Protective effect of three different fluoride pretreatments on artificially induced dental erosion in primary and permanent teeth

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Abstract: Objective: To assess the effect of acidulated phosphate fluoride gel (APF), sodium fluoride varnish (NaF) and casein phosphopeptide-amorphous calcium phosphate fluoride paste (CPP-ACPF) on the dental erosion produced by coca cola in primary and permanent teeth. **Design:** Sixty extracted human primary molars (n = 30) and young permanent premolars (n = 30) were used in this study. The coronal portion of each tooth was sectioned mesio-distally. Specimens were prepared by embedding the crown sections in acrylic resin blocks leaving the enamel surfaces exposed. Specimens were ground, polished and randomly assigned to one of three groups each of 10 according to the protective agent used: APF gel (1.23% F), NaF varnish (0.1%F), and CPP-ACPF paste (0.2%F). Half of the exposed enamel surface was protected with adhesive tape during the treatment of the remaining surface according to their group. Six daily demineralization–remineralization cycles of 5 minutes of immersion in a cola drink (pH 2.3) and 30 minutes in artificial saliva were conducted for 14 days. Surface Vickers Micro-hardness readings were recorded at baseline and 14 days later for both halves. Percentage surface microhardness reduction (%SMHR) was then calculated. Data were analyzed using ANOVA and Duncan's post-hoc test ($p < 0.05$). **Results:** All of the tested fluoride treatments were able to reduce erosive enamel loss in both primary and permanent groups. In primary teeth only APF gel showed significantly higher anti-erosive effect than both fluoride varnish and CPP-ACPF paste. In permanent teeth both CPP-ACPF paste and APF gel showed significantly higher protective anti-erosive effect than fluoride varnish. **Conclusions:** under the conditions of this study, all of the tested fluoride treatments were able to reduce erosive enamel loss in both primary and permanent teeth. Primary and permanent enamel substrates reacted differently to different fluoridated compounds. CPP-ACPF paste is a promising remineralizing material.

[Sherine B Y Badr[1], Mohamed A Ibrahim. Protective effect of three different fluoride pretreatments on artificially induced dental erosion in primary and permanent teeth. Journal of American Science 2010;6(11):442-451]. (ISSN: 1545-1003).

Keywords: dental erosion, fluoride varnish, fluoride gel, CPP-ACPF paste, microhardness, primary, permanent.

1. Introduction

Erosive tooth wear or dental erosion has gained more attention from the dental profession since the decline in dental caries in many industrialized countries. Dental erosion is a localized loss of the tooth surface by a chemical process of acidic dissolution of nonbacterial origin. [1]This process may be caused by extrinsic or intrinsic agents. Extrinsic agents include acidic foodstuffs, beverages, snacks and may also occur following environmental exposure to acidic agents.[2,3] One of the most common extrinsic factors that cause dental erosion is the excessive consumption of acidic food and beverages. [4,5] Intrinsic erosion is associated with gastric acid which may be present intra-orally following vomiting, regurgitation, gastro-oesophageal reflux.[6]

Lifestyle changes and a rise in the consumption of acidic foods and beverages have led to an increase in the prevalence of dental erosion around the world in recent years. High prevalence numbers ranging from 30%[7] to 68%[8] have been reported, especially among children and adolescents,[9] while the prevalence and etiology of dental erosion have been the focus of innumerable papers in the last two decades, studies regarding the prevention of this disease by chemical substances are still needed.

Several studies have reported the effectiveness of topical fluoride as a cariostatic agent in enhancing enamel remineralization.[10-14]A similar anti-erosive capability of different topical fluorides was tested.[15-17]

In recent years casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes have also been demonstrated to have anticariogenic[16-21] as well as anti- erosive properties.[22-24]

Current recommendations in many recent studies for management of demineralized lesions include the use of oral products containing CPP-ACP (e.g., Tooth Mousse and Recaldent) and fluoride,[18,19,22,24] thus MI paste plus was recently introduced in the market combining both. MI paste plus is water based, lactose free cream containing casein phosphopeptide and amorphous calcium phosphate fluoride (CPP-ACPF). The level of fluoride is 0.2% w/w (900 ppm) which approximates that of adult toothpastes. When CPP-ACPF is applied in the oral environment, it will bind to biofilms, plaque, bacteria, hydroxyapatite and soft tissue localizing bio-available calcium, phosphate and fluoride.

