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An *in vitro* Assessment of Remineralization Capacity of MI Varnish, Whey Extract, and Xylitol Mouth Wash on Demineralized Enamel

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ABSTRACT

Introduction: The remineralization of the initial carious area can be accomplished by therapeutic or biological methods rather than the conventional surgical approaches. One of the key fundamentals of a biological approach is the practice and application of remineralizing agents to the tooth. Objective: To assess the remineralizing potential of natural product, whey extract, and to compare it with the commercially available MI varnish and Xylitol mouthwash.

Methods: 60 maxillary premolar teeth indicated for orthodontic extraction purpose was subjected to demineralization and were randomly divided into 3 groups of 20 samples in each group: Group 1 – Whey Extract, Group 2 – MI Varnish and Group 3 – Xylitol Mouthwash. The whole experimental process was standardized by taking great attention on sectioning and regarding the treatment of groups with respective remineralizing agents and. All the samples were analyzed by using CFLSM (Confocal Laser Scanning Microscope). The results obtained were evaluated statistically.

Results: The present study displayed that remineralization potential was maximum in Group 2 followed by Group 1 and finally Group 3 during 1st week and 2nd week but the remineralization potential during 4th week was improved in Group 1 followed by Group 2 and 3.

Conclusion: Whey extract can be used as a natural remineralizing agent as a substitute to fluoride and has long-lasting remineralization potential compared to MI varnish and Xylitol mouth wash.

Key Words: Enamel, Confocal Laser Scanning Microscope, Remineralization, Whey extract, Xylitol

INTRODUCTION

Dental caries is a most communal microbial disease affecting mankind. It is a burden on public health worldwide and affecting various rural and urban communities.¹ The prevalence of dental caries in developing countries like India has shown results ranging from 31.5% to 89%.² The earliest sign of a new carious lesion is the appearance of a chalky 'white spot' on the tooth surface, indicating an area of demineralization of enamel.³

The remineralization of early carious lesions can be done by biological or therapeutic approaches rather than the traditional surgical approaches. One of the important elements of a biological approach is the practice and application of remineralizing agents to the tooth. These agents aim at controlling the demineralization and remineralization cycles, dependent upon the microenvironment around the tooth.⁴ Naturally available dairy products such as yoghurt, bovine milk, and cheese extract are proved to be effective in preventing dental caries.⁵ Their main function is to stabilize

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high concentrations of calcium and phosphate ions at the tooth surface binding to pellicle and plaque which provides a highly effective means of elevating calcium levels in dental plaque, something which is desirable for enhancing remineralization.⁶⁻⁸

Therefore, this study was done to identify the remineralizing potential of natural product, whey extract, and to compare it with the commercially available MI varnish and Xylitol mouthwash.

MATERIALS AND METHODS

This *in vitro* study was done in the Department of Pedodontics and Preventive dentistry after obtaining approval from the institutional ethics committee. Sixty maxillary premolar teeth indicated for orthodontic extraction purpose with sound enamel and dentin without any structural defects and caries lesions were included and teeth with white spot lesion, initial cavitated lesions and with cracks and other external structural defects were excluded. The roots of the selected teeth samples were dissected using a diamond disc hand motor. The crown portions were then sectioned mesiodistally into buccal and lingual halves to obtain 90 samples. A window of 4x4 mm was created by on the centre of enamel surfaces of the sectioned samples. The remaining surfaces were coated with an acid-resistant nail varnish and allowed to dry. After drying the samples were placed in a demineralization solution in 4 days. 60 teeth subjected to demineralization followed by remineralisation with respective a remineralising agents with 20 samples in each; Group 1 – Whey Extract, Group 2 – MI Varnish AND Group 3 – Xylitol Mouthwash. Commercially available MI Varnish and Xylitol Mouthwash were used for the study. Whey Extract was prepared manually.

Demineralization solution

The solution used was made up of analytical grade chemicals and distilled water. It contains 2.2 mM calcium chloride ($\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$), 2.2 mM monosodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot 7 \text{H}_2\text{O}$) and 0.05 M lactic acid. The final pH was made to 4.5 with 50% sodium hydroxide (NaOH).

