

Remineralisation Potential Of Grape Seed, Ginger Honey - An In vitro Study

Research Article

Vijayapriyanga Senthilkumar¹, Sindhu Ramesh^{2*}

¹ Postgraduate Student, Department of Conservative Dentistry and Endodontics, Saveetha Institute Of Medical And Technical Science, Saveetha Dental College, Saveetha University, 162, Poonamallee High Road, Chennai 600077, Tamil Nadu, India.

² Professor and Head, Department of Conservative Dentistry and Endodontics, Saveetha Institute Of Medical And Technical Science, Saveetha Dental College, Saveetha University, 162, Poonamallee High Road, Chennai 600077, Tamil Nadu, India.

Abstract

Introduction: Remineralization is an effective treatment that may reverse or stop the caries progression in earlier stage. In recent years, the grape seed extract is the potential remineralising agent under investigation.

Materials and Methods: The 60 extracted tooth were obtained, a window of 3mm×4mm was prepared at the cervical portion of the tooth. The roots were sectioned and sealing of the tooth except for window is done with nail varnish. Demineralization was done with lactic acid and remineralization protocols were done with respect to their groups CPP-ACP, Gingerpowder+ honey and grape seed extract with 20 tooth per group. Then teeth were subjected to microhardness test and imaging was done with fluorescence spectroscopy.

Results: The results were analysed using one-way ANOVA with dunnett's test. The results showed that grape seed extract was significantly better than other groups, followed by ginger powder and honey and CPP-ACP paste.

Keywords: Caries; Demineralization; Ginger Honey; Grape Seed; Microhardness; Remineralisation.

Introduction

Minimal invasive dentistry is a conservative approach to preserve the remaining tooth structure as much as possible, in that path of conservative approach comes the remineralisation of caries [1, 2]. Caries starts as a demineralization and then which is followed by organic matter and invasion of microorganisms [3-7]. Despite of world wide improvements in oral health, dental caries is the major problem in most of the countries which affects about 60-90% of school children. The current concept in cariogenesis is that due to continuous alteration in PH the demineralization-remineralisation cycle takes place.

Remineralisation is possible only when we are able to control the pH in favour of remineralisation, that is the resting PH should be stable [8, 9]. This induce the partially dissolved crystals to grow by accretion of calcium and phosphate ions from the remineralising agent. It is usually the natural repair process that counteracts the

cariogenic progress [9, 10].

Various agents are implemented in past few decades to remineralize, minimize or delay the progression of caries like fluoride, Casein Phosphopeptide - Amorphous Calcium Phosphate(CPP-ACP), tri calcium phosphate [11-14] etc. Due to various other factors influencing on caries, permanent solution for caries is still not achievable. Caries is one of the main factor which is progressing to other pulp and periapical diseases. If we could able to stop or remineralize the caries, the progression of caries diseases could be kept in control, there by preventing the tooth from undergoing deep caries leading to pulpal involvement and increasing the oral hygiene etc.

The fluoride reacts with oral fluids and combine with calcium and phosphate ions to form fluorapatite crystals, the anticaries effect of fluoride depends on the use of concentration and frequency of application.

*Corresponding Author:

Sindhu Ramesh,

Professor and Head, Department of Conservative Dentistry and Endodontics, Saveetha Institute Of Medical And Technical Science, Saveetha Dental College, Saveetha University, 162, Poonamallee High Road, Chennai 600077, Tamil Nadu, India.

Tel: +919840136543

Email Id: drsinsushil@gmail.com

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In the year 1998, Casein Phosphopeptide -Amorphous Calcium Phosphate(CPP-ACP) was introduced as a remineralizing agent. It is a milk protein combined with ACP, which promotes remineralisation by inhibiting the colonization of cariogenic bacteria [14-17].

Natural products are used in folk medicines for thousands of years, in recent years most of the researchers are concentrating on natural products for treating the diseases. As of researches done so far in all fields of treating the oral diseases, it proves to work out with the natural agents with addition of few initiators to enhance the effectiveness. Only a very few studies are reported with natural products on the demineralization and remineralization of dental hard tissues.

