The effect of acidulated phosphate fluoride (APF) gel against erosion on enamel microstructure of primary teeth which soaked with Citrus aurantifolia

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ABSTRACT

Introduction: Erosion is a common dental problem that is especially prevalent in children due to the increasing consumption of acidic food and beverages. APF gel is believed to be effective in reducing the demineralization effect of the teeth as well as escalating the remineralization process. This research aims to determine and evaluate the effectiveness of 1.23% APF gel as a preventive agent for the erosion of tooth enamel, specifically in deciduous teeth. **Methods:** This type of research is true experimental research. The population of this research would be primary teeth extracted from children, using a purposive sampling technique with inclusive and exclusive criteria. A total amount of 5 maxillary primary central incisors were extracted and cleaned. APF gel was applied repetitively on the left side of the teeth for 4 minutes every hour, whereas the right side was left as it is. Samples were then analyzed using a scanning electron microscope (SEM). A qualitative and subjective analysis regarding the micromorphological features of the erosion can be done. **Result:** The side of teeth without APF gel application has a greater erosion rate compared with teeth with APF gel application. The tooth which is demineralized shows a honeycomb structure of the interprismatic enamel. As for the dentinal tubules, teeth without APF gel application have a bigger opening of dentinal tubules, going up to 7.868 micrometers in size compared to teeth with APF gel application (1-2 micrometers). **Conclusion:** The application of professional acidulated phosphate fluoride (APF) gel on deciduous teeth even when soaked in Citrus aurantifolia, is proven to be effective in preventing dental erosion.

Keywords: acidulated phosphate fluoride (APF) gel; citrus aurantifolia; dental erosion; primary tooth

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INTRODUCTION

Fluoride is a natural mineral that can be found naturally in soil, food, and water. In some countries, a small amount of fluoride with a concentration of 1 part per million (1 ppm) is added to public water supplies.[1] Since the introduction of water fluoridation, fluoride supplements, and topical fluoride have been introduced in the market and have achieved a significant level of popularity

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among the public in preventing and controlling dental caries.[2] Professional acidulated phosphate fluoride application is one of the most well-known methods used for dental caries prevention. It was introduced in the form of a solution or gel in the 1960s and can be applied with a paint-on technique whereby the fluoride material is applied on the tooth with a cotton applicator or brush or applied with the tray technique in which a small amount of fluoride material is added to a tray and then inserted in the patient's mouth.[3]

The efficacy of professional acidulated phosphate fluoride is recognized from an evidence-based perspective as it has been convincingly demonstrated that the recent decline in caries prevalence is primarily attributed to the increased use of professional acidulated phosphate fluoride application.[4] Clinical trials have also shown that the use of acidulated phosphate fluoride (APF) gels containing 1.23% fluoride (F-) on a twice-annual basis is effective in preventing dental caries. The landmark Cochrane review, published in 2002 and based on 25 studies involving over 7,000 children, displayed a prevented fraction of caries of 28%. A recent study by the American Dental Association Council on Scientific Affairs has also concluded that the application of fluoride gel is effective in preventing caries in school-aged children.[5] Based on Izzudin's research, the application of 1.23% APF gel on high viscosity GIC reduces the microhardness level compared to the control group.[6]

However, besides being a carious agent, there are evidence of the superiority of APF gels containing similar concentrations of fluoride ions concerning the uptake and penetration depth of fluoride ions into the enamel and their ability to protect against highly acidic challenges to tooth surface enamel.[7] The exposure of the enamel and dentin to nonbacterial acidic dissolution to tooth surface enamel causes a localized loss of tooth surface. This is known as dental erosion. Dental erosion can be caused by either intrinsic or extrinsic factors. The most common source of intrinsic acid is hydrochloric acid produced by the stomach, frequently observed in patients with bulimia or alcoholism which promotes chronic vomiting. On the other hand, extrinsic factors include excessive intake of acidic beverages and snacks which is the most common cause of dental erosion.[8]

Due to the dramatically increased consumption of acidic beverages such as citrus drinks in children and adolescents, erosive tooth wear or dental erosion has gained more attention from the dental profession.[9] In 2000, according to the National Diet and Nutrition Survey in the UK, 58% of 4-6-years-old and 42% of 11-14-years-old were affected by dental erosion. In another research, the erosion problem in Saudi Arabia was reported to be 34% and 26% of children and adolescents, respectively, and an overall 47% were reported to be affected in Ireland.[10]

According to some in vitro studies, the role of fluoride in the prevention of erosion is has been reported to be a limited erosion-inhibiting effect from the application of topical professional acidulated phosphate fluoride (APF) gel[10], however it has been proven that the application of fluoride can inhibit the demineralization process as well as promote the remineralization process of the tooth structure, and the application of fluoride has been suggested as a treatment option in the prevention of erosion. To further observe the effect of fluoride on a tooth that is soaked in Citrus aurantifolia, a scanning electron microscope (SEM) is used. With this method, a qualitative and subjective analysis regarding the micromorphological features of the erosion can be done.[11] Features of erosion under SEM include enamel prism dissolution, enamel porosity, intercrystalline space, and opening of dentinal tubules.[12] The purpose of this research is to describe the micromorphological structural change of primary tooth after being soaked in citric fruit when applied with 1.23% phosphate fluoride (APF) gel.

