REMINERALIZATION EFFECT OF HEXAMET APHOSPHATE COMBINED WITH LOW FLUORIDE TOOTHPASTE ON ENAMEL SURFACE OF PRIMARY TEETH (IN VITRO STUDY)

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ABSTRACT

INTRODUCTION: Investigators have been searching for alternative remineralizing compounds that have superior properties to those conventionally used; in order to reduce the risk of fluorosis in children.

OBJECTIVES: To evaluate the anti-caries effect of hexametaphosphate (HMP) combined with low-fluoride toothpaste on primary teeth enamel surface using fluoride uptake analysis.

MATERIALS AND METHODS: Seventy five freshly extracted anterior primary teeth were sectioned into two halves in a labiolingual direction (150 specimens). One half of each tooth remained untreated and served as control and the other corresponding half that was treated with the remineralizing toothpaste served as test. Specimens were assigned into subgroup IA (untreated) n=25, subgroup IB (250ppm F + 0.5% HMP) n=25, subgroup IIA (untreated) n=25, subgroup IIB (500 ppm F) n=25 and subgroup IIIA (untreated) n=25, subgroup IIIB (1000 ppm F) n=25. Specimens were subjected to pH cycling for five days and immersed in remineralizing solution for an additional two days. The enamel fluoride uptake analysis of specimens was evaluated quantitatively using an ion specific electrode. Data were analyzed using Mann-Whitney U, Kruskal-Wallis and Post-hoc pair-wise test.

RESULTS: There was a significant high median enamel fluoride uptake between test subgroups IB (250ppm F + 0.5% HMP), IIIB (1000 ppm F) and their controls where p=0.008 and p=0.008 respectively, while there was no significant difference in median enamel fluoride uptake between the test subgroup IIB (500 ppm F) and its control subgroup IIA where p=0.690. Pair-wise comparison of enamel fluoride uptake in the three test subgroups IB, IIB and IIIB showed significant high median value of subgroup IB than subgroup IIIB where p=0.027.

CONCLUSIONS: A toothpaste containing 250ppm F + 0.5% HMP has a high anti-caries potential in comparison to pediatric 500 ppm F and standard 1000 ppm F toothpastes on primary teeth.

KEYWORDS: Demineralization, fluoride, hexametaphosphate, enamel, pH cycling, toothpastes.

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INTRODUCTION

Despite many years of research and the availability of novel anti-caries products, dental caries is still one of the most prevalent chronic diseases affecting humans of all ages, especially children (1). The prevention and treatment of early caries lesions is a constant challenge in dentistry, especially in patients at high risk. Therefore, efforts have been directed to search advances in technologies to promote remineralization of early caries lesions in enamel, as well as to prevent the caries process at the earliest possible stage (2). Although caries incidence and prevalence have decreased significantly over the last few decades due to the wide spread use of fluoride, an increase in the prevalence of dental fluorosis has been reported (3).

The occurrence of dental fluorosis lesions is associated with excessive fluoride intake during the period of tooth development (4, 5). Its severity depends on the dose, duration, and timing of intake (6). The total amount of fluoride consumed from all sources during the critical period of tooth development is the most important risk factor in determining fluorosis occurrence (7). In order to reduce the risk of fluorosis in children, toothpaste formulations with a reduced concentration of F have been recommended (8, 9). Since this could have a negative impact on the anticaries effect of fluoride toothpastes, providing low levels of calcium and phosphate in conjunction with minimal amounts of fluoride may improve remineralization of the tooth and overcome risk of fluorosis (10).

The cariostatic effect of phosphate salts has been studied since the 1960s. Numerous laboratory and animal studies have compared ortho-, meta-, pyro- and polyphosphate (11, 12). The pyrophosphates and polyphosphates belong to a class of compounds called 'calcium phosphate surface active builders' (CPSABs).

The chemistry of CPSABs requires them to have a strong binding affinity for surfaces of sparingly soluble calcium salts. This binding may interfere with crystal growth and/or dissolution. The first CPSAB successfully incorporated into dentifrices was soluble sodium pyrophosphate in the mid-1980s (13).

