Assessment of Remineralisation Potential of Cranberry Extract and Grape Seed Extract Dentifrice on Primary Teeth Using Laser Fluorescence Device

C. Pushpalatha, N. Sneha, R. Deveswaran, and Latha Anandakrishna

Abstract Early detection of dental caries with optical instruments and promoting remineralisation is one of the measures in the prevention of early carious lesion. The present study was conducted to assess and compare the remineralisation potential of cranberry extract and grape seed extract dentifrice on primary teeth using DIAGNOdent pen. 20 over retained non-carious primary teeth with intact crown were extracted and sectioned into coronal and root part at cementoenamel junction. They were placed in demineralising solution for 96 h. The demineralized teeth were randomly divided into the following four groups. Group 1 was control group, Group 2 was Cranberry extract dentifrice, Group 3 was Grape seed extract dentifrice and Group 4 was Fluoridated dentifrice. Demineralised enamel blocks were then brushed with their respective dentifrice twice daily at an interval of 12 h for 2 min using soft bristle toothbrush over the demineralised area for 7 days. Remineralisation was assessed using DIAGNOdent pen after 7 days and subjected to statistical analysis using one-way ANOVA test and Tukey's HSD Post Hoc analysis. Results revealed that cranberry extract dentifrice (4.2 ± 0.4) had maximum remineralisation of demineralised primary enamel followed by grape seed extract dentifrice (4.8 ± 0.8) and then fluoridated dentifrice (5.0 ± 1.2). Hence cranberry and grape seed extract dentifrice are promising novel natural non-fluoridated remineralizing dentifrice for primary teeth.

Keywords DIAGNOdent · Cranberry extract · Grape seed extract

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1 Introduction

Fluoride in the form of dentifrice, rinses and varnish has declined dental caries by promoting remineralisation of tooth structures by utilizing calcium and phosphate ions present in the saliva. Fluoride in the early stages of caries development reduces demineralization and enhances remineralisation by crystal growth and formed crystal is more resistant to acid attack. However, the ingestion of fluoride in preschool children and in children with special health care needs is used in caution due to inability to expectorate as a result of poor development of oral musculature, this leads to excessive fluoride in the blood plasma resulting in dental and skeletal fluorosis where the outer portion (enamel) of the teeth and bones in the body becomes brittle and weak if consumed over a long period of time [1–3]. Therefore, several new non-fluoridated remineralising agents have been studied since last decade [4]. Literature supports that herbal extracts rich in proanthocyanidins are gaining a lot of attention in the prevention of dental caries especially grape seed extract and cranberry extract. These proanthocyanidins rich extract are reported to possess antibacterial, antioxidant, anti-adhesion and anti-inflammatory activities. Grape seed extract has remineralisation properties on both enamel and dentine in primary and permanent teeth [4–7]. Whereas remineralisation potential of cranberry extract is still not explored either in primary or permanent teeth. There are no study reports of grape seed extract and cranberry extract in dental formulation like dentifrice. Hence the present study was designed to assess and compare the remineralisation potential of cranberry extract and grape seed extract dentifrice on primary teeth using DIAGNOdent pen (KaVo Germany).

2 Materials and Methodology

2.1 Materials

Cranberry extract powder and Grape seed extract powder were procured from HerboNutra, Delhi. Carbopol 140 and Xanthan gum were purchased from Micromaster Pvt Ltd., Mumbai. Ethanol was purchased from Rankem, Mumbai. All other reagents used were of analytical grade.

2.2 Preparation of Enamel Blocks

The current in vitro study was approved by institutional ethics committee. The teeth samples of 20 over retained non-carious primary teeth with intact crown which were extracted after informed consent from patient for therapeutic purpose were collected. Teeth with any dental anomalies and cracks were excluded from the study. Teeth were sectioned into coronal and apical halves. The coronal part of the tooth was used for the study. The teeth were cleaned and polished with pumice using prophylactic brush.

The teeth were then randomly divided into 4 experimental groups of 5 teeth in each group. The groups were Group 1 (Control), Group 2 (Cranberry extract dentifrice), Group 3 (Grape seed extract dentifrice) and Group 4 (Fluoridated dentifrice). The samples were covered with two coats of acid resistant nail varnish except for a window of 3 × 3 mm on the buccal surface [8, 9].

2.3 Preparation of Demineralization Solution

The demineralizing solution of pH 4.5 was prepared by dissolving one pH buffer capsule of pH 4.0 in 100 ml of deionized water by stirring until the capsule got completely dissolved. Digital pH meter was used to check the pH value.

