

Effect of Various Agents on Remineralization of Artificial Carious Lesions on Primary Molar: An Invitro Study

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Abstract

Introduction: Minimal invasive dentistry has evolved as a boon for pediatric dentistry. With the progress of understanding carious process, Remineralisation concept bridges the traditional gap between preventive and restorative procedures. There are various chemical and herbal remineralising agents available for suppressing cariogenic microorganisms and growth useful in remineralization.

Aim and objective: To evaluate and compare the effect of fluoridated toothpaste, casein phosphopeptide -

amorphous calcium phosphate (CPP-ACP), grape seed extract and ginger-honey mixture on artificial carious lesions in primary molars.

Methodology: A total 40 primary molar teeth were selected for the study. Baseline Vicker’s microhardness of sound enamel was checked using Vicker’s microhardness tester. Artificial carious lesions were created on each enamel specimen by placing them in lactic acid for demineralization. Then all the teeth were divided into the two groups (20 each) and again subdivided into two groups (10 each); each group was subjected to different

surface treatment as follows; Group A - Commercially available remineralizing agents; Group B - Herbal available remineralizing agents; Group 1A - Fluoridated toothpaste; Group 1B - CPP-ACP; Group 2A - Grape seed extract Group 2B - Ginger-honey mixture.

Result: Grape seed extract and CPP-ACP showed statistically significant difference after remineralization.

Conclusion: GSE helps in remineralization of artificial carious lesions on primary molars making it a promising naturally occurring remineralizing agent and can be used as an alternative to CPP-ACP or fluoride.

Keywords: Grape seed extract, primary molars, remineralization

Introduction

Dental caries is a common problem in most developed and developing countries. It is a dynamic process that is initiated by provoking the enamel lesion by periods of demineralization and remineralization. It is caused by intimate contact of the enamel with dental biofilm influenced by the oral environment. [1] Various factors contribute to the severity and complexity of the environment such as several factors such as the surface of the enamel, saliva and the presence of dental biofilm, among others, modulate the severity and the development of caries lesions in an individual. Remineralisation is nothing but the net gain in minerals at the surface of enamel, which were lost due to demineralization. [2,3] Early intervention of incipient caries lesion aims at remineralization of an active non cavitated subsurface lesion and thereby preventing cavitation by further mineral loss. In last decade, various topical gels, varnishes, mouthwashes and dentifrices containing fluoride being used by dentists. Flouridated toothpastes helps in reducing demineralization by formation of fluorhydroxyapatite crystals which in turn enhancing remineralization.[4] It has been also suggested in the

literature that Tooth mousse contains nanocomplexes of milk protein casein phosphopeptide (CPP) with amorphous calcium phosphate (ACP). It has been suggested that casein phosphopeptide (CPP) has the ability to stabilize calcium phosphate in solution by binding amorphous calcium phosphate with their phosphoserine residues, thereby allowing formation of small CPP - ACP clusters and which promotes remineralization.[4,5] Now a days natural products have been used as folk-medicines for thousands of years, and are promising agents. Some authors have reported about the effects of grape seed extract and ginger & honey combination on the demineralization and remineralization processes of dental hard tissues.

Grape seed extract contains proanthocyanidins which is said to strengthen the collagen tissues and promotes mineral deposition on superficial layer of lesion which positively affects remineralization. Also Ginger- honey mixture has antimicrobial activities and high content of fluoride. Honey is a supersaturated sugar solution with low water activity that does not allow the growth of bacteria thus showing bacteriostatic effect. [6,7]

Thus the present study was undertaken to determine and compare the remineralization potential of fluoridated toothpaste, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), Grape seed extract and Ginger-honey mixture of artificial carious lesions on primary molars.

Materials and methods

The present study was comparative study conducted in the Department of Pediatric & Preventive Dentistry, Bharati Vidyapeeth Dental College and Hospital, Sangli. Sample size was calculated as 40 from article published by, Gocmen GB et al in 2016. [7] Effectiveness of some herbals on initial enamel caries lesion 40 non carious

extracted primary molars were collected and stored in 0.10% thymol solution.

Inclusion criteria

Primary molars indicated for extraction having sound enamel (over retained teeth or teeth indicated for serial extraction) were included in the study.

Exclusion criteria

Carious teeth, discoloured teeth having hypoplastic defects, fractured or cracked tooth were excluded from the study.