Missel et al (14) demonstrated that the addition of phosphate in the form of sodium trimetaphosphate (TMP) to toothpaste containing (250ppm) fluoride can reduce

enamel demineralization to levels similar to those seen for conventional toothpaste containing (1100ppmF) fluoride.

Such effects occur due to specific characteristics of this phosphate, such as its capacity to form complexes with metal ions (Ca+) and its ability in reducing enamel solubility (15, 16).

Beside TMP sodium hexametaphosphate (HMP) has also been shown to enhance the anti-caries effect of toothpastes containing (250 ppm F), or (1100ppmF) on bovine enamel (17-19); as the complexing ability of phosphates is proportional to the total number of phosphorus atoms in the polyphosphate (13). The HMP molecule is widely used in the food industry as an antimicrobial agent owing to its ability to increase the permeability of the bacterial outer membrane (20).

Since the combination of hexametaphosphate /fluoride showed great potential as an anticaries treatment on bovine enamel, it would be interesting to verify whether a similar synergistic anticaries effect exists between fluoride and 0.5 %HMP on human primary enamel, and this triggered the interest to carry out this study. The proposed null hypothesis assumed that toothpastes with low fluoride concentration combined with 0.5 %HMP would have similar enamel remineralizing effect as that of standard F toothpaste.

MATERIALS AND METHODS

The study was an experimental in vitro study. The minimal sample size was calculated based on a study aimed to evaluate the anticaries effect of low fluoride toothpaste combined with hexametaphosphate (HMP) on enamel surface. Da Camara et al. in 2014 (17), reported that the smallest difference in enamel surface hardness (SH) values +139.9-55.6 =84.3kgf/mm^2).

This data resulted in large effect size (f=0.40), which when adopted resulted in a minimum required sample size per group of 22 teeth (number of groups=3) (total sample size needed=66 teeth) is the enough required sample to detect a standardized effect size of 0.40 of the primary outcome, as statistically significant with 80% power and at a significant level of 95% (alpha error accepted =0.05). Sample size per group was increased to 25 per group (total sample size needed=75 teeth) (+13.36%) to control for attrition bias.

Seventy-five freshly extracted non carious anterior human primary teeth were collected after the approval of the Ethics Committee from the outpatient clinic of El Anfoshy Pediatric Hospital, Ministry of Health, Alexandria Governorate. The study was performed at the Dental Materials Department Faculty of Dentistry, Environmental Studies Department at the Institute of Graduate Studies and Research, and the Geology Department at the Faculty of Science, Alexandria University.

The teeth were cleaned from debris and blood, washed, dried and then stored in normal saline solution at room temperature. All teeth were randomly allocated using permuted block randomization technique (21) into three groups according to the type of toothpaste used. Each tooth in each group (25 teeth/ group) was longitudinally sectioned in a labiolingual direction with a diamond disc in two halves (each half was considered as a specimen, amounting to 150 specimens). The mesial halves remained untreated with toothpastes and served as control specimens (subgroups: IA, IIA, and IIIA), whereas the distal halves were subjected to treatment by different regimens of toothpastes and served as test specimens: subgroup IB: 250ppm F + 0.5 % HMP toothpaste, subgroup IIB: pediatric 500 ppm F toothpaste and subgroup IIIB: standard 1000 ppm F toothpaste.

Specimens were subjected to 5 days pH cycling and treatment with toothpaste slurries then immersed in remineralizing solution for an additional two days, after which enamel fluoride uptake analysis was assessed. (17)

Toothpaste formulation

The experimental toothpaste contained: water, sorbitol, glycerin, sodium lauryl sulphate, flavor strawberry, flavor optamintol, 0.5% concentration of Hexametaphosphate (SANTA CRUZ Biotechnology, CAS Number 68915-31-1 USA) and 250 ppm F in the form of Sodium Fluoride (NaF) (toothpaste prepared at the Swiss Egyptian Company for Oral Care Products and Cosmetics (SESIC), Alexandria, Egypt). The pediatric toothpaste contained 500 ppm F (Signal Kids, Unilever Mashreq-Personal Care Company (S.A.E) 6th of October City, 4th industrial, Egypt) and the standard 1000 ppm F toothpaste (EMOFORM-F, SESIC, Alexandria, Egypt) were used to compare them with the experimental toothpaste.