A controversial issue in dental erosion literature involves differences in erosion progression rates between primary and permanent teeth. Some authors stated that primary enamel is more susceptible to erosion than permanent enamel[25-27] while others found no differences between these two types of substrates.[28-30] However, the protective effect of different remineralizing agents especially CPP-ACPF on both primary and permanent enamel did not receive much attention.

Softening of the enamel surface is an early manifestation of the erosion process. Reduced surface hardness which accompanies erosion of the enamel surface by acidic beverages can be assessed using a physical measurement such as the hardness test.[17, 23, 31]

In view of the above considerations, the present paper aimed to investigate the protective effect of single application of 0.1% NaF varnish, 1.23% APF gel and CPP-ACPF on artificially induced dental erosion in human primary and permanent enamel. Thus the null hypothesis tested was that the different fluoride treatments will not exhibit different protective potential on enamel erosion.

2. Material and Methods

Sample preparation

Sixty human primary molars (n = 30) extracted from children 10- 12 years old and young permanent premolars (n = 30) extracted for orthodontic purpose from children 12-14 years old were used in this study. Enamel specimens were prepared by sectioning the coronal portion from the radicular portion of each tooth using a diamond bur in a high-speed handpiece with an airwater spray. The crowns were then transversely sectioned from the mesial to distal surface through the center of the crown using a high-speed saw (Buehler Int., Evanston, IL) cooled with water. The enamel sections that were free of any caries or any enamel defects were embedded in acrylic resin with the outer buccal or lingual enamel surface exposed. The enamel surfaces were ground wet using 600-2000 grit silicon carbide abrasive paper (Buehler, Lake Bluff, IL) and polished with 1.0 and 0.05 mm alumina suspension (Buehler) to expose flat enamel for microhardness measurments. Test specimens were randomly assigned to one of three groups each of 10 according to the protective agent used: 1.23% APF gel (dentsply professional 1301 Smile Way, York PA 17404) , 0.1 % NaF varnish (flour protector, Ivoclar Vivadent AG, FL - 9494 Schaan Liechtenstein), and 0.2% CPP-ACPF paste (Prospec™ MI Paste, GC Corporation, Tokyo, Japan).

Fluoride treatment

Before topical fluoride application, the specimens were thoroughly rinsed with deionized water and delicately dried using a paper towel. Half of the exposed enamel surface of each specimen was protected with adhesive tape while the remaining surface was treated according to their group. APF gel and CPP-ACPF paste were applied for 4 minutes with the aid of a cotton tip and were later removed by squirting deionized water to rinse thoroughly. The NaF varnish was applied using a microbrush, left to act at the enamel surface for 12 hours to simulate clinical topical fluoride application[10], and then delicately removed using cotton tips immersed in deionized water. No chemical substances were used for the removal of the varnish in order not to alter the enamel surface. All specimens were then stored in artificial saliva overnight.

pH cycling

Six daily demineralization–remineralization cycles of 5 minutes of immersion in a cola drink (pH 2.3) and 30 minutes in artificial saliva were conducted for 14 days. All specimens were stored in artificial saliva between and after cycles.

During demineralization cycles, the specimens were immersed in cola drink (Coca-Cola) of pH 2.3; at room temperature for 5 minutes in separate containers (15 ml/specimen) hermetically sealed.[32] The cola drink was changed every cycle. After thorough rinsing with deionized water and careful drying, the specimens were stored in artificial saliva solution during the 30 minutes resting intervals and overnight. The artificial saliva solution was changed every 2 days.

Hardness assessment

Enamel demineralization was measured as surface softening. The surface Micro-hardness (SMH) of the specimens was determined using Digital Display Vickers Micro-hardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd. China) with a Vickers diamond indenter and a 20X objective lens. A load of 200 g was applied to the surface of the specimens for 15 seconds. Five indentations were equally placed over a circle of 1-mm diameter at the middle third of the specimens. The diagonal length of the indentations was measured by built in scaled microscope and Vickers values were converted into micro-hardness values. SMH was obtained using the following equation:
$HV=1.854\ P/d^2$ where, HV is Vickers hardness in Kgf/mm^2, P is the load in Kgf and d is the length of the diagonals in mm. The surface Micro-hardness of the specimens was measured once at baseline, and after 14 days of the six daily demineralization–remineralization cycles for both treated and untreated teeth halves. Additionally, the percentage reduction in surface microhardness (% SMHR) was calculated as:

$$\frac{\text{Microhardness (at base line)} - \text{Microhardness (After)}}{\text{Microhardness (at base line)}} \times 100$$

Statistical analysis

Data were presented as means and standard deviation values. Analysis of Variance (ANOVA) was used to compare between means of the three groups. Duncan's post-hoc test was used to determine significant differences between the means when ANOVA test result is significant. Paired t-test was used to compare between mean microhardness values before and after treatment within each group.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with Statistical Package for Scientific Studies (SPSS 16.0, SPSS, Inc., Chicago, IL, USA.) for Windows.