METHODS

The inhibitory effect of 1.23% acidulated phosphate fluoride (APF) gel against tooth erosion was done on 5 primary maxillary central incisors. The population of this research would be primary teeth extracted from children. The purposive sampling technique was used, in which the inclusive criteria included sound tooth of primary maxillary central incisors free from caries, discoloration, or defects such as enamel hypoplasia, and white spot lesion or abrasion whereas the exclusive criteria were fractured, eroded, or abraded tooth. They were labeled A, B, C, D, and E. All labial parts of the

tooth were divided into two parts by drawing a fine line on the crown (labial) of the teeth using a permanent marker. The right side of the labial part of the teeth for all samples was applied with APF gel for 4 minutes every hour for 12 hours. However, for samples A, B, and C, the left side of the crown was left untreated whereas, for samples D and E as control positive, the left side of the crown was applied with acid-resistant nail varnish instead. This procedure is done for 12 hours to see how the effect of consuming citric food or beverages in large amounts frequently will cause erosion of the teeth of individuals.

The effect of 1.23% APF gel against erosion, these teeth were soaked in 10 mL of lime extract of 20% concentration for 12 hours and then examined using a scanning electron microscope (SEM). SEM is used to observe and analyze the microstructure of the tooth by comparing the difference in the morphological structure of the teeth between samples with the treatment of 1.23% APF gel and samples without the treatment of 1.23% APF gel after being soaked in the lime extract of 20% concentration. Lime (*Citrus aurantifolia*) extract was used as the fruit extract because it is among the lowest pH level with a pH of 2.71 as compared with other citric fruits such as lemons and oranges. This type of research is a true experimental study and qualitative assessment. Ethical clearance of research has been approved by the Health Research Ethics Committee, Medical Faculty, Universitas Padjadjaran No. 83/UN6. C1.3.2/KEPK/PN/2016.

RESULTS

Figure 1 shows the microstructure of enamel which was protected with a layer of acid-resistant nail varnish when soaked in Citrus aurantifolia.

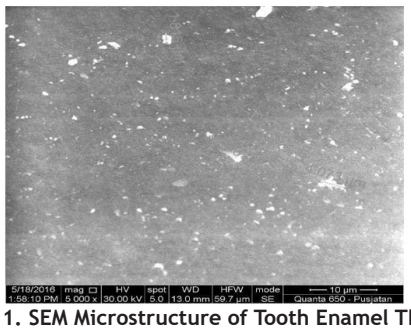

Figure 1. SEM Microstructure of Tooth Enamel That Did Not Undergo Erosion (applied with nail varnish) Visualized under SEM with A Magnification of 5000x (sample A).

The enamel surface is generally smooth and homogenous without any pits or opening of dentinal tubules showing that the tooth did not undergo any erosion.

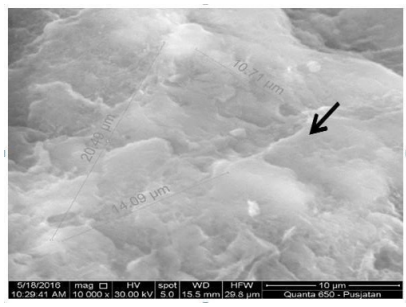

Figure 2. SEM Microstructure of Tooth Enamel after Being Soaked in Citric Fruit Extract (with the application of APF gel) Visualized under SEM with A Magnification of 10000x (sample B).

Figure 2 shows the image of the enamel surface slightly demineralized by soaking in the lime extract. Pits are obtained by chemical attack of the Citrus aurantifolia even when applied with acidulated phosphate fluoride of 1.23%. The distance from one enamel pit to the other is about 10.71 micrometers (Arrows).

