International Journal of Dental Research
www.dentaljournal.net
Online ISSN: 2664-9055, Print ISSN: 2664-9047
Received: 07-08-2024, Accepted: 06-09-2024, Published: 21-09-2024
Volume 6, Issue 3, 2024, Page No. 50-52

Evaluation of the Remineralizing potential of pine bark extract, Herbal Oil (Arimedadi Thailam) and Caesin Phosphopeptide-amorphous calcium phosphate on enamel: An *in vitro* study

Dr Aju Anto Puthur[1*], Dr Moksha Nayak[2], Dr Rohini B[1], Dr Gagan Gopal G[3]
[1] Department of Conservative Dentistry & Endodontics, KVG Dental College, Sullia, Karnataka, India
[2] Principal, Department of Conservative Dentistry & Endodontics, KVG Dental College, Sullia, Karnataka, India
[3] House Surgeon, Department of Conservative Dentistry & Endodontics, KVG Dental College, Sullia, Karnataka, India

Abstract
Aim: To compare remineralizing potential of novel agent- Pine bark extract and herbal oil-arimedadi thailam with conventional remineralizing agent.
Material and Methods: An *in vitro* study was done to compare remineralizing potential of Pine bark extract and herbal oil-Arimedadi thailam with conventional remineralizing agent Caesin phosphopeptide-amorphous calcium phosphate (CPP-ACP). Forty human premolars were covered with nail varnish except for 4 mm × 4 mm exposed window on the facial aspect, which was treated with 37% phosphoric acid for 30 s. Samples were then randomly divided into four groups (n = 10): Group I –Pine Bark extract, Group II –Arimedadi thailam, Group III – Casein phosphopeptide-amorphous calcium phosphate, and Group IV – Control. The samples in each group were treated with the remineralizing agent, after which they were rinsed with deionised water, dried, and stored in artificial saliva. The same procedure was repeated for the span of 14 days. Depth of demineralization and remineralization was analysed using Raman microscopy. Data were analysed using the Statistical Package SPSS 22.0 and level of significance was set at $P < 0.05$. ANOVA test was used to analyse the depth of demineralization and remineralization.
Results: The highest remineralization was observed in Group 2 (Herbal Oil), followed by Group 1 (Pine Bark Extract) and Group 3 (CPP-ACP Toothpaste), while the lowest remineralization potential was noted in Group 4 (Control).
Conclusion: Herbal oil- Arimedadi Thailam used in this study showed better remineralizing potential than pine bark extract and conventional remineralization agent. However, further clinical studies with greater sample size have to be performed to invite greater research about the use of this novel agent in dentistry.

Keywords: Remineralizing agent, herbal oil, pine bark extract, CPP-ACP

Introduction

Dental caries is a biofilm-mediated, diet modulated, multifactorial, noncommunicable, dynamic disease resulting in the net mineral loss of dental hard tissues. [1]
Cariogenic bacteria, fermentable carbohydrates, and salivary dysfunction are scientifically proven as the important pathological factors. [2]
Demineralization and remineralization are balanced processes that normally occur in the oral cavity. Diet variations, oral hygiene, or microbial activity can lead to the predominance of demineralization. Remineralization is favoured by the buffering action of saliva, permitting calcium, and phosphate ions to precipitate onto the tooth and form new mineral. Therefore, modulation of the demineralization-remineralization balance is the key to prevention of dental caries. [3]
Various remineralizing agents such as fluorides, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), self-assembling peptides are used to revert initial noncavitated carious lesion. The direction toward herbs and their products is the basis for many unique pharmaceuticals. [4]

Proanthocyanidins (PAs) are substances that have been the target of recent studies aiming to control or treat carious lesions. They are naturally presented as a metabolite widely available in fruits, vegetables, nuts, seeds, flowers and bark. [5]
PA increases the synthesis of collagen, accelerates the conversion of insoluble collagen to soluble collagen during development and decreases the rate of enzymatic degradation of the collagen matrix. [6] Bedran-Russo, *et al* (2008) showed that a 6.5% grape seed extract solution could interact with demineralized dentin and increase its modulus of elasticity and strength, due to the stability and increase in the amount of cross-linked collagen [8]. Furthermore, another study about the mandibular bone formation in rats showed that PAs are necessary antioxidants for calcium absorption. [9] In this regard, considering a biomimetic approach using selective natural agents may be a novel approach to stabilize and strengthen the exogenous dentin, inducing collagen crosslinking through interaction with non-collagen protein and by remineralization, possibly decreasing the biodegradation rate and increasing the mineral nucleating [7]
The anticarcinogenic potential and remineralizing properties of CPP-ACP have been exhibited *in vitro* and in situ studies. CPP-ACP is a technology-based on ACP stabilized by CPPs. The beneficial effect obtained from CPP-ACP is associated with its ability to localize calcium and phosphate in dental plaque in the proximity of the tooth, thus facilitating remineralization. [10]
The aim of this study was to compare remineralizing potential of novel agent- Pine bark extract and herbal oil-Arimedadi thailam with conventional remineralizing agent CCP-ACP.