Treatment procedure (17)

Each specimen was incubated in an individual container and subjected to 5 pH cycles at 37º C for 5 days.

For the control subgroups (IA, IIA, and IIIA) each pH cycle included: immersion in freshly prepared remineralizing solution for 18h (pH=7) (1.5 mmol/L calcium nitrate (Ca $(NO3)_2$), 0.9 mmol monosodium phosphate (NaH_2PO_4), 150 mmol potassium chloride (KCl), 0.02 mmol cacodylic buffer to adjust pH 7.0 and 0.05 µg F/mL as sodium fluoride (NaF), 1.1 ml/mm^2); rinsed with deionized water and re-immersed in freshly prepared demineralizing solution for 6h (pH=4.7) (2.0 mmol calcium nitrate (Ca $(NO_3)_2$), 2.0 mmol monosodium phosphate (NaH_2PO_4), 75 mmol acetate buffer to adjust the pH to 4.7 and 0.04 mg F/ml as sodium fluoride (NaF), 2.2 ml/mm^2) and rinsed with deionized water.

For the test subgroups (IB, IIB, and IIIB) each pH cycle included: immersion in toothpastes slurries (toothpaste suspended in deionized water 1:3 weight/weight) for 1 min; rinsed with deionized water; re-immersed in freshly prepared remineralizing solution (pH=7. 0) for 18h; rinsed with deionized water; and re-immersed in toothpastes slurries for 1 min; rinsed with deionized water; and finally re-immersed in freshly prepared demineralizing solution (pH=4.7) for 6h and rinsed with deionized water.

For an additional 2 days, all specimens of both groups were immersed in a freshly prepared remineralizing solution and prepared for evaluation.

Enamel fluoride uptake analysis (22-24)

One layer of enamel was removed by drilling the proximal surface with a 0.68-mm flat end diameter diamond bur mounted on low speed handpiece. The enamel powder was collected in a separate plastic jar. 1.0 mL of 1.0 mol/L HCl was added to the enamel powder and the mixture was kept under constant stirring for 1 h prior to the addition of 1.0 mL of 1.0 mol/L NaOH to adjust the pH to 7 for enamel fluoride analysis.

For enamel fluoride analysis in (mV), an ion specific electrode (94-09BN, Orion Research Inc. Products gp.529 Main St. Boston MA 02129 USA) was calibrated by using standard fluoride solutions of 0.1,1,10 and 100 ppm. Samples and standards were buffered with TISAB II at a 1:1 ratio (sample/ TISAB II). The millivoltage (mV) readings

were then entered in to a computer program (Excel) that mathematically established the part per million (ppm) values for each mV unit, using the fluoride slope curve.

Statistical analysis

Data were collected and entered to the computer using SPSS program for statistical analysis (ver 21) (25). Data were described using minimum, maximum, median and inter-quartile range. Mann-Whitney U test was carried out to compare between control and test subgroups. Comparison was carried out between test subgroups using Kruskal-Wallis test. As Kruskal-Wallis test was significant, Post-hoc pair-wise comparisons was carried out using Dunn-Sidak test for multiple comparison. An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%. Box and whisker graph was used as the data was not normally distributed.

RESULTS

The median enamel fluoride uptake value of (250ppm F + 0.5% HMP) treated specimens (subgroup IB) was significantly higher than untreated specimens (subgroup I A) where p=0.008, Figure (1).

