth without abrasions, caries, and erosions were prepared. One premolar was used as a positive control, one premolar was used as a negative control, one premolar had SDF applied, and the last premolar had Propolis fluoride applied. The positive control was soaked in water. The negative control was demineralized using acetic acid for 2 d. The SDF tooth was demineralized beforehand using acetic acid for 2d, followed by application of SDF for 2d. The Propolis fluoride tooth was also demineralized for 2 d prior to the application of Propolis fluoride. After all the treatments were done for each tooth, all teeth were analysed with SEM and EDX.

To compare the ability of Propolis fluoride and SDF on arresting dental caries, an in vivo study was conducted with 167 children aged 6–7. The data for thein vivo study were secondary data. Then, the data were analysed by SPSS 22 using the Mann-Whitney U test, Wilcoxon test, and Chi square test.

Table 1. Comparison of bacteria counts among Propolis fluoride, SDF, and control groups.

Bacteria Count (CFU/mL)			***P-value* (Mann-Whitney U Test)**		
Propolis fluoride	SDF	Control	C Vs Propolis fluoride	C Vs SDF	Propolis fluoride Vs Propolis fluoride
0.531	0.184	65.503			
0.352	0.173	56.387			
0.324	0.149	55.481			
0.271	0.123	49.764	0.001	0.001	0.109
0.110	0	45.002			
0.089	0	42.535			
0.075	0	40.235			

C=Control

Results

Comparison of Bacterial Properties between Propolis fluoride and SDF

Evaluation was done 2 d after the bacteria were inoculated to the agar plate. The bacteria count decreased from the average of 50,701 in the control group to 0.250 CFU/mLin the Propolis fluoride group and 0.089 CFU/mL in the SDF group. When the Propolis fluoride and SDF groups were compared with the control group, the p-value was 0.001 in the Propolis fluoride and SDF groups. Both p-values are below 0.05 which was interpreted as a significant difference between the control and Propolis fluoride groups, and a significant difference between the control and SDF groups. When SDF and Propolis fluoride groups were compared, the p-value was above 0.05 which means that there isno significant difference between Propolis fluoride and SDF groupsin terms of their antibacterial ability.

Comparison of Remineralization Ability between Propolis fluoride and SDF

SEM was done in Laboratorium Pusat Forensik POLRI. The result was a micrograph photo of each premolars' surfaces.

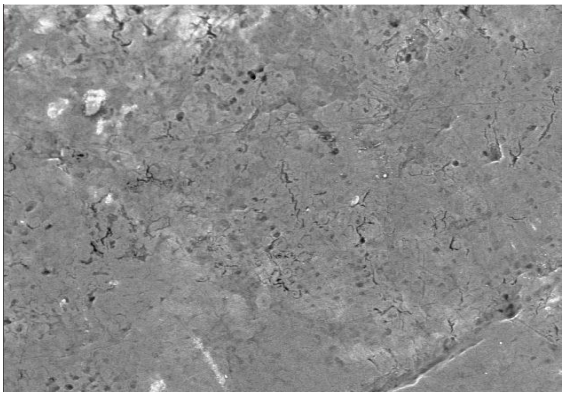

Figure1

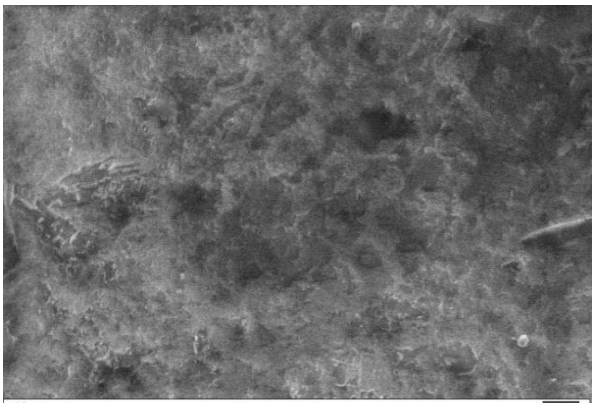

Figure 2

Figure 1, 2: Micrograph photo of premolars' surfaces; 1. Micrograph photo of positive control. 2. Micrograph photo of negative control.

