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To evaluate and compare the remineralizing potential of CPP-ACP based experimental herbal pediatric dentifrice with commercially available herbal pediatric dentifrice on artificially demineralized human enamel -an in-vitro study

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Abstract

Background: Carious process involves a cycle of both reversible and irreversible stages.[4] Demineralization at subsurface level increases porosity that eventually changes the optical properties of the teeth further leading to cavitations if no treatment is undertaken. The main purpose of Remineralization is a novel non invasive management of early carious lesions, which bridges the traditional gap between preventive and invasive dentistry. The demineralization process continues till the pH raises causing remineralization to occur with deposition of calcium, phosphate, and fluoride ions (fluoroapatite), which are more resistant to crystals' dissolution by organic acids as fluoroapatite crystals have advancing growth pattern resulting in a formation of large crystals with hexagonal outlines. Therefore, the best method for caries control is to focus on the ways to improve the remineralizing process with the help of remineralization products.

Aim: To evaluate & compare the remineralizing potential of Herbal based experimental pediatric

dentifrice with commercially available herbal pediatricdentifrice incorporated with CPP-ACP complex

Materials and method: Thirty sound primary extracted anterior teeth were divided into 2 groups and a 4 by 4 mm window was created on buccal surface isolating the rest using nail varnish. The tooth specimens were demineralised for 96 hours, dried and sectioned. The sectioned specimens were then evaluated under stereomicroscope at 20x magnification for demineralisation. Teeth in each group were treated with their respective remineralizing agents using a rubber cup for two minutes every 24 hours for 21 days. The remineralization status was then assessed under a stereomicroscope at 20x magnification. Samples were scored for demineralisation and remineralisation using Img J Software. Statistical analysis was done using Shapiro wilk test and paired t test. The data was analysed by SPSS (21.0 versions).

Results: Significant remineralization was seen with in both the group with $p<0.05$. The highest value for remineralization was observed with Experimental herbal pediatric toothpaste when incorporated with Cpp-Acp, had better remineralizing efficacy when compared to Himalaya herbal pediatric toothpaste incorporated with Cpp-Acp complex.

Conclusion: Experimental Herbal Pediatric Dentifrice proved to a better remineralizing agent when incorporated with Cpp-Acp complex. Further specific experimental quantification and evaluations are to be carried out in future to develop this toothpaste as a product to be benefitted by the society.

Keyword: CPP-ACP, Demineralization, Pediatricdentifrice, Remineralization

Introduction

The global prevalence of dental caries, erosion and hypersensitivity have increased over the last two decades This has sparked a greater interest in creating more effective regimens for these ailments with prevention and treatment.

Rather than one-way demineralization process, the current knowledge of the caries process is based on the accumulation of several episodes of demineralization and remineralization.

Demineralization is the process by which organic acids produced by plaque bacteria remove the mineral content of hydroxyapatite crystals from their surface. The most stable form of hydroxyapatite found in the environment has a pH of 7.4. Calcium hydroxyapatite ($Ca10(PO_4)6(OH)_2$) in enamel and calcium hydroxyapatite dissolved in the plaque biofilm are in a state of continuous chemical balance. When the plaque's pH drops below 5.5, mineral crystals dissolve.

The first macroscopic signs of enamel caries are enamel white spot lesions, where the surface layer of enamel remains intact throughout, underlying demineralization but cavitation occurs in the absence of intervention.

Since saliva has a limited capacity to remineralize teeth, intraoral remineralization aids have been created to help treat and prevent dental caries and erosion.[1]

It has been discovered that the casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) nanocomplex, which is present in casein milk protein, remineralizes subsurface lesions in human enamel.[2]

There is a dearth of information regarding pediatric herbal dentifrices containing Cpp-Acp concentration, and even fewer have examined the effects of herbal concentration dentifrices on primary teeth. Hence, the subsequent study was contemplated to assess and compare the remineralizing potential of experimental herbal dentifrice with commercially available herbal pediatric dentifrice incorporated with Cpp-Acp complex.

Material and Method

Study design and location: The present prospective in-vitro experimental comparative study was carried out in the Department of Paediatric and Preventive Dentistry, Darshan Dental College and Hospital, Udaipur, Rajasthan. This study was approved by the Institutional Ethical Committee (Protocol no. 2020-21/10

Source of Data: Thirty sound human extracted primary anterior teeth with intact crowns free from caries, abrasions, stains and restorations were included in the study whereas restored teeth, fractured teeth, endodontically treated teeth and carious crown portion of tooth were excluded from the study.

Methodology: Casein was isolated from milk and Cpp-Acp complex was formulated.

Fig. 1: Caesin isolation and Cpp-Acp complex preparation

Fig.2: Maintaining the pH using PH meter

Preparation of Samples

The Selected teeth underwent a thorough cleaning process, including washing and disinfection with 5.25% sodium hypochlorite. The ultrasonic scaler was used effectively, eliminating all the calculus and soft tissue debris from the teeth. Subsequently, the teeth were stored in regular saline until the experiment commenced. To prepare the samples, the apical 1/3rd of the root was carefully removed using a safe-sided diamond disc under a water spray. A 4 x 4 mm window was created on the buccal surface using nail varnish. Artificial saliva was used to store the samples.

The specimens were then divided randomly in two groups of 15 each (n=15):

1) Experimental herbal pediatric dentifrice + cpp-acp complex,

2) Commercially available herbal pediatric dentifrice + cpp-acp complex.

Lesion formation: All specimens were placed in custom made demineralizing solution for 96 hours to produce artificial carious lesions of approximately 100 µm depth .This solution's pH was kept constant at 4.5. The teeth were carefully sectioned buccolingually. To assess the demineralization status of the specimen, a stereomicroscope was utilized. Subsequently, the teeth were immersed in artificial saliva to mimic real intraoral conditions.