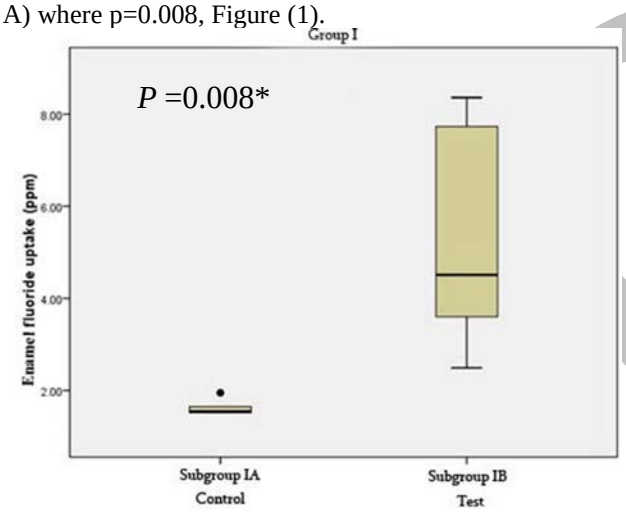

Figure (1): Box and whisker graph of Enamel fluoride uptake (ppm) in group I between subgroup IA (control) and subgroup IB (0.5HMP+250ppmF) (test), the thick line in the middle of the box represents the median, the box represents the inter-quartile range (from 25th to 75th percentiles), the whiskers represents the minimum and maximum after excluding outliers (black-filled circles).

*: Statistically significant (p<0.05).

The median enamel fluoride uptake value of (500 ppm F) treated specimens (subgroup IIB) was not significantly different from untreated specimens (subgroup IIA) where p=0.690, Figure (2).

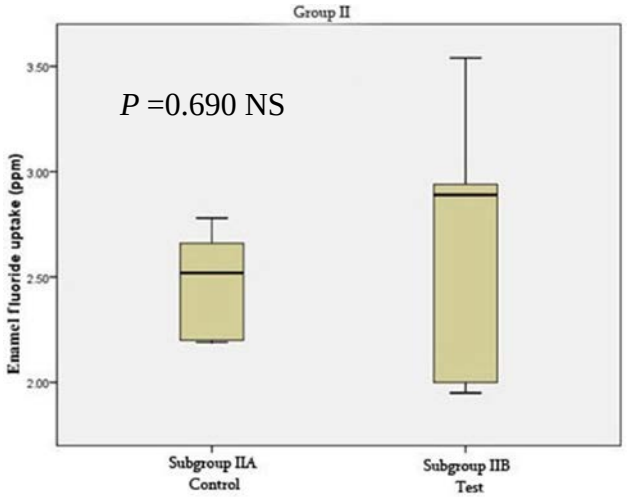

Figure (2): Box and whisker graph of Enamel fluoride uptake (ppm) in group II between subgroup IIA (control) and subgroup IIB (500ppmF) (test), the thick line in the middle of the box represents the median, the box represents the inter-quartile range (from 25th to 75th percentiles), the whiskers represents the minimum and maximum..

NS: Statistically not significant (p≥0.05).

The median enamel fluoride uptake value of (1000 ppm F) treated specimens (subgroup IIIB) was significantly higher than untreated specimens (subgroup IIIA) where p=0.008, Figure (3).

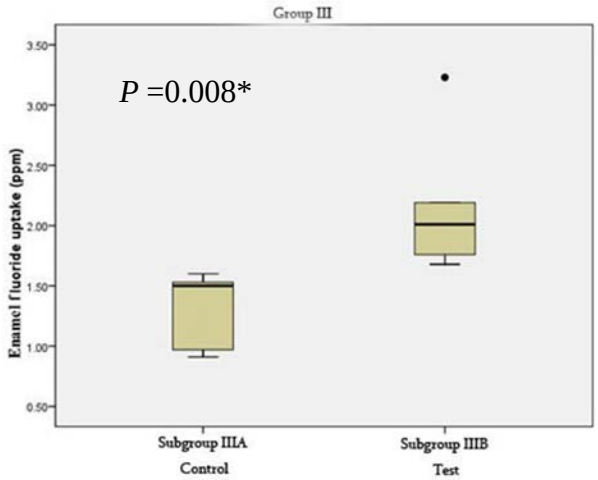

Figure (3): Box and whisker graph of Enamel fluoride uptake (ppm) in group III between subgroup IIIA (control) and subgroup IIIB (1000ppmF) (test), the thick line in the middle of the box represents the median, the box represents the inter-quartile range (from 25th to 75th percentiles), the whiskers represents the minimum and maximum after excluding outliers (black-filled circles).

.*: Statistically significant (p<0.05).