Artificial saliva used was made up of, 0.381 g/L sodium chloride, 2.200g/L gastric mucin, 0.213g/L calcium chloride, 1.114 g/L potassium chloride and 0.738 g/L potassium hydrogen phosphate. 0.01 mM of Rhodamine B dye was prepared by adding 23.95 mg of Rhodamine B into 500 ml of distilled water. The remineralisation capacity of each agent was evaluated at 1st, 2nd and 4th days after remineralisation using Confocal Laser Scanning Microscopic evaluation (CFLSM).

Whey Extract (Supernatant)

Whey was prepared from yoghurt by centrifugation at 4000 rpm at 25°C for 10 min. After 3 rounds of centrifugation, yo-

ghurt was separated into 2 fractions. The insoluble fraction at the bottom is yoghurt precipitate and the soluble fraction containing CPPs remains in the suspension as whey (supernatant).

Confocal Laser Scanning Microscopic evaluation

The teeth samples from experimental groups were kept in Rhodamine B dye for 24 hours. Then the stained sections were placed on the microscopic glass slide, and by using a 10x objective. Cross-sectional analysis of enamel lesions and the remineralized samples with Confocal Laser Scanning Microscopic (CFLSM) were based upon digital images taken at specific controlled conditions. Analysis of all samples was done with a Carl-Zeiss CFLSM. The accompanied software (Image J program) calculated image-based parameters of selected lesion zones.

Sound enamel (untreated specimen) records near-zero fluorescence (grayscale ~ 0) and appears pitch black. Lesions (demineralized specimens) slight autofluorescence but the inhibition of Rhodamine B dye (0.1mM) allows the porous demineralized layer to fill and appear with considerable contrast (higher gray values) which are revealing of less porosity and dye penetration or more mineral.

The obtained data were statistically analyzed using SPSS version 20.0. Qualitative data will be expressed in percentage and quantitative as mean (SD). One way ANOVA followed by Tukey's post hoc analysis was done for comparison of three groups. The paired t-test was used for within-group comparison at various intervals. a p-value less than 0.05 was considered to be statistically significant.

RESULTS

Table 1 indicates the remineralisation potential of various groups at the time interval of 1st, 2nd and 4th week. Group I why extract showed a mean value of 54.35, 54.52, and 48.42 respectively at 1st, 2nd and 4th-week duration, similarly group II MI varnish showed 64.56, 56.29 and 40.57 respectively. Group III showed 50.34, 47.07 and 39.57 at 1st, 2nd and 4th week. It indicates that Why extract was better than the other two groups after 4 weeks in remineralization potential. The difference was statistically significant at 1st week (0.01) but insignificant at 4th week. The present study showed that remineralization potential was maximum in Group 2 followed by Group 1 and finally Group 3 during 1st week and 2nd week but the remineralization potential during 4th week was better in Group 1 followed by Group 2 and 3.

Table 2 indicates the Comparison of intragroup remineralization potential in Group I. The difference was significant between 1st and 4th week of remineralization potential ($P < 0.05$).

DISCUSSION

In the present study was done to evaluate and compare the remineralization potential of Whey Extract which is a diary product containing CPP-ACP (Casein Phosphopeptide-Amorphous Calcium Phosphate), MI varnish and Xylitol mouthwash, and they exhibited remineralization potential which is in concurrence with the other studies on whey extract,^{5,7,8} MI varnish⁹ and xylitol mouthwash showing their remineralization potential on artificially demineralized enamel.^{12,13}

In our study, the remineralization potential of CPP-ACP and CPP-ACPF decreased gradually from 1st week to 4th week. They are found to act by binding at a location inside the enamel subsurface lesion as well as at the surface of the lesion.^{10,11} Once it is present in the enamel subsurface lesion, the CPP-ACP and CPP-ACPF would release weakly bound calcium, phosphate and fluoride ions, which would then deposit into crystal voids. The release of calcium and phosphate ions would be thermodynamically driven. The CPPs have a high binding affinity for apatite; therefore, on entering the lesion, the CPPs would bind to the more thermodynamically favoured surface of an appetite crystal face. The CPPs, once bound to apatite crystals in the enamel subsurface lesion, may have an important role in regulating anisotropic crystal growth and also inhibiting crystal demineralization.^{14,15} Xylitol also showed a reduction in remineralization potential from 1st to 4th week.