Study design

Specimen Preparation

A total 40 primary molar teeth were selected for the study and stored in 0.10% thymol solution immediately after extraction. They were cleaned using ultrasonic scaler and polished using gamma alumina polishing gel and were sectioned 1 mm below cemento-enamel junction followed by mounting on cold cure acrylic blocks with surface facing buccally.[7] [Fig 1]



Figure 1: Primary molar mounted on acrylic rods

Phase I Vicker’s micro-hardness of sound enamel specimen

Baseline Vicker’s microhardness of sound enamel was checked using Vicker’s microhardness tester (Omni Tech, Model-S.Auto,Pune) and was measured using the Vicker’s microindenter A force of 200g for 15 seconds was applied to the enamel surface by a pyramid diamond indenter at three points at a distance of 100 micrometer between them and mean average of these values was considered as baseline surface hardness value.[7]

Phase II Demineralization phase

Artificial carious lesions were created on each enamel specimen by placing them in lactic acid as 0.1 molar amount and Carbopol 907 as 0.2%, 50% hydroxyapatite saturated in volume and adjusted to pH 5 using NaOH at 37° temp. in an incubator for 72 hrs, simulating an active area of demineralization. Vicker’s microhardness testing of the demineralized specimens were checked using same criteria as used earlier and indentations were made in the similar way below the initial hardness indentations. Mean average of these values were considered as final value for vicker’s microhardness after demineralization [7,8]

Phase III Remineralization phase

Then all the teeth were divided in to the two groups (20 each) and again subdivided into two groups (10 each); groups were subjected to different surface treatment as follow: [Table 1]

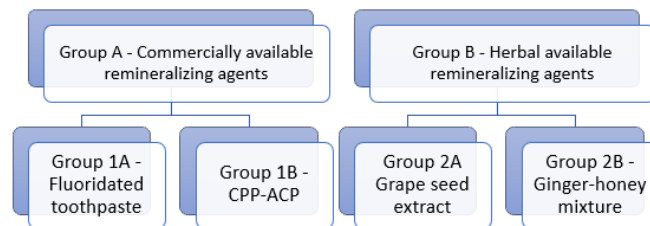


Table 1: Remineralization phase samples subjected to various groups.

Re mineralizing regimens were prepared: [7]

In Group 1A; Slurry of Fluoridated toothpaste (colgate strong teeth (1000ppm)) was prepared by mixing 15g of toothpaste in 45 ml of deionized water in the ratio of 1:3.

In Group 1B; Slurry of CPP-ACP (GC tooth mousse recaldent TM,GC company) was prepared by mixing 15g of CPP-ACP in 45 ml of deionized water in the ratio of 1:3.

In Group 2A; Grape seed extract was used directly as available (Nutribiotics Vegan GSE In Unfiltered Formula, Dietary Supplement)

In Group 2B; 8 mg of ginger powder (“our organic tree” PURE ginger) was mixed into 1 mg of honey (True elements, Organic honey) and applied on demineralized enamel specimen using microbrush.

All 10 samples were immersed in each respective group. All remineralizing regimens were prepared freshly at each application and pH cycling was done for 21 days.

After pH cycling on 21st day, Vicker’s microhardness of the remineralized specimens were checked using same criteria as used earlier while checking for baseline microhardness and the average value was considered as mean micro hardness of the corresponding specimen denoted as VHN for remineralization. [7] [Fig 2]

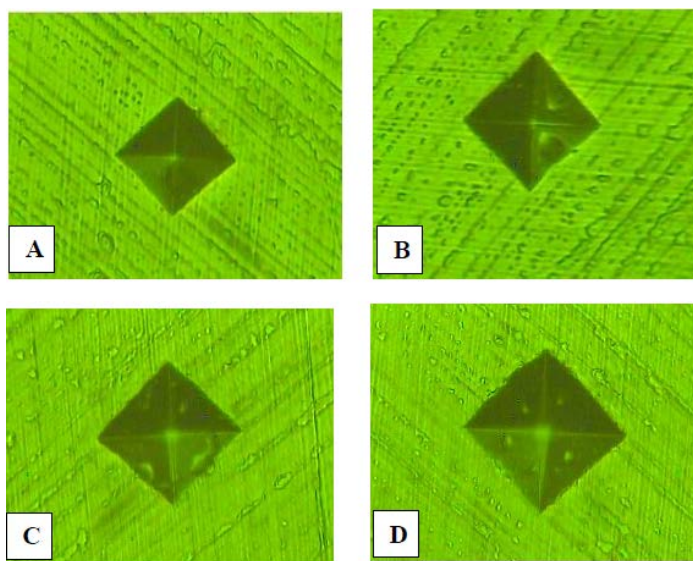


Figure 2: Microscopic image of specimen showing

indentation by micro indenter after remineralization A) Sodium fluoride B) CPP-ACP C) GSE D) Ginger-honey.