Figure 1 shows enamel surfaces of the positive control. Radiopaque opacity was evenly distributed in the tooth surface, which shows the dense crystalline structure of the teeth before being demineralized. Figure 2 shows the enamel surface of the negative control, which was demineralized using acetic acid 98% for 2 d. Radiolucency was visible throughout the tooth surface, which indicated the loss of crystalline structure of the tooth surface after the demineralization process.

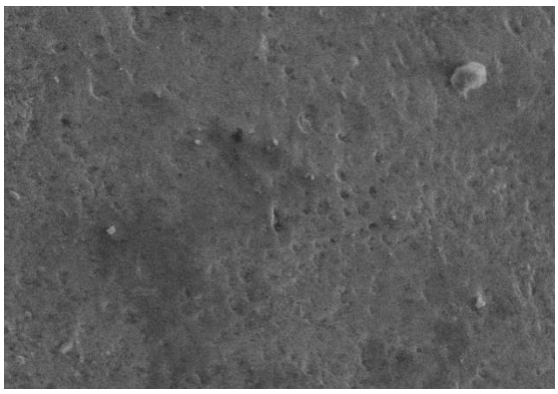

Figure 3

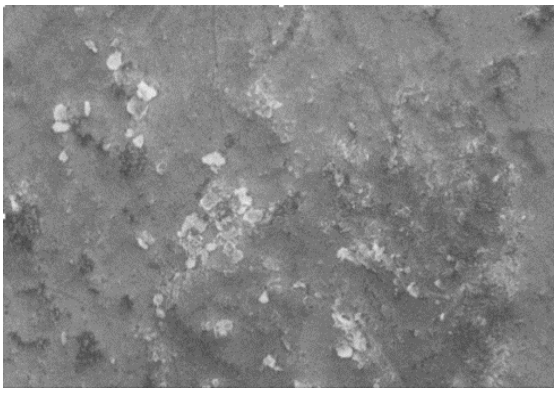

Figure 4

Figure 3,4: Micrograph photo of premolars' surfaces. 3. Micrograph photo of enamel surface after Propolis Fluoride application. 4. Micrograph photo of enamel surface after SDF application

Figures 3 and 4 are micrograph photos after the remineralization process using Propolis fluoride and SDF for 2 days. After remineralization, premolar surfaces that had Propolis fluoride and SDF applied showed evenly distributed opaque colours on the micrographs and a decrease in radiolucency, indicating an increase in mineral density compared to the negative control specimen.

Electron Dispersive Xray (EDX) analysis was done in the centre of Police forensic laboratory. EDX analysis shows the mineral content of the premolar surfaces. The focus of the analysis was the fluorine content in the enamel to understand Propolis fluoride and SDF's ability to precipitate fluorine on the enamel surface (Table 2).

Tabel 2. Mineral Content of enamel surface on control and treatment group.

Unsur	% percent		
	Negative Control	PpF	SDF
Carbon	6.19	6.81	7,46
Oxygen	28.42	14.62	27.07
FLUORINE	***5.35***	***21.19***	***23.43***
Sodium	0.37	0.75	0.62
Magnesium	0.33	0.23	1.01
Phosphor	13.32	11.69	8,33
Chlorine	0.54	0.49	0.40
Calcium	35.11	37.25	30.60
Silver	0	0	1.04

Based on EDX analysis, both PpF and SDF groups exhibited increases in flourine content, from 5.35% in the negative control group to 21.9% in the PpF treatment group and 23.43% in the SDF treatment group.