Materials and methodology

Forty human premolars extracted for orthodontic purpose and free of caries or cracks were selected for the study. Teeth were cleaned with ultrasonic scaler and placed in distilled water until use. Teeth were covered with nail varnish except for 4 mm × 4 mm exposed window Artificial caries-like lesion was produced by treating the exposed window with 37% phosphoric acid for 30 s Demineralized samples were randomly divided into four groups consisting of 10 samples in each group:

Group 1

Samples were treated with pine bark extract for 5mins

This duration of application was chosen to match the time span of other conventional remineralizing agents.

Group 2

Samples were treated with Herbal oil -Arimedadi thailam for 5mins

Group 3

Samples were treated with CPP- ACP tooth paste for 5mins

Group 4 (Control group)

Samples were not treated with any remineralizing agent.

The samples in each group were treated with the remineralizing agent, after which they were rinsed with deionized water, dried, and stored in artificial saliva

The same procedure is repeated for the span of 14 days, as it has been previously reported by Burwell *et al*. and Vashisht *et al*. that the average time for remineralization after acid exposure is 14 days. [11,12]

The samples are then subjected to confocal laser scanning microscope (CLSM) to analyze for the depth of remineralization.

Data was analysed using the Statistical Package SPSS 22.0 and level of significance was set at $P < 0.05$. ANOVA test was used to analyse the depth of demineralization and remineralization.

Results

Table 1: Comparison of depth of demineralisation between the groups (microns)

	Mean	Minimum	Maximum	P
Group 1 (Pine Bark)	1675.763	1380.97	1860.71	<0.56*
Group 2 (Herbal Oil)	1692.049	1401.1	1840.22	
Group 3 (Toothpaste)	1662.76	1450.07	1798.01	
Group 4 (Control)	1687.35	1398.98	1866.5	

Table 1 shows the depth of demineralization after the use of 37% phosphoric acid for 30 s in all the samples.

Table 2: Comparison of depth of remineralisation between the groups (microns)

	Mean	Minimum	Maximum	P
Group 1 (Pine Bark)	1008.25	214.55	2152.56	<0.05*
Group 2 (Herbal Oil)	2071.57	904.03	2317.54	
Group 3 (Toothpaste)	958.46	768.67	1039.56	
Group 4 (Control)	413.56	65.45	678.25	

Table 2 demonstrates the depth of remineralization after the use of various remineralizing agents in each group.

Highest remineralization was seen in Group 2 (Herbal Oil) followed by Group 1 (Pine Bark Extract) and Group 3 (CPP -ACP Toothpaste). The least remineralization potential was seen in Group 4 (Control).

There were statistically significant differences seen in between all the experimental groups.

Discussion

The dynamic nature of dental caries requires timely and precise management, as early diagnosis allows for reversal in its initial stages, while later stages are often irreversible. Remineralization can be achieved when pH levels are neutralized, aided by the presence of calcium and phosphate ions, which play crucial roles in restoring enamel. [2] Novel treatments focus on halting or reversing non-cavitated, demineralized lesions by using topical remineralizing agents that create a favourable environment for enamel repair.

This study aimed to evaluate the remineralization potential of pine bark extract, herbal oil (Arimedadi Thailam), and CPP-ACP toothpaste.

According to the findings, herbal oil Arimedadi Thailam exhibited the highest remineralization potential, followed by pine bark extract, and lastly, conventional CPP-ACP toothpaste. Previous studies on sesame seed oil by Faieze Hamze and Leila have highlighted its ability to remineralize enamel, a property linked to Arimedadi Thailam's high effectiveness since sesame oil is a key ingredient. [14]

Pine bark extract, rich in proanthocyanidins (PA), has been shown to enhance collagen synthesis and accelerate the conversion of soluble to insoluble collagen, which plays a crucial role during tooth development. Additionally, PA-treated collagen matrices are non-toxic and inhibit the enzymatic activity of glucosyltransferase, F-ATPase, and amylase, enzymes produced by *Streptococcus mutans* that polymerize sucrose and starch into glucans. This glucan formation is essential for *S. mutans* colonization on tooth surfaces, thus contributing to plaque formation and the development of caries. By inhibiting glucosyltransferases, PA can effectively prevent caries progression.