Statistical Analysis

Data obtained was compiled on a MS Office Excel Sheet and was subjected to statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM). Inter group and intra group comparison (>2 groups) was done using one way ANOVA followed by pair wise comparison using post hoc test. For all the statistical tests, $p < 0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

Results

Intergroup shows the comparison of mean values for all groups at different phases i.e. initial hardness, after demineralization and post pH cycling (Remineralization) reveals that, there was a statistically significant difference between the groups after remineralization. ($p < 0.01, 0.05$). For Baseline to demineralization with higher difference in the values for group 1B and least in group 2A and demineralization to remineralization with higher difference in the values for group 2A and least in group 2B. [Fig 3]

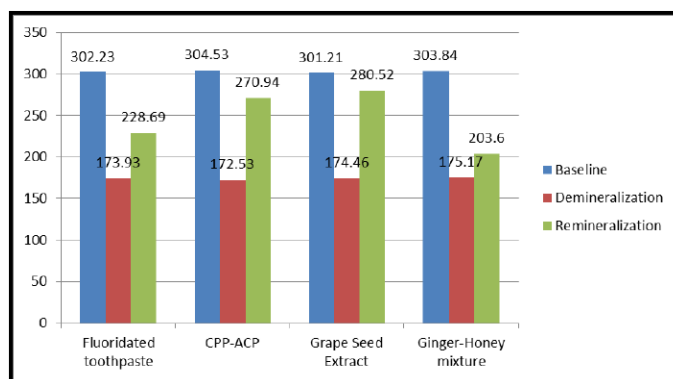


Figure 3: Inter group comparison of Vickers’s microhardness at different phases (Baseline, demineralization and remineralization)

In Intragroup comparison in Group 1 between mean of Fluoridated toothpaste group (Group 1A) and CPP-ACP

group (Group 1B) at different phases shows that there was a statistically significant difference ($p = 0.001$) After remineralization mean values for fluoridated toothpaste and CPP-ACP were 228.69 and 270.94 unit respectively. Hence it can be inferred that, remineralization has occurred more in the CPP-ACP group (Group 1B)

Similarly, Intra group comparison of Vickers micro hardness between Group 2 (Grape seed extract and Ginger- honey mixture) after remineralization. The mean remineralization for Grape seed extract and Ginger- honey mixture were 280.52 and 203.6 unit respectively. The t-value was 77.36 for the mean difference between Grape seed extract and Ginger- honey mixture were significant ($p = 0.001$). Hence it shows that, remineralization has occurred more in the Grape seed extract group. (Group 2A)

Inter group comparison of Vickers micro hardness between Fluoridated toothpaste and Ginger- honey mixture after remineralization showed statistically significant difference ($p = 0.001$) showing more occurrence of remineralization in the Ginger- honey mixture group (Group 1A)

However, intergroup comparison of CPP-ACP and Grape seed extract were non significant ($p = 0.07$). Thus states that remineralization has occurred more in the Grape seed extract group (Group 2A)

Discussion

‘Minimal intervention dentistry’ has been gaining immense topic of interest especially when it comes to remineralization of early carious lesions, advocating a biological or therapeutic approach for early surface lesions.[9]

As we all know demineralization mainly occurs when tooth is exposed to an acid attack causing dissolution of minerals by transportation of calcium and phosphate ions to the plaque till equilibrium. As H^+ ion concentration

gradient is the main driving force for the demineralizing process, an increase in pH of the demineralizing medium will reduce the driving force for H^+ transport and hence could diminish the rate of demineralization. Thus the caries can be arrested or repaired.[10]

Remineralization is a process, whereby calcium and phosphate ions are supplied from an external source to the tooth, thereby converting ion deposition into crystal voids in demineralized enamel, thus producing net mineral gain.

[2] Thus it can be possible with a variety of remineralizing agents such as fluoride, CPP-ACP, self-assembling peptide, calcium glycerophosphate (CaGP), bioactive glass, tricalcium phosphate, xylitol and among natural food sources, ginger and honey mixture and rosemary or some medicinal plant extracts like grape seed extract which are natural herbals helping in remineralization of teeth.