Comparing median values of enamel fluoride uptake in the three test subgroups IB, IIB and IIIB using Kruskal-Wallis test showed significant difference between the three subgroups where p=0.027, Table (1).

Table (1) Median values of enamel fluoride uptake in the three test subgroups IB, IIB and IIIB.

Enamel fluoride uptake (ppm)	**Subgroup IB** 0.5HMP+250ppmF **(test)**	**Subgroup IIB** 500ppmF **(test)**	**Subgroup IIIB** 1000ppmF **(test)**
Min-Max	2.49-8.36	1.95-3.54	1.68-3.23
Median (IQR)	4.51 (3.60-7.73)	2.89 (2.00-2.94)	2.01 (1.76-2.19)
Kruskal-Wallis test	**$X^2_{(df=2)(KW)}$=7.220** ***p*=0.027***		

*****: Statistically significant (p<0.05).
$X^2_{(df=2)(KW)}$: X^2value for Kruskal-Wallis test.

Post-Hoc pair-wise comparison of enamel fluoride uptake between the three test subgroups IB, IIB and IIIB using Dunn-Sidak test to adjust the significant values, showed a high significant difference between the test subgroup IB (0.5HMP+250ppmF) compared to the test subgroup IIIB (1000ppmF) where p=0.027. Subgroup IB (0.5HMP+250ppmF) showed high enamel fluoride uptake compared to subgroup IIB (500ppmF) with no significant difference where p=0.198**,** Table (2)

Table (2) Pair wise comparison of enamel fluoride uptake between three test subgroups IB, IIB and IIIB.

	Subgroup IB 0.5HMP+250ppmF **Test**	**Subgroup IIB** 500ppmF **Test**	**Subgroup IIIB** 1000ppmF **test**
Standard Test Statistic	2.616		
P1	0.027*****		
Standard Test Statistic		0.778	
P2		1.000	
Standard Test Statistic	1.838		
P3	0.198		

P1: P value for Post-Hoc pair-wise comparison of enamel fluoride uptake between subgroup IB and subgroup IIIB using Dunn-Sidak test.
P2: P value for Post-Hoc pair-wise comparison of enamel fluoride uptake between subgroup IIB and subgroup IIIB using Dunn-Sidak test.
P3: P value for Post-Hoc pair-wise comparison of enamel fluoride uptake between subgroup IB and subgroup IIB using Dunn-Sidak test.
*****: Statistically significant (p<0.05)

DISCUSSION

The results of the present study rejected the null hypothesis assumed that toothpastes with low fluoride concentration combined with 0.5 %HMP would have similar enamel remineralizing effect as that of standard F toothpaste.

The addition of sodium hexametaphosphate (HMP) to toothpastes and gels with reduced fluoride concentration has shown to promote a synergistic protective effect against enamel demineralization on bovine enamel in vitro (17,26), as sodium hexametaphosphate interferes with the enamel de-remineralization processes due to its ability to bind to the enamel surface and reduce its solubility. Therefore, the evaluation of the remineralization effect of low fluoride toothpaste (250 ppm F) combined with (HMP) on primary natural enamel as well as comparing it with pediatric formula (500 ppm F) and standard formula (1000 ppm F) was a strong motive to undertake the current study.

In the present study to mimic the environment of the oral cavity as regards the dynamics of mineral loss and gain, involved in caries formation, the pH-cycling model was used. This model requires a small sample size and offers higher standardization of variables (27).

The use of fluoride ion selective electrode in conjunction with TISAB II is a reliable and most common method of testing fluoride. TISAB II is used during almost all fluoride testing procedures which creates a constant background ionic strength for fluoride measurements and stabilizes the pH. It also prevents any interference of foreign ions and de-complex the fluoride ions making them available for measurements (28).

The 0.5% concentration of sodium hexametaphosphate used in the current study was based on da Camara et al 2014 (17) in vitro study. They revealed that the addition of 0.5% HMP to the low-fluoride toothpastes (250 ppm F) resulted in the lowest mineral loss among all studied groups. High concentrations of HMP (2% and 3%) have been shown to impair the effect of fluoride; as adsorption of polyphosphate is very fast, HMP sequesters calcium ions from hydroxyapatite as a result of its strong ability to complex metal ions and competes with the adsorption of ionic fluoride which results in increased demineralization (29). However, 0.5% and 1% concentration HMP allows an opportunity for strong complex with calcium in medium-like saliva (17). This provides higher calcium availability during demineralization and remineralization processes and reduces the precipitation rate of calcium phosphate on the enamel surface (30, 31).