Naturally occurring plant metabolite Proanthocyanidins(PA) are widely available in vegetables, fruits, nuts, seeds, flowers and barks. Studies have shown that PA increased the collagen synthesis and accelerates the conversion of soluble collagen to insoluble collagen during development. Thus remineralization with grape seed extract brings changes in the organic matrix by the presence of newly induced collagen cross links [18-21]. It has biocompatibility and many beneficial biological properties, including anticariogenic, antibacterial, anti-inflammatory and immunostimulatory effects [22-25].

Zingiberofficinale (or ginger, Ginger rhizome) is most traditionally used herbs in India, this natural food source possesses antimicrobial and antifungal activities. Few studies have also reported its effect on streptococcus mutans [26-29]. One more traditionally used with herbal medicines in India is honey. It has a PH of about 3.9, which have bacteriostatic effect on pathogens. Studies have shown its efficacy on oral pathogenic bacteria [30, 31].

We have numerous highly cited publications on well designed clinical trials and lab studies [32-45]. The aim of this study is to evaluate the efficiency of remineralisation among chemical and natural remineralising agents.

Materials And Methods

Specimen collection

Extracted 60 single rooted teeth were collected. The teeth were cleaned, and the contaminants were removed with an ultrasonic scaler. The root fragments from below the cemento-enamel junction were sealed with an acid-resistant nail varnish except for 3 mm × 4 mm window and 100µm lesion was done using microtome. They were divided into three groups 20 per group, group 1 - CPP-ACP, group 2- ginger powder(1%) with honey(1ml), group 3- grape seed extract (6.5%)

Grape seed extract preparation

Grape seeds were powdered, then 100 gm were extracted with ethanol to water ratio of 70:30 v/v by maceration method. Then the extracts were filtered.

The total phenol content of grape seed extract was estimated by Folin-Ciocalteu method and processed by adding one ml of GSE

solution in acetone/water (6/4) in test tube and mixed with 0.2ml of Folin-Ciocalteu reagent for 3min. Then 1 ml of 2% (w/v) sodium carbonate was added to it. This mixture is agitated with a vortex mixer and kept in dark for 30mins then they were centrifuged at 1200g for 5 min. the absorbance of this GSE extracts and the prepared blank were measured at 750nm using spectrophotometer. The total phenolic content of GSE was 70 g of gallic acid equivalents (GAE)/100 g.

Demineralization with lactic acid

The baseline microhardness value was measured. Three groups (n=20) in each group was treated with a demineralizing solution containing undersaturated 0.1-M lactic acid buffer solution (pH 4.75) for 30 min and then placed in artificial saliva at 37°C between treatments. These procedures were conducted for 7 days.

Treatment protocol

The specimens in Group1 (n=20) were painted with CPP-ACP paste. The specimens in Group 2 (n=20) were applied with Ginger powder + honey. The specimens in Group 3 (n=20) were applied with grape seed extract for 5 minutes twice a day for 7 days. These specimens were then placed in artificial saliva throughout the duration of study to stimulate oral condition. The solution was kept at room temperature. After that each tooth was rinsed with de-ionized water for two minutes, then teeth were restored in glass container filled with (20 ml) of de-ionized water and stored at a temperature of 37°C in the incubator for the next day. The procedure was repeated daily for seven days.

Surface microhardness measurement

Surface microhardness of specimens was measured at baseline using Vickers microhardness tester. The microhardness tester is equipped with a 400X magnification lens that enables a clear view of the indentation created by the microprobe. The created indentation has 2 diameters of X and Y that are precisely measured by the device and reported as d1 and d2. The microhardness is calculated using the mean d1 and d2 and the formula below:

$D =$ The microhardness number is inversely correlated with the d value.

The greater the d1, d2 and consequently the total d value, the higher the penetration of indenter into the surface and the lower the microhardness number of the object and vice versa.

In order to confirm the accuracy of the obtained microhardness value, each specimen was tested 5 times and the mean of all values was reported as the microhardness number. During the experiment, the solutions had a temperature equal to the room temperature (approximately 30°C). After immersion for 5min, the specimens were rinsed with saline solution, dried and their surface microhardness was measured again. The specimen image was taken after microhardness measurement using fluorescence spectroscopy (Figure 1).

Statistical Analysis

Therefore, surface microhardness was measured and the data were analyzed using one-way ANOVA with Dunnett's T3 test.

p<0.05 was considered statistically significant.

Results And Discussion

The results of this *in vitro* study are shown in table 1.