In the present study Fluoridated toothpaste {colgate strong teeth (1000ppm)} was chosen as it has been seen that the cariostatic efficacy was declined by use of flourides. Fluorides act by increasing enamel resistance, increased rate of maturation, remineralization of incipient lesions, interference with microorganisms and improved tooth morphology.[5] The effectiveness of topical sodium fluoride (NaF) as a cariostatic agent has been established well, and professional topical NaF applications are commonly used to arrest the progression of active caries.[5]

Also anticariogenic properties of milk and milk products such as cheese have been shown in human and animal models.[10-12] and has been suggested that milk derived, Casein phosphopeptides (CPP), have the ability to stabilize calcium phosphate in solution through binding amorphous calcium phosphate (ACP) with their multiple phosphoserine residues allowing the formation of small CPP-ACP clusters which have an anti-caries protective

effect, thus suppressing demineralization, enhancing remineralization, or possibly both.[13]

In the current study 33% of GSE was used which consists of proanthocyanidin (PA). It is commonly used as natural antioxidants and free radical scavengers. GSE is a rich source of PA and has been reported to strengthen collagen-based tissues by increasing collagen cross-links. It contributes to not only the deposition of mineral on the superficial layer of the lesion but also may interact with the organic portion of enamel and dentin through PA-collagen which interacts stabilizing the exposed collagen matrix. It also inhibits the surface-adsorbed glucosyltransferases and acid production by *S. mutans*. [14,15,16]

Thus the present study focused on comparing the remineralizing efficacy of Fluoridated toothpaste Casein phosphopeptide amorphous calcium phosphate phosphate Grape seed extract, Ginger and Honey mixture as there are no studies comparing these four agents.

The surface microhardness was measured using Vickers micro hardness tester (Omni Tech, Model-S.Auto, Pune) as it is quick, easy, feasible and very simple to perform and is cost effective as compared to other techniques.[17] Also the demineralization solution used was lactic acid as 0.1 molar amount and Carbopol 907 as 0.2%, 50% hydroxyapatite saturated in volume and adjusted to pH 5.0 using NaOH at 370 C for 72 hrs which was in accordance with studies by Silva A P et al in 2015 [8] and White DJ in 1987 [18], have shown that demineralization of tooth with this solution for continuous exposure for 3 days, would stimulate a severe demineralization and magnifying the effects of each treatment on enamel [8,19]

Reynolds et al in 1997 [11] stated that CPP-ACP are able to consume the acid generated during enamel lesion remineralization by generating more calcium and

phosphate ions thus maintaining the high concentration gradient into the lesion thus helping in remineralization.

A study conducted by Agnihotri Y et al in 2012 compared the remineralization efficacy of CPP-ACP and fluoride and showed both can inhibit demineralization and enhance remineralization with CPPACP having more remineralizing potential which were in accordance with the present study results.[20]

Kumar VL et al (2008) [21] compared CPP-ACP containing Tooth Mousse on the remineralization of enamel lesions and comparing its efficacy to that of fluoride-containing toothpaste on molars. They stated that CPP-ACP has more remineralization capacity as compared to sodium fluoride. This was in accordance with the present study results. Also, Similar was found in study conducted by Yamaguchi K et al (2006) [13] and Jo SY et al (2014) [22]

Mirkarimi M et al (2013) conducted study to evaluate the effect of grape seed extract (GSE) on artificial enamel caries in primary human teeth and stated that GSE enhanced the remineralization process of artificial enamel lesions.[17] They found that the morphology of GSE treated enamel was clearly different and there were deposits of scaffolding insoluble complexes on the enamel surface. Similarly in the study by Xie Q et al (2008) [16] evaluated remineralization effects of grape seed extract on artificial root caries and concluded that 6.5% GSE is more effective in remineralization when compared with 1000 ppm fluoride (NaF) in accordance with the present study.

In the current study ginger-honey mixture has shown minimum remineralization or less than sodium fluoride and any other groups which may be because addition of honey, the content of fluoride gets decreased to 23.7 mg/kg which indirectly decreases the capacity for remineralization. [23,24]

Conclusion

Considering the results of this study it can be concluded that the GSE helps in remineralization of artificial carious lesions on primary molars making it a promising naturally occurring remineralizing agent and can be used as an alternative to CPP-ACP or fluoride. Also, CPP-ACP is a good remineralizing agent as it has shown significant remineralization as compared to sodium fluoride and Ginger-honey mixture.

Limitations

This study was done under *in vitro* conditions which may not simulate the complex oral conditions where demineralization and remineralization is a constant cyclic process. Thus further studies should be conducted using *in vivo* or *ex vivo* models for a closer simulation of oral environment to draw firm conclusion concerning the efficacy of these agents in natural oral conditions.

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