When comparing untreated specimens with those treated by 0.5% HMP+ 250 ppm F and 1000 ppm F toothpastes, a higher enamel fluoride uptake was recorded, whereas the untreated specimens and those treated by pediatric formula showed no remarkable uptake. This endorses the fact that addition of 0.5% HMP to fluoride concentration as low as 250 ppm results in an uptake that offers higher prevention.

In the current study, 0.5 HMP+ 250 ppm F toothpaste test subgroup showed the highest fluoride uptake when compared with 500 ppm F and 1000 ppm F toothpastes. The high deposition of fluoride on enamel in specimens treated with 0.5 HMP+ 250 ppm F toothpaste can be explained by the ability of HMP to form strong complexes with metal ions (crosslinking) leading to the reticular formation on enamel surfaces ‘‘coats’’. After toothpaste application this protective layer on the enamel surface hinders acid diffusion. Moreover, the presence of 0.5 HMP provides a synergistic effect for enamel fluoride uptake by holding the pores on the enamel surface open and enhancing the diffusion of Ca and F inward to the deeper layers of enamel (13, 32). However, 500 ppm F and 1000 ppm F toothpaste effect was related to a greater effect of fluoride on the outer enamel surface (33).

The 30 µm depth prismless outer enamel layer that is present in almost 100% of primary (34) teeth may also restrict the effect of the 500 ppm F and 1000 ppm F toothpastes to the superficial layer of enamel; as it acts as a barrier against mineral diffusion to deeper enamel layers.

While, HMP has a direct impact on incorporation of fluoride and calcium at the depth and reduces the acid diffusion into enamel by its selective permeability action as discussed (13, 32), thus it enhances the effect of the toothpaste from the outer layer to more deeper layers of enamel.

The results of the present study are in agreement with da Camara et al (17) who determined the fluoride uptake by bovine enamel of 15 groups that were treated with several concentrations and formulas of toothpaste.

They revealed that the addition of 0.5% HMP to the low-fluoride toothpastes (250 ppm F) resulted in the lowest mineral loss among all groups, but HMP concentrations higher than 0.5% impaired the effect of fluoride.

On the other hand, da Camara et al in 2016 (19) found that similar amounts of enamel fluoride uptake were observed for the 1100 ppm F and 1100ppm F combined with 1% HMP toothpastes, the effect of the 1100 ppm F toothpaste (without HMP) was more marked at the outer parts of the lesion. At variance, addition of 2 % HMP reduced the presence of fluoride in enamel when compared to the 1100 ppm F group. This aspect reinforces the concept that high percentage of HMP in the medium can supersaturate the enamel surface and further sequester calcium ions from hydroxyapatite due to its strong ability to complex metal ions (31). These results declared that the enamel fluoride diffusion of HMP combined with fluoride toothpastes depend on the appropriate molar ratio of the two active ingredients.

One of the limitations of this study is that results from in vitro experiments cannot be directly extrapolated to clinical situations. It is, therefore, crucial to mention that cariogenic bacteria and different salivary compounds will influence the outcome during application of a particular fluoride product. In addition, the present study was limited to a 5-day period, while the oral de/remineralization processes are long-term ones.

Within the limitation of the current study, it is evident that toothpastes with reduced F concentrations but containing HMP showed better remineralization capacity than standard F toothpastes. This novel component would be particularly effective in controlling caries for high risk preschoolers. Further studies should be conducted to evaluate clinically the remineralization efficiency of low fluoride concentration combined to HMP in high risk children.

CONCLUSION

Based on the results of the present study, it is concluded that toothpastes containing 250ppm F with 0.5% HMP produced significant anti-caries effect relative to pediatric 500 ppm F and standard 1000 ppm F toothpastes.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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