Figure 3 shows the microstructure of the tooth enamel being demineralized after being

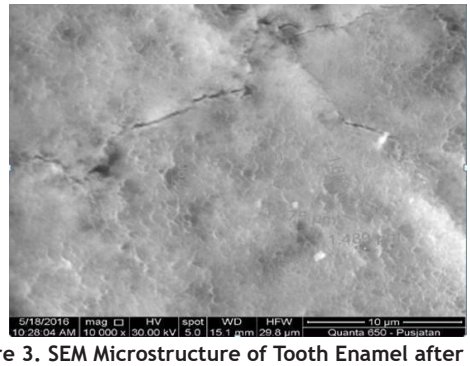

Figure 3. SEM Microstructure of Tooth Enamel after Being Soaked in Citric Fruit Extract (with the application of APF gel) Visualized under SEM with A Magnification of 10000x (sample C)

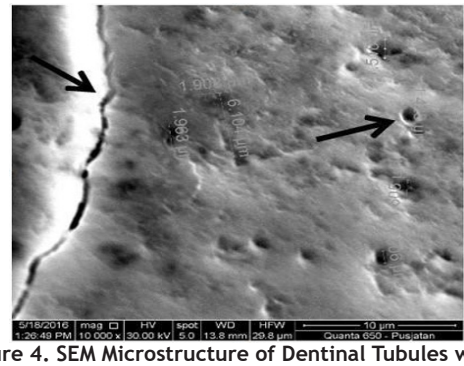

Figure 4. SEM Microstructure of Dentinal Tubules with A Magnification of 10000x (sample C).

soaked in citric fruit extract even when applied with acidulated phosphate fluoride (APF) gel of 1.23%. The honeycomb structure of the enamel as seen in the figure is formed due to the protrusion of the inter-prismatic enamel. This is seen only when a tooth undergoes slight demineralization. Figure 4 shows the opening of the small dentinal tubules due to the effect of erosion. The size of the dentinal tubules ranges from 1–2 micrometers. There is a crack line on the image of the microstructure of enamel as well probably due to the weak structure of the tooth (Arrows).

Figure 5 shows the microstructure of the tooth enamel of sample A in which the tooth is soaked in citric fruit extract without the treatment of acidulated phosphate fluoride gel whereby it undergoes pure erosion. In Figure 5. a under a magnification of 5000x, the enamel is fully eroded leaving only about 38.43micrometers of enamel left protruding on the tooth surface with the opened dentinal tubule of 7.868 micrometers. Figure 5. b, under a magnification of 1000x, it is seen that there are only small pits of enamel left on the tooth surface. There is some opening of

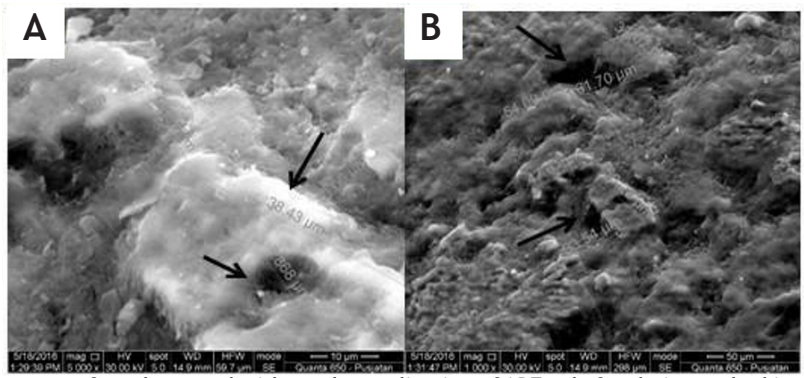

Figure 5. SEM microstructure of tooth enamel without the application of APF gel after being soaked in citric fruit extract with a Magnification of 5000x and b) 1000x (sample C).

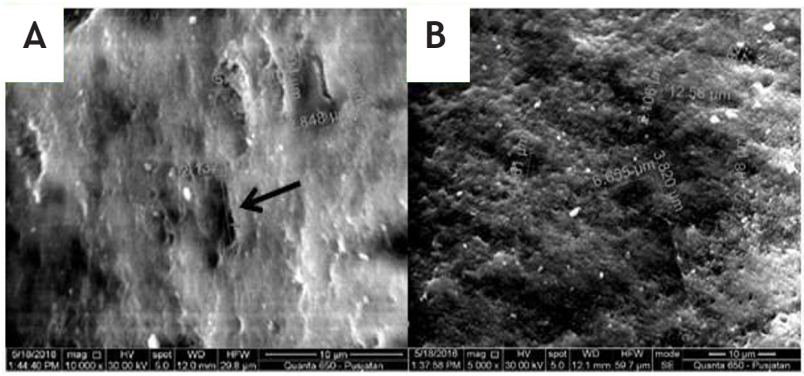

Figure 6. Comparison between the microstructure of the Tooth (a) with the application of 1.23% APF gel and (b) without the application of 1.23% APF gel after being soaked in citric fruit extract (sample D).