3. Results

Primary teeth:

The mean (±SD) values of SMH in the different groups at base line and after 14 days of demineralization-remineralization cycles for treated and untreated teeth halves were respectively (263.4± 2.6, 213.5±6.6 and 184.8± 19.5), (277.7±73,191.3±23.4 and176.9±11.5) and (285.5±37.9, 196.9±28 and 184.9±32.5) for the gel, varnish and paste groups respectively, table (1). As regards the mean (±SD) %SMHR of treated and untreated teeth halves, were (18.6 ±3.3 and 29.6±6.7), (29.3 ±9.6 and 33.9±12.2), and (29.8 ± 15.4 and 34.8±12.2) for the gel, varnish and paste respectively, table (2) and fig. (1).

Statistical analysis showed that all of the three treatments were able to diminish the amount of enamel hardness loss and provide a significant protective effect against erosive enamel loss than the untreated teeth halves with no statistically significant difference between untreated halves. Moreover, APF gel group showed the statistically significantly lowest mean % SMHR (i.e. APF gel showed the highest protective effect against erosive enamel loss) compared to fluoride varnish and CPP-ACPF paste with no statistical significant difference between them.

Permanent teeth:

The mean (±SD) values of SMH in the different groups at base line and after 14 days of demineralization-remineralization cycles for treated and untreated teeth halves were respectively (268.4±41.4, 187.4±18.7 and 170.8±22.62), (336.5±25.7, 191.9±9 and 169.8±15.9), and (297.5± 32.3, 225.9±1.6 and185.7±28.1) for the gel, varnish and paste group respectively, table (1). As regards the mean (±SD) %SMHR of treated and untreated teeth halve**s**, were (28.7 ±16.3 and 35±16), (42.9±2 and 49.6±1.6) and (23.6 ±8.8 and 36.7±16.3) for the gel, varnish and paste respectively, table (2) and fig. (1).

Similar to primary teeth statistical analysis showed that all of the three treatments were able to diminish the amount of enamel hardness loss and provide a significant protective effect against erosive enamel loss than the untreated teeth halves with no statistically significant difference between untreated halves. Moreover, there was no statistically significant difference between APF gel and CPP-ACPF paste groups which showed lower means % SMHR (i.e. highest anti-erosive protective effect) than the fluoride varnish group.

Comparing primary and permanent teeth, in primary teeth only APF gel showed statistically significantly the lowest mean % SMHR compared to the CPP-ACPF paste and varnish groups. However, in the permanent teeth both APF gel and CPP-ACPF paste showed statistically significantly the lowest mean % SMHR compared to the fluoride varnish group.

Table (1): Means and standard deviation (SD) values of surface microhardness (SMH) in the different groups at base line and after 14 days of demineralization-remineralization cycles for both treated and untreated teeth halves in primary and permanent teeth**.**

Teeth	Group / Treatment	APF gel		fluoride varnish		CPP-ACPF paste	
		Mean	SD	Mean	SD	Mean	SD
primary	Before (at base line)	262.4	2.6	277.7	73	285.5	37.9
	After (Treated half)	213.5	6.6	191.3	23.4	196.9	28
	After (Untreated half)	184.8	19.5	176.9	11.5	184.9	32.5
Permanent	Before (at base line)	268.4	41.4	336.5	25.7	297.5	32.3
	After (Treated half)	187.4	18.7	191.9	9	225.9	1.6
	After (Untreated half)	170.8	22.6	169.8	15.9	185.7	28.1

Table (2): Means (+SD) values and results of ANOVA test for the comparison between (% SMHR) of the treated and untreated teeth halves in primary and permanent teeth.