The superior remineralization potential of MI varnish i.e., fluoride enriched CPP-ACP might be due to the availability of calcium, phosphate and fluoride in one product. The CPP-ACPF acts by its synergistic effect of CPP-ACP and fluoride in reducing caries experience attributable to the formation of CPP stabilized ACP, resulting in the increased incorporation of fluoride ions into plaque, together with increased concentrations of bioavailable calcium and phosphate ions.⁹ A similar study reported by Reynolds et al. showed that a dentifrice formulation containing 2% CPP-ACP nano complex plus 1100 ppm fluoride (CPP-ACPF) had shown to be superior to dentifrice containing only 1100 ppm F in the remineralization of enamel subsurface lesions in situ with a mineral that was more resistant to acid challenge.⁸

Following MI varnish, whey extract (CPP-ACP) showed better remineralization potential. CPP-ACP a nano complex derived from bovine milk protein (casein calcium) was first postulated as a remineralizing agent by Reynolds in 1988.¹⁶ The anti-cariogenic mechanism of this nano complex is achieved by incorporation of ACP into plaque and on to the tooth surface. The CPP acts as an ACP carrier localizing the highly soluble calcium phosphate phase on the tooth.^{15,16}

Rezvani et al.⁵ done a comparative study to assess the effects of enamel microhardness using whey extract and tooth

mousse and found that the microhardness increased in each group and between the groups and they concluded that the effect of whey on increasing the enamel microhardness was more than that of tooth mousse.

Xylitol is the other agent used in the study which also exhibited remineralization potential, as it cannot be converted into harmful lactic acid by cariogenic bacteria. Thus the harmful effects of the bacteria and the metabolic product (lactic acid) can be significantly reduced allowing the mouth to naturally remineralize damaged teeth with less interruption.¹⁷ Miake et al. done a study on demineralized teeth and observed that xylitol can help in remineralization which is consistent with the result obtained from this study. In that study, 1 week of xylitol chewing gum consumption has significantly increased the mineral content in enamel subsurface lesions.¹⁸

Conversely, this in vitro study had certain drawbacks such as difficulty in simulating the oral environment due to variable level of salivary proteins and salivary flow rate, lack of bacteria in artificial saliva solution and a harsher acidogenic challenge used for a shorter period. Even though MI varnish has got better remineralization potential over whey extract and xylitol mouth wash, long term in vivo studies needed to be carried out to reconfirm the results.

CONCLUSION

It was concluded that MI varnish, Whey extract and Xylitol mouthwash are effective in remineralizing demineralized enamel surfaces. Whey extract can be used as a natural remineralizing agent as an alternative to fluoride and has long-lasting remineralization potential compared to MI varnish and Xylitol mouth wash.

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Author contribution

1. Shreya N Shah- editing
2. Vinola2, - final approval, drafting manuscript
3. Suresh Kumar Vasaviah3,- manuscript preparation
4. Baby John J - data evaluation
5. Kavya Bhola- analysis
6. Prabu Mahin Syed Ismail- editing

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Table 1: Remineralization potential of all 3 groups during 1st, 2nd and 4th week

Materials	1 st week		2 nd week		4 th week	
	Mean	SD	Mean	SD	Mean	SD
Group ₁ (Whey Extract)	55.43	7.83	54.56	9.63	49.03	7.94
Group ₂ (MI Varnish)	62.68 ^a	9.58	56.29	10.34	41.04	12.58
Group ₃ (Xylitol MouthWash)	50.57 ^a	7.67	48.08	6.16	39.51	7.12
p value	0.01 [*]	-	0.11	-	0.07	-

Table 2: Comparison of remineralization potential of Group₁ (Whey Extract)

Intra group comparison	Mean difference	p-value
1 st week -2 nd week	0.84	0.72
1 st week -4 th week	6.30	0.02 [*]