The results were expressed as Mean ± SD (n=20). The *p<0.001 statistically significant as compared with after demineralization groups. #p<0.05 statistically significant as compared with CPP-ACP treatment. ap<0.001 statistically significant as compared with CPP-ACP treatment.

This study results show that all group are statistically significant with their demineralizing groups. Group 2 is statistically significant when compared to CPP-ACP (p<0.05). Grape seed extract (6.5%) is statistically significant to CPP-ACP (p<0.001).

In this study, grape seed extract of 6.5% has more remineralisation potential than the commonly used CPP-ACP, followed by ginger powder and honey combination.

Mahkameh Mirkarimi et al demonstrated that grape seed has positive effects in remineralization in an *in vitro* study conducted in human primary teeth. It is a non invasive therapy of carious lesions [19].

Cheng-fang Tang et al., demonstrated that transient GSE bio-modification may promote remineralization (mostly HA crystals) on the superficial surface of acid- etched demineralized dentine. 15% of GSE preconditioner, without PH adjustment gives the best results, that may be ascribed to its higher polyphenolic content [46].

Shiny Benjamin et al., demonstrated the mechanism of grape seed extract with the PH cycling and confocal scanning microscopy. Grape seed extract is better than fluoride and 0.5% CaGP [47].

Grape seed extract contains 98% of proanthocyanidin (PA). This proanthocyanidin is seen widely in fruits, nuts, seeds, flowers, bark and vegetables. This is a natural plant metabolite, antioxidant and a free radical scavenger. The large molecule structure of PA, bioflavinoid consists of flavin. This PA is found to produce acid production against streptococcus mutans and also increase collagen synthesis by preventing conversion of soluble collagen to insoluble collagen [46, 48].

Ginger rhizome (ginger) has been used as a food spices and medicinal plants for many centuries as tradition in India. Among all the natural food sources, ginger is found to have the more antimicrobial activities. It is a natural material showing no toxicity and are considered 'generally recognized as safe' (GRAS) by the US Food and Drug Administration (FDA). The pungent oil component in it consist a series of polyphenolic ketones with many pharmacological activities [49].

Honey has an antibacterial affect on S.mutans, L. acidophilus, A.viscosus, Paeroginosa, V.alcaligens and S.aureus. The PH of honey is 3.9, hence it is acidic and can inhibit the growth of pathogens because most thrive PH 4.0-4.5. Anyway dilution of honey with saliva will increase the PH and reduce its effect. This increases the enzyme activity and the action of enzyme glucose oxidase and production of hydrogen peroxide, which is an oxidizing agent, will increase [50, 51]. Further studies have to be conducted with incorporation of few agents in the natural remineralizing agents and to provide for clinical usage in future.

Conclusion

Within the limitations of the study, the remineralization of these study groups is greater in grape seed extract, CPP-ACP and ginger honey combination. These study shows that remineralization is achievable even with natural products like grape seed extract

Figure 1. Microhardness test fluorescent image, a diamond pyramidal indentation for Vickers hardness measurement was observed on the enamel surface.

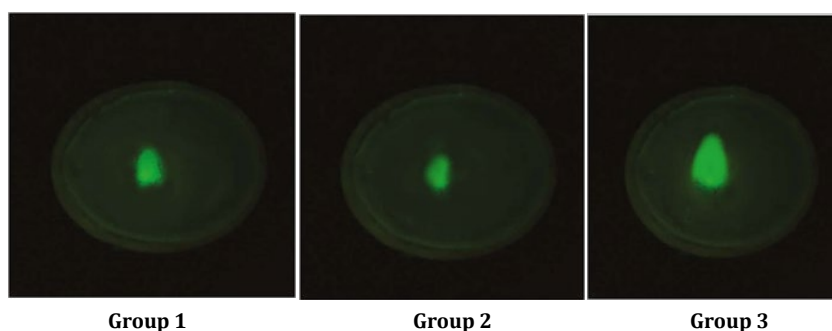


Table 1. This table shows the Micro hardness testing results of treatment groups.

Groups	Vickers hardness number (VHN)	
	After Demineralization	After Remineralization (7 days)
CPP-ACP	38.56±2.5	45.69±3.3*
Ginger powder (1%) + honey (1ml)	35.15±2.4	42.31±3.2*#
Grape seed extract (6.5%)	36.48±1.9	56.12±2.9*a

and ginger honey.

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