Teeth	Group / Teeth halves	APF gel		fluoride varnish		CPP-ACPF paste		P-value
		Mean	SD	Mean	SD	Mean	SD	
primary	Treated halves	18.6 [b]	3.3	29.3 [a]	9.6	29.8 [a]	15.4	**0.034***
	Untreated halves	29.6	6.7	33.9	12.2	34.8	12.2	**0.872**
Permanent	Treated halves	28.7 [b]	16.3	42.9 [a]	2	23.6 [b]	8.8	**0.045***
	Untreated halves	35	16	49.6	1.6	36.7	16.3	**0.386**

*: Significant at $P \leq 0.05$, Means with different letters are statistically significantly different according to Duncan's test

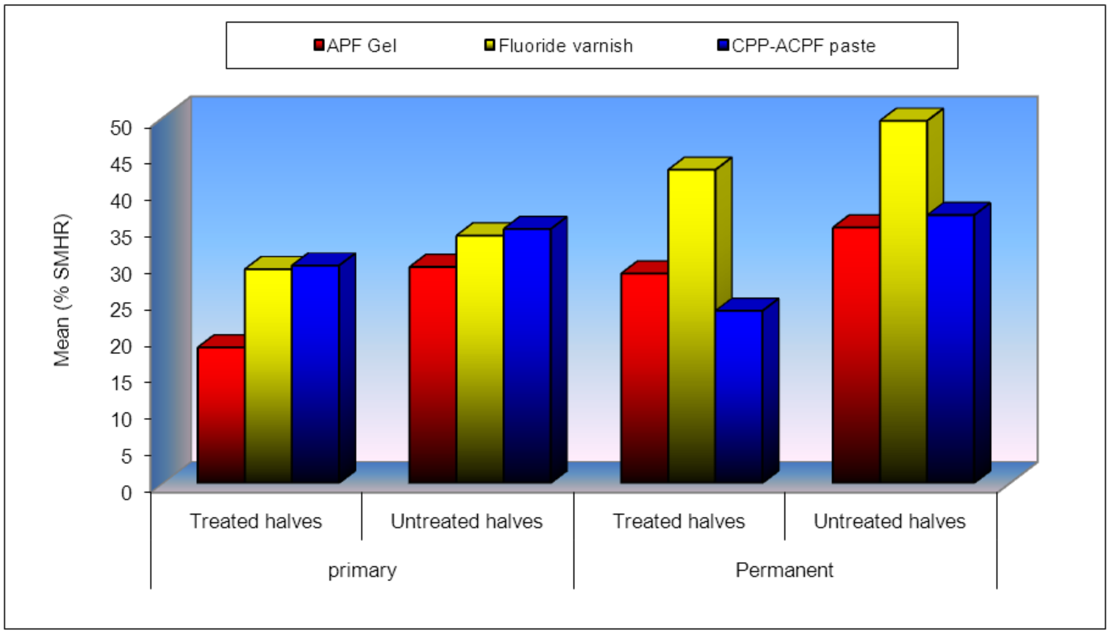

Fig. (1): Means (% SMHR) of both treated and untreated teeth halves in primary and permanent teeth

4. Discussion:

Epidemiological studies have reported that dental erosion is common in adolescents, 37% in the UK and 41% in the US, [33] and that its incidence is increasing with time.[34] Erosion may not only cause direct loss of surface enamel or dentin, but also renders tooth structures more susceptible to caries.[35] The 1993 UK Child Dental Health survey[36] reported that over half of five and six years-old children had eroded surfaces on one or more primary incisors. Among children aged 11 years or older, a quarter or more were found to have some erosion of the palatal surfaces of the upper permanent incisors. Erosion appears to have increased in children particularly from the higher socioeconomic groups.[37, 38]

Recently a new material CPP-ACPF paste was introduced to the market combining both CPP-ACP and fluoride. None of the studies up to our knowledge studied the protective anti-erosive effect of this material on both primary and permanent teeth. Moreover, in this study this effect was compared to two of the most commonly used fluoride products in dental practice nowadays as 2.26% NaF varnish and 1.23% APF gel.

Cola was used in this study to induce artificial erosive effect as in other studies[23, 31] since; it is one of the most commonly consumed acidic beverages. The cola drink was changed every cycle to ensure that it was carbonated and to reduce the buffering effect from ions dissolved from the enamel surface. Cola containers were hermetically sealed because removal of gas from the drink may increase its pH and decrease its potential of dissolving hydroxyapatite

Indentation hardness testing with either Knoop or Vickers indenter has been used for the measurement of initial enamel hardness, enamel softening as an early manifestation of the erosion process, as well as enamel hardening after remineralization. Both indenters are suitable for hardness testing of non-metallic materials. [10,17,23,39, 40,41] Vickers hardness test was chosen in this study with the 200 g load because it provided the appropriate size of indentations for accurate measurement with the available equipment and the present experimental design. SMH readings were recorded at baseline, and after 14 days of the six daily demineralization–remineralization cycles for both treated and untreated teeth halves to determine minor changes due to erosive enamel loss. Additionally, the % SMHR was then calculated to refer this minor reduction in SMH to the initial readings at base line of the same teeth.