2.4 Demineralization of the Samples

Baseline values of all the samples were assessed with DIAGNOdent pen. Samples were then immersed individually into four separate 500 ml circular polyvinyl containers each containing 100 ml demineralizing solution for a period of 96 h at 37 °C.

2.5 Preparation of Cranberry Extract and Grape Seed Extract Dentifrice

Required quantity of xanthan gum was mixed with glycerine and water, stirred for 5 min and set aside for 30 min. Grape seed extract powder and cranberry extract powder were taken separately in a pestle and mortar. Calcium carbonate and Dicalcium phosphate was added to this and triturated till fine powder was obtained. To this fine powder, glycerine mixture was added and blended evenly to form a paste. Methylparaben and flavouring agent were added to the paste and mixed thoroughly. Required quantity of glycerine was added to obtain desired consistency. A total of 6 formulations were prepared for both cranberry and grape seed extract and the one that had better consistency was chosen for further studies.

2.6 Remineralisation of Teeth

The demineralized samples were mounted on the acrylic resin blocks. The readings of demineralised blocks were examined using DIAGNOdent pen. Enamel blocks were brushed with their respective toothpaste twice daily for 2 min at an interval of 12 h using soft bristle toothbrush in a circular motion over the demineralised area. Dentifrice was washed from the sample thoroughly with water. The samples were dried. This procedure was carried out for 7 days. At the end of the 7th day, remineralisation was checked using DIAGNOdent pen for all the groups.

3 Statistical Analysis

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) of Windows Version 22.0. One-way ANOVA test followed by Tukey's HSD post hoc analysis was used to compare the mean moment values between the study groups. The level of significance was set at $P < 0.05$. Bonferroni's Post hoc Analysis was used to compare the mean moment values between different time intervals in each study group.

4 Results and Discussion

See Tables 1, 2 and 3.

Formulation F3 of Cranberry and F6 of grape seed extract were chosen for the study as they had better consistency in comparison to other formulations. To detect and quantify the carious lesions on occlusal and smooth surface, the in vitro studies have shown that original laser device (DIAGNOdent 2095) has good performance and reproducibility [10]. The literature review on Laser fluorescence device suggests that sensitivity values for detection of caries ranged from 0.19 to 1.0 which is high. Whereas, the sensitivity values ranged from 0.52 to 1.0 have shown that laser fluorescence device exhibited almost high sensitivity and almost low specificity in comparison to visual assessment method. The latest version of laser fluorescence pen (DIAGNOdent pen) showed sensitivity value of 0.67 and specificity was 0.79 for enamel and dentinal caries at cut-off value of 25 which was a threshold value.

Table 1 Composition of formulations

Ingredients	Grape seed extract dentifrice			Cranberry extract dentifrice		
	Qty in g			Qty in g		
	F1	F2	F3	F4	F5	F6
Grape seed extract powder	10.0	10.0	10.0	–	–	–
Cranberry seed extract powder	–	–	–	10.0	10.0	10.0
Xanthan gum	1.0	1.25	1.5	1.0	1.25	1.5
Calcium carbonate	5.0	5.0	5.0	5.0	5.0	5.0
Glycerine (ml)	5	5	5	5	5	5
Flavour	0.5	0.5	0.5	0.5	0.5	0.5
Methyl paraben	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	q.s to 50 g	q.s to 50 g	q.s to 50 g	q.s to 50 g	q.s to 50 g	q.s to 50 g

Table 2 Mean DIAGNOdent pen moment values for different study groups at different treatment procedures

Time	Groups	*N*	Mean ± SD	*P*-value
Baseline	Group 1	5	6.6 ± 1.7	0.689
	Group 2 (F3)	5	6.2 ± 1.6	
	Group 3 (F6)	5	7.0 ± 2.1	
	Group 4	5	5.8 ± 0.8	
After demineralisation	Group 1	5	12.2 ± 2.6	
	Group 2 (F3)	5	10.4 ± 1.3	0.529
	Group 3 (F6)	5	11.4 ± 2.3	
	Group 4	5	10.8 ± 1.5	
After remineralisation	Group 1	5	10.2 ± 0.4	<0.001*
	Group 2 (F3)	5	4.2 ± 0.4	
	Group 3 (F6)	5	4.8 ± 0.8	
	Group 4	5	5.0 ± 1.2	