Discussion

Based on the study, PpF and SDF can decrease bacteria growth significantly from 50,701 CFU/mLin the control group to 0.250 CFU/mL in the PpF group and 0.089 CFU/mLin the SDF group. SDF has superior antibacterial ability to PpF's; this can be caused by the concentration of active material content in SDF that reaches 38%, as oppose to PpF, which only contains 10% active material. Another cause of PpF's inferior antibacterial ability is the unstable nature of the PpF mixture. Nevertheless, based on the Mann-Whitney U test, there is no significant difference between SDF and PpF's antibacterial ability ($p>0.05$). SDF's antibacterial property is mainly due to silver. SDF can inhibit biofilm formation, especially *Streptococcus mutans* and *Actinomycetes Naeslundii*.[11] *S. mutans* is known to be the most important cause of caries initiation.[11-12] However, *A.naeslundii* plays an important role in collagen degradation on dentin caries. SDF 38 can inhibit 3 MMPs in dentin. MMP-8 (neutron filkola genase) can degrade triple-helical fibrilar collagens into three-quarter and one-quarter fragments. MMP-2 and MMP-9 are gelatinase, which cause collagen type IV degradation.[13] SDF and $Ag(NH_3)_2$ F can react with hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, to produce calcium fluoride, CaF_2, and silver phosphate, Ag_3PO_4, which will protect tooth structures by forming a black impermeable layer.[14] This layer can cover dentin tubules and lower loss of calcium and phosphor loss from demineralization. Silver can inactivate GTF enzymes, which prevent the formation of biofilm.[15]

Propolis works by disrupting bacteria membranes and cytoplasm, causing partiallysis of bacteria membrane, and inhibiting bacteria

protein synthesis.[16] Propolis's antibacterial role was played by flavonoid content within propolis itself. Flavonoid compounds in propolis have been proven to reduce gingivitis, stomatitis, and other oral problems.[17] Propolis can inhibit the formation of biofilm by inactivation of glucosyl transferase (GTF). GTF is an enzyme produced by *S.mutans*, and catalyses production of glucan from sucrose. Glucan plays an important role in bacteria adhesion and accumulation, and thus the formation of dental biofilm.[18] Beside that, hesperidin content of propolis has also been proven to obstruct collagen degradation in dentin.[19] According to Graça (2011), propolis can cause a few side effects, such as contact dermatitis and oral mucositis caused by the 3-methyl-2-butenyl caffeate and phenylethyl caffeate compounds. However, these compounds cannot be found in propolis produced in tropical areas.[17] Besides its remineralization ability, fluoride has antibacterial properties.[20] Fluoride can disrupt bacteria metabolism through glycolysis inhibition[21] by interrupting enolase enzymes to decrease piruvate and ATP synthesis, causing a drop in lactic acid production.[21,21,23] Fluoride can prevent bacteria adhesion to tooth surfaces by decreasing surface tension between bacteria and host surfaces, via calcium channel competition.[24]

Negative control specimens demineralized using acetic acid show the destruction on their crystalline structures. This structural damage was translated as an increase in radiolucency on the micrograph. This radiolucency structure is caused by the increase of surface porosity. On the remineralised specimens using PpF or SDF, the micrograph from each specimen appeared more radiopaque compared to the negative control specimen. This increase in radiopacity is caused by the escalation of fluoride content in the tooth surfaces. This escalation showed that remineralization processes do occur within the specimen after the application of SDF and PpF.

Based on EDX analysis, fluorine content within PpF- and SDF-applied specimens was shown to be escalated compared to the negative control. This increase in fluorine content demonstrated that both SDF and PpF can precipitate fluorine into the tooth structure. Fluorine content within SDF and PpF mixtures is able to obstruct demineralization by its ability to form fluoroapatits, which have higher tolerance to acid challenges (lower critical pH than hydroxyapatite).[25,26] Fluoride can also act as an intraoral reservoir after being topically applied and released slowly through saliva.[27] Fluoride can remain on the oral hard tissue, teeth, oral mucosa, and dental plaque.[27] Fluoride retention within dental plaque can lower the demineralisation process, especially when the pH level has dropped.[27] The lethal dose for fluoride itself is 0.1–0.3 mg/kg body weight.[27]

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