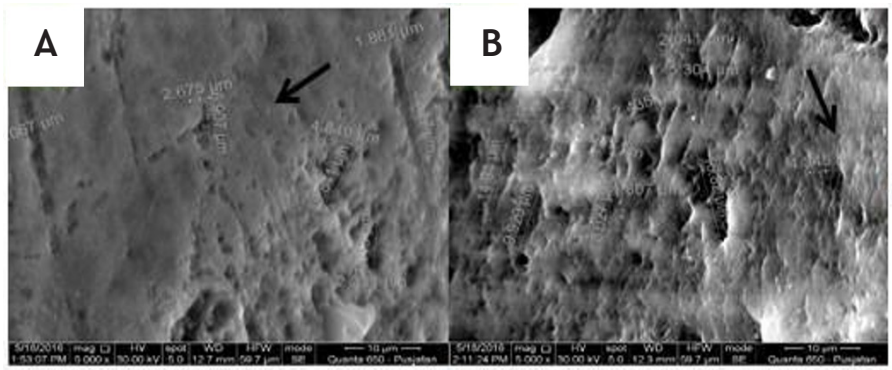

Figure 7. The Difference between the microstructure of The Tooth (a) applied with APF gel and (b) not applied with APF gel after being soaked in citric fruit extract (sample E).

dentinal tubules as well as closed dentinal tubules. This figure shows that the tooth is severely eroded when not applied with any fluoride treatment when soaked in Citrus aurantifolia.

Figure 6 shows the difference between the degree of erosion of the tooth structure with and without the treatment of acidulated phosphate fluoride gel. Figure 6. shows the enamel structure with the application of APF gel. In this figure, there is moderate erosion of the enamel with some openings of the dentinal tubules. The sizes of the dentinal tubules are about 2 micrometers. However, in Figure 6. b, we can see that the enamel surface is severely eroded as there is less enamel structure left on the tooth. Similar to the results shown in figure 6, the severity of erosion is greater in figure 7. b whereby they are not treated with APF gel than in Figure 7. a whereby they are treated with APF gel.

DISCUSSION

Based on the results obtained from the experiment, we can observe that there is a significant difference in the enamel and dentin microstructure of the teeth when applied and not applied with acidulated phosphate fluoride (APF) gel of 1.23% after being soaked in Citrus aurantifolia. Figure 3 shows a close-up image of chemically eroded enamel displaying the typical honeycomb structure. In enamel, acid attacks due to immersion of specimens in erosive solutions lead to surface etching patterns with an exposition of enamel prisms to an extent depending on the severity of the erosive challenge. Therefore, the typical honeycomb structure as seen in Figure 3 is usually only seen when the prismatic enamel is eroded, leaving the inter-prismatic enamel protruding showing that demineralization has occurred.[12]

Figure 4 shows the opening of the dentinal tubules due to the effect of erosion. The size of the dentinal tubules ranges from 1 – 2 micrometers. The openings of these dentinal tubules prove that this is an advanced lesion caused by demineralization of the tooth surface.[13]

Figure 5 shows the microstructure of the tooth enamel of sample A in which the tooth is soaked in Citrus aurantifolia without the treatment of acidulated phosphate fluoride gel whereby it undergoes pure erosion. Figure 5.a under a magnification of 5000x, the enamel is fully eroded leaving only about 38.43 micrometers of enamel left protruding on the tooth surface with the opened dentinal tubules of 7.868 micrometers. In Figure 5. b, under a magnification of 1000x, there are only small pits of enamel left on the tooth surface. There is some opening of dentinal tubules as well as closed dentinal tubules and the collagen matrix between them is eroded and demineralized. This figure shows that the tooth is fully eroded without any fluoride treatment of 1.23% APF gel when soaked in Citrus aurantifolia.

Similar to Figure 5, there is a difference in the microstructure of the teeth when applied with APF gel and without the application of APF gel. Figure 6 shows the comparison between the degree of erosion of the tooth structure with and without the treatment of acidulated phosphate fluoride gel. Figure 6 shows the enamel structure with the application of APF gel. There is moderate erosion of the enamel with some openings of the dentinal tubules.