The results of our study showed that all of the three fluoride treatments were able to diminish the amount of enamel hardness loss and provide protective effect against erosive enamel loss in the treated teeth halves than the untreated halves in both primary and permanent teeth. These results are in accordance with those of previous studies that investigated the effect of APF gel[17, 42] and NaF varnish[17, 43, 44] on dental erosion. Moreover similar to our study, these studies showed a reduction of hardness in all treated groups indicating that, although fluoride products can inhibit dental erosion, they do not completely prevent it. Contradictory results ranging from no or limited protection of topical fluoride against dental erosion[38, 45, 46] up to almost complete protection[47] are found in literature. This may be due to differences in study design, particularly regarding the type of dental substrate, the frequency of application, the pH and concentration of the different fluoridated substances used.[17, 48]

In primary teeth our 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 bases that the acidic pH of APF gel may have etched the enamel surface and helped to increase the incorporation of fluoride into enamel. Another explanation may be that the free negative fluoride ions become more reactive in acidic media, thus enhance the formation of CaF_2. Several studies agreed with the enhancement of the formation of the CaF_2 layer under acidic conditions when comparing neutral to acidic fluoride solutions[49] and when comparing neutral to acidic fluoride gels.[50-51] limited studies compared the APF gel to the varnish due to difference in application techniques.[10,17] However, our result that the APF gel offer higher protective anti-erosive effect than the fluoride varnish comes in agreement with Lee etal., 2010 [10] who found that APF gel showed the better effect in terms of fluoride uptake. Moreover, Murakami etal., 2009[17]stated that although the fluoride varnish used in his study had a greater concentration of fluoride (2.26%F) and was left to act in contact with the teeth for a longer period of time, the APF gel showed similar protective anti-erosive effect. However, in our study the significantly higher anti-erosive effect of the APF gel over the varnish can be attributed to the lower concentration of fluoride in the varnish used in our study (0.1%F).

In the permanent teeth, although all of the three treatments were able to diminish the amount of enamel hardness loss and provide protective effect against erosive enamel loss than the untreated teeth halves similar to primary teeth. Yet, the protective effect offered not only by APF gel but also by the CPP-ACPF paste was significantly higher than that of the fluoride varnish. Thus CPP-ACPF paste showed significantly high erosive protection potential in permanent teeth. This can be attributed to the formation of a stabilized amorphous calcium fluoride phosphate phase. These results come in agreement with several studies that proved that the combined effect of CPP-ACP and fluoride as two separate products was beneficial in enhancing remineralization and anticariogenic effect, [19, 52] as well as improving acid-resisting effect. [52, 53]

Our results can be justified on bases that this recently introduced material combines both fluoride and CPP-ACP in one product thus offering the protective effect of both of them.

The protective effect of fluoride is mainly attributed to the formation of a CaF_2 like layer on the tooth surface, which acts as a fluoride reservoir. During an acidic attack, fluoride released from the CaF_2 deposit can be incorporated into the mineral by forming fluoroapatite or fluorohydroxyapatite resulting in a decreased susceptibility to further dissolution. A similar mode of action is assumed for the anti-erosive capability of fluorides. Additionally, the CaF_2 layer might act as a mechanical barrier hampering the contact of the acid with the underlying enamel or as a mineral reservoir, which is attacked by the erosive challenge, thus leading to a buffering or depletion of hydrogen ions from the acid. The formation of the CaF_2 layer depends on the pH and the concentration of the fluoride agent and the duration of application.[54] As high concentrated fluoride agents or a prolonged application time might lead to a thicker and more stable CaF_2 precipitate, an intensive fluoridation is considered as most effective for prevention of erosive enamel loss.[55, 56]