In comparison, CPP-ACP (Group III) demonstrated the least depth of remineralization among the tested groups. However, when compared to the untreated control group, it showed a notable increase in remineralization. These findings are consistent with similar studies by Girish Babu, Indrapriyadharshini, and Morales-Vadillo, which compared fluoride varnish with CPP-ACP. [13] Confocal Laser Scanning Microscopy (CLSM) analysis revealed that Arimedadi Thailam-treated specimens (Group II) had significantly greater remineralization depth than other groups, affirming its superior efficacy.

This discussion highlights the potential of alternative, herbal-based treatments like Arimedadi Thailam in promoting enamel remineralization, offering a promising approach to managing early-stage caries and improving patient outcome.

References

1. Machiulskiene V, Campus G, Carvalho JC, Dige I, Ekstrand KR, Jablonski Momeni A, *et al*. Terminology of dental caries and dental caries management: consensus report of a workshop organized by ORCA and Cariology Research Group of IADR. Caries Res,2020:54:7-14.

2. Oliveira GM, Ritter AV, Heymann HO, Swift E Jr., Donovan T, Brock G, *et al*. Remineralization effect of CPP ACP and fluoride for white spot lesions *in vitro*. J Dent,2014:42:1592-602.
3. Soares R, De Ataide IN, Fernandes M, Lambor R. Assessment of enamel remineralisation after treatment with four different remineralizing agents: a scanning electron microscopy (SEM) study. J Clin Diagn Res, 2017, 11.
4. Khan SA, Khan AM, Karim S, Kamal MA, Damanhouri GA, Mirza Z. Panacea seed "Nigella": a review focusing on regenerative effects for gastric ailments. Saudi J Biol Sci,2016:23:542-53.
5. Joshi SS, Kuszynski CA, Bagchi D. The cellular and molecular basis of health benefits of grape seed proanthocyanidin extract. Curr Pharm Biotechnol,2001:2:187-200.
6. Aldini G, Carini M, Piccoli A, Rossoni G, Facino RM. Procyanidins from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: new evidences for cardio-protection. Life Sci,2003:73:2883-98.
7. Bedran-Russo AK, Pashley DH, Agee K, Drummond JL, Miescke KJ. Changes in stiffness of demineralized dentin following application of collagen crosslinkers. J Biomed Mater Res B Appl Biomater,2008:86:330-4.
8. Macedo GV, Yamauchi M, Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res,2009:88:1096-100.
9. Ishikawa M, Maki K, Tofani I, Kimura K, Kimura M. Grape seed proanthocyanidins extract promotes bone formation in rat's mandibular condyle. Eur J Oral Sci,2005:113:47-52.
10. Savas S, Kavrík F, Kucukyılmaz E. Evaluation of the remineralization capacity of CPP ACP containing fluoride varnish by different quantitative methods. J Appl Oral Sci,2016:24:198-203.
11. Premnath P, John J, Manchery N, Subbiah GK, Nagappan N, Subramani P. Effectiveness of theobromine on enamel remineralization: a comparative *in vitro* study. Cureus, 2019, 11.
12. Chaudhury T, Ananthakrishna S, Kumari RV, Kour S, Syam A. Comparative study of remineralization potential of three different remineralizing agents on demineralized enamel using light fluorescence and confocal fluorescence microscope: an *in vitro* study. Int J Prev Clin Dent Res,2018:5:45-9.
13. Kumar NK, Naik SB, Priya CL, Merwade S, Brigit B, Guruprasad CN, Prabakaran P. Evaluation of the remineralizing potential of Nigella sativa, sodium fluoride and casein phosphopeptide-amorphous calcium phosphate on enamel: an *in vitro* study. J Indian Assoc Public Health Dent,2020:18(4):313-7.
14. Hamze F, Ghasemi L, Kamalinejad M. Evaluating the effect of an experimental sesame gel on human enamel: atomic force microscopy study. J Dent,2022:23(1 Suppl):169.