The widths of the dentinal tubules are about 2 micrometers which is wider than the openings of dentinal tubules in figure 4. However, in Figure 6. b, when the tooth is left soaked in lime extract without any fluoride treatment applied to the tooth, it can be observed that the enamel surface is fully eroded. In enamel, the lesion primarily develops in the prism sheath areas, followed by the dissolution of prism cores. Eventually the prismatic areas are also affected and this stage of erosion is seen in Figure 6. However, due to the fact that the tooth is left untreated and soaked in lime extract for 12 hours, the erosive demineralization results in the exposure of an outer layer of the fully demineralized organic matrix as seen in Figure 6.b.[14]

In Figure 7, both sides of the labial part of the tooth underwent erosion. However, the severity of erosion without treatment of APF gel in Figure 7. a is higher as compared to the severity of erosion when applied with the treatment of APF gel in Figure 7. b. Figure 7. a shows the microstructure of the teeth with part of the enamel still intact but with moderate erosion as there still some dental enamel left on the tooth structure. However, when compared with Figure 7. b, the enamel is worn out with the wider opening

of dentinal tubules showing a more severed and advanced erosion.[15]

Based on the images obtained from the scanning electron microscope, the decrease in mineral loss observed on the structure of tooth which received APF gel treatment indicates that fluoride gel or varnishes such as APF gel is able to significantly inhibit erosion of the tooth surface. However, it does not completely stop erosion from happening but instead decreases the effect of erosion.[3,9,13] Erosion usually happens when the tooth is in contact with acidic food or beverages with a critical pH of 5.5 or lower. During erosion by acid and/or chelators, these agents interact with the surface of the mineral crystals, only after they diffuse through the plaque, the pellicle, and the protein/lipid coating of the individual crystals themselves. The direct attack by the hydrogen ion combines with the carbonate and/or phosphate releasing all of the ions from that region of the crystal surface leading to direct surface etching, therefore, leading erosion.[16]

In an in vitro study to look at the effects of pH and concentration of citric acids, malic acids, and lactic acids on enamel, numerical data and contour plots for each acid showed a similar pattern for increasing erosion with decreasing pH and increasing concentration and vice versa for decreasing erosion.[17] Nevertheless, when the samples are soaked in the lime extract of 20% concentration with a pH of 2.71, the samples undergo severe destruction of the enamel tooth structure whether the samples were treated with APF gel or untreated with APF gel. Although the severity of the erosion of the enamel tooth's structure is significantly less severe with the treatment of APF gel as compared with the samples without treatment of APF gel. This is due to the fluoride content in the APF gel helping in inhibiting the demineralization of the tooth enamel.

Many experiments using high fluoride dosages have demonstrated promising results in increasing enamel resistance and decreasing the rate of dental erosion development. Such protection against erosion may result from the formation of the CaF2 layer offering additional minerals to be dissolved during the erosive challenge before the subjacent enamel is attacked. Thus, more acidic and concentrated fluoride preparations, which form thicker CaF2 layers, offer a greater protection against dental erosion. Besides that, during an acid attack, the fluoride released from CaF2 deposit can be incorporated into the mineral by forming fluorapatite or fluoride-hydroxyapatite, resulting in a decreased susceptibility to further dissolution.[9,18]

Additionally, the fluoride content in the acidulated phosphate fluoride (APF) gel helps to prolong the contact time between fluoride and tooth surface since it attaches to the tooth surface in a thin layer for a long period (12 or more hours). This excess fluoride acts as a slow-release reservoir of fluoride and helps in remineralization when the pH of the plaque on the teeth starts decreasing.[2] In another research done by Badr[8], primary teeth shown from their results showed that APF gel provided the highest protective effect against erosive enamel loss compared to fluoride varnish and CPP-ACPF. This can be explained on the bases that the acidic pH of the APF gel may have etched the enamel surface and helped to increase the corporation of fluoride into the enamel. Another explanation may be that the free negative fluoride ions become more reactive in acidic media, thus enhancing the formation of CaF2 and therefore, the mechanism explained above can help in the remineralization of the teeth.

From Figure 1 to Figure 7, there is a significant difference between the microstructure of the primary maxillary central incisor when soaked in the lime extract of 20% concentration with and without treatment of acidulated phosphate fluoride (APF) gel, as well as enamel microstructure without enamel erosion. We can conclude that the application of APF gel does help in inhibiting dental erosion but they do not completely prevent it.[9]

With scanning electron microscopy (SEM), surface alterations after erosive attacks are qualitatively examined and estimated. Grading of the severity of surface alteration could be on individually adapted scales. In a journal done by Benedetto, the report states that SEM study and analysis provide a complete overview of the distinctive morphological characteristics and the microwear features of the dental wear lesions, clarifying their clinical and diagnostic presentations and possible significance.[2]

CONCLUSION

The application of fluoride (acidulated phosphate fluoride gel of 1.23 %) on tooth surfaces does help in preventing or decreasing the severity of erosion of the tooth but does not completely stop tooth erosion from happening.

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