*Statistically significant

Table 3 Comparison of DIAGNOdent pen moment values for all the three groups at baseline, after demineralization and after remineralisation

Group	Time	*N*	Mean ± SD	*P*-value[a]	Sig. diff	*P*-value[b]
Group 1	BL	5	6.6 ± 1.7	0.03*	BL versus AD	0.11
	AD	5	12.2 ± 2.6		BL versus AR	0.04*
	AR	5	10.2 ± 0.4		AD versus AR	0.39
Group 2 (F3)	BL	5	6.2 ± 1.6		BL versus AD	0.001*
	AD	5	10.4 ± 1.3	<0.001*	BL versus AR	0.14
	AR	5	4.2 ± 0.4		AD versus AR	0.001*
Group 3 (F6)	BL	5	7.0 ± 2.1		BL versus AD	0.003*
	AD	5	11.4 ± 2.3	<0.001*	BL versus AR	0.12
	AR	5	4.8 ± 0.8		AD versus AR	0.003*
Group 4	BL	5	5.8 ± 0.8		BL versus AD	0.006*
	AD	5	10.8 ± 1.5	0.001*	BL versus AR	1.00
	AR	5	5.0 ± 1.2		AD versus AR	0.01*

BL: Baseline, *AD*: After Demineralisation, *AR*: After Remineralisation
*Statistically significant

These laser devices are used to supervise the carious lesion and to check the effect of preventive interventions [11]. Hence for the present study, the latest version of laser fluorescence device, i.e. DIAGNOdent pen was used to assess the outcome of the remineralisation potential of novel dentifrices on initial caries lesion. In the present era of minimal intervention of dentistry, a variety of natural compounds have been

refined to develop remineralizing agents in prevention of caries. Proanthocyanidins is a natural plant metabolite present in fruits, vegetables, nuts, seeds, flowers and barks. Proanthocyanidins have been certified to be safe as a dietary supplement and in different clinical applications. Proanthocyanidins from cranberry extract and grape seed extract have shown to have antibacterial, anti-inflammatory and antioxidant properties [5]. Grape seed extract also exhibits remineralizing action on the superficial layer of the lesion through mineral deposition by combining with the calcium at pH of 7.4 leading to the formation of insoluble complex [7]. Since there were no studies on remineralisation of potential of cranberry extract. Hence in the previous study, the concentration of cranberry extract and grape seed extract in intracanal medicament was determined and assessed for antibacterial and surface microhardness. The results showed 95% bacterial reduction in cranberry and grape seed extract groups where chlorhexidine group (control) showed 99% bacterial reduction. There was an increase in microhardness in both cranberry extract and grape seed extract intracanal medicament groups in comparison to chlorhexidine group showing no change [12]. In the present study, the same concentration of cranberry extract and grape seed extract was used to formulate novel dentifrices. In the present experimental study, DIAGNOdent measurement was obtained at three different time intervals before demineralisation, after demineralisation and after remineralisation. The baseline data using DIAGNOdent pen the mean moment value for Group 1 was 6.6 ± 1.7, Group 2 was 6.2 ± 1.and Group 3 was 5.8 ± 0.8 showing statistically no significant difference ($P < 0.05$). Whereas in Group 1, there was no statistical significance difference ($P < 0.05$). The results of the present study suggest that after 7 days of application of cranberry extract dentifrice, Grape seed extract dentifrice and fluoridated dentifrice onto the tooth surface, there was a noticeable remineralisation of initial carious lesion. This suggests that cranberry extract dentifrice and Grape seed extract dentifrice helps in mineral deposition on the superficial layer of the lesion. The present study correlates with our previous study in which there was a 95% increase in microhardness in both intracanal medicaments containing cranberry extract and Grape seed extract [12]. The results of the present study indicate that both cranberry extract and grape seed extract dentifrices can be used for enamel remineralisation in the case of initial caries lesion. However, these novel dentifrices should be used with caution clinically since in vitro remineralisation is different when compared with dynamic complex biological system.

5 Conclusion

In conclusion, this work assessed and compared the remineralisation potential of cranberry extract and grape seed extract dentifrice on primary teeth using DIAGNOdent pen. Both cranberry extract dentifrice and grape seed extract dentifrice are promising alternatives to enhance the remineralisation of artificially induced enamel caries lesion in primary teeth. These novel dentifrices might be an effective natural dentifrice for non-invasive therapy for the reduction of initial enamel caries.

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