The protective effect of CPP-ACP lies in the fact that it provides a reservoir of neutral ion pair that inhibits enamel demineralization and promotes remineralization.[18, 57] Calcium and phosphate ions are building blocks for the remineralization process, and are found in saliva. Casein phosphopeptide amorphous calcium phosphate complex (CPP–ACP) has been introduced as a supplemental source of calcium and phosphate ions in the oral environment. The amorphous calcium phosphate is biologically active, and is able to release calcium and phosphate ions to maintain saturation levels of calcium and phosphate at the tooth surface. It is hypothesized that, in addition to the prevention of erosive demineralization, CPP-ACP also remineralizes (repairs) eroded enamel and dentine crystals. This hypothesis is supported by an observation that superficial granular structures, probably representing remineralized enamel crystals were formed on the enamel surface after exposure to a sports drink containing CPP-ACP.[58]

Comparing primary and permanent teeth, in primary teeth only APF gel showed statistically significantly the lowest mean % SMHR compared to the CPP-ACPF paste and varnish groups. However, in the permanent teeth both APF gel and CPP-ACPF paste showed statistically significantly the lowest mean % SMHR compared to the fluoride varnish group. This can be attributed to two factors, first to the structural difference between both primary and permanent enamel. Deciduous teeth demonstrate a higher degree of enamel porosity[59] and a lower degree of mineralization[60] than permanent teeth. This was attributed to greater density of the interprismatic fraction and the prism-junction in deciduous enamel than its permanent analogue. [61]This difference in porosity might contribute, at least in part, to the observed variation to the response to various protective agents. Other differences between deciduous and permanent tissues may also be of importance. For example deciduous enamel has a higher content of carbon dioxide and carbonate, as well as a lower content of phosphorous and calcium phosphate than the permanent tissue in its composition.[29,62,63] Primary enamel has less organized microcrystals[64] and a greater diffusion coefficient.[28] Furthermore, primary teeth possesses an aprismatic layer on its outer surface, which erodes in a highly irregular manner and is probably not as liable to erosive destruction when compared to prismatic enamel.[24, 65]

A second explanation to the significantly high anti-erosive effect of CPP-ACPF paste on permanent enamel and not on primary one may be the increased reactivity of the permanent teeth. In this study different primary and permanent substrates, at different developmental stages and with different post-eruptive ages were used. Thus, an ‘‘older’’ tooth that has been exposed to the oral environment and in contact with the acids and fluoride for longer periods of time during its life cycle than a newly erupted ‘‘young’’ tooth is expected to be more mineralized and more acid resistant.[66, 67] In the present study, exfoliated human primary molars and young premolars extracted for orthodontic purpose from children aged 12-14 years old were used. The exfoliated primary molar specimens had been exposed to the oral environment for a much longer period of time, adding more acid-resistant fluoridated crystals to its enamel’s composition when compared to young permanent premolars. Thus it can be anticipated that it may differ than permanent teeth in its response to different remineralizing agents.

Our results that CPP-ACPF paste showed statistical significant low mean % SMHR as APF gel in the permanent teeth has shown that CPP-ACPF is a very promising remineralizing material. In study by Schupbach et al.,1996 [68] it was demonstrated that CPP-ACPF could be incorporated into the pellicle in exchange for albumin and that it inhibits the adherence of S. mutans and S. sobrinus. Therefore, CPP-ACPF can be expected to be effective intraorally on both permanent and primary teeth in high-risk patients. The fact that this paste can be self-applied as recommended by the manufacturer renders it available in the oral cavity for longer periods, thus saliva will enhance the effectiveness of CPP-ACPF and the flavor will stimulate the saliva flow. The longer CPP-ACPF and saliva are maintained in the mouth, the more effective the expected results. Although our study could not simulate the complex oral environment, it showed the potential of CPP–ACPF paste for reversing the harmful effect of a cola drink on tooth surfaces especially in permanent teeth. It is speculated that the effect of CPP-ACPF paste will be enhanced under oral conditions in the presence of biofilm which can bind to casein phosphopeptide and act as a reservoir for calcium and phosphate ions, thus enhance remineralization. It also neutralizes the acid challenges from acidogenic bacteria in plaque and from other external and internal acid source.[23]

5. Conclusion:

Under the conditions of this study, these conclusions can be derived:

1. All of the tested fluoride treatments positively reduced erosive enamel loss in both primary and permanent teeth.
2. In primary teeth only APF gel showed effective anti-erosive effect.
3. In permanent teeth both CPP-ACPF paste and APF gel showed effective protective effect against dental erosion.
4. Primary and permanent enamel substrates reacted differently to different fluoridated compounds.
5. CPP-ACPF paste is a promising remineralizing material.

Recommendations:

Future studies are needed to determine which topical fluoride agent exerts the most protective anti-erosive effect intraorally.

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9/28/2010