Remineralization: Over a span of 21 days, each group of teeth received the appropriate remineralizing agent once every 24 hours using a rubber cup for 2 mins. Following this, the specimens were dried and cleaned with deionized water before being evaluated under a stereomicroscope to determine their remineralization status.

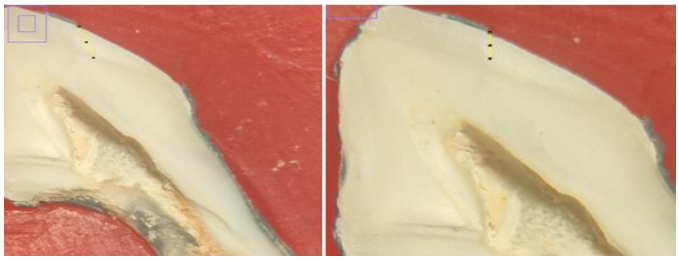

Fig. 3: A) Demineralisation and B) Remineralisation viewed under stereomicroscope

Data Analysis

The data was analysed by SPSS 21.0 version. Shapiro Wilk test was used to check which all variables were following normal distribution. Data was normally distributed, bivariate analyses were performed using the Independent t test and Paired t test. Level of statistical significance was set at p-value < 0.05.

Results

No significant difference was seen in the mean difference of depth of demineralisation and remineralization when compared among two study groups as p>0.05.

Significant remineralization was seen with in both the groups when comparison was made from the difference in depth of demineralisation to remineralisation as p<0.05 , remineralisation tendency was seen to be more of Experimental Herbal Pediatric toothpaste when compared to commercially available Pediatricdentifrice +cpp-acp complex.

Table 1: Comparision between depth of demineralisation to remineralisation.

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Group - I Herbal experimental Pediatricdentifrice +cpp-acp complex	0.18312	0.008879	0.002292	0.178202	0.188037	79.876	14	.001*
Group - II Commercially available Herbal Pediatricdentifrice + cpp-acp complex	0.16514	0.013538	0.003495	0.157642	0.172637	47.241	14	.001*

Discussion

Dental caries can result from even a slight imbalance in the process of demineralization and remineralization, which happen simultaneously in the oral cavity.[3] The earliest indication of dental caries, white spot lesions, can be reversed if the balance tips in favour of remineralization.[4] An essential part of the demineralization process is played by the metabolic by-products of oral cavity-dwelling bacteria.[5]

Enamel caries in a primary tooth spreads quickly into the underlying dentine, this is most likely caused by the enamel's relative thinness, lower mineral concentration, and higher organic content in primary tooth enamel as opposed to permanent dental enamel.

Numerous studies have repeatedly highlighted the negative effects of typical nonherbal toothpaste ingredients such as sodium lauryl sulphate and fluorides (Lipper, 2013)[7], Orisakwe et al., 2016)[6]

Many investigations have been carried out to confirm the effectiveness and longevity of remineralizing substances that are sold commercially for white spot lesions; however, there is little data on the use of pediatric herbal dentifrices containing Cpp-Acp concentration.

Isolating Casein was done using a slightly modified method that was taken from a study done by Muhammad Sahlan (2012).[8] The formula for CPP-ACP was derived from a World Intellectual Property Organisation patent, which was submitted by Eric C. Reynolds and has the US publication number 2005/0037948 for Calcium Phosphopeptide Complexes.[9]

Torwane et al. (2015) established the role of herbal toothpaste in reducing dental caries by finding that the development of dental caries was reduced in both herbal and commercial dentifrices.[10]

Formulation excipients used in the Experimental herbal pediatric dentifrice

Sn.	Excipients	Concentration Range	Type
1.	Abrasives	7-11%	Punica granatum
2.	Humectants	37-45%	Glycerine
3.	Binding agents	0.8-2.5%	Chitosan
4.	Antibacterial agent	0.05-0.5%	Propolis Chitosan

5.	Foaming agent	1-2%	Quillaja Saponaria Sodium coccyl glutamate
6.	Flavour	1-6%	Mentha Arvensis
7.	Sweetner	18-24%	Xylitol Honey
8.	Remineralizing agent	1-20%	CPP-ACP

With a few concentration adjustments, the formulation concentration of the herbal toothpaste is as specified by PCT, Publication number 2014/191009/A1, WO2021/046047/A1.

Punica granatum was used in our study as a useful herbal ingredient for reducing the adhesion of different bacteria in the mouth. Since studies have shown that Punica Grantaum has antibacterial, antioxidant, and anti-inflammatory qualities, we included it in our investigation.[11]

A study by Bhatt et al. (2015)[12] concluded Salvadorapersica's antimicrobial properties were found to be effective against a variety of oral pathogens and were categorized as broad-spectrum antimicrobials by Balto et al. and Al-Hazmie et al. (2017)[45].

As the remineralizing effect of Himalaya herbal kids toothpaste was not previously discovered in the literature, it is possible to argue that the study's findings are groundbreaking; as a result, there are no supporting or contradicting data.

In our study, maxillary and mandibular primary incisors were selected due to large, flat enamel surface area on the labial surface and little variation in each tooth's morphology.

Similarly, Rirattanapong P, et al in 2016 used primary incisors in their study to evaluate demineralisation and remineralisation of artificial enamel lesions.[13]

In our study, the selected specimen samples were covered in an acid-resistant nail varnish, resulting in two 4 mm by 4 mm square windows on the buccal surface. One benefit of this design was that it allowed for baseline measurements of the lesion depth in any tooth, which minimized specimen-to-specimen variations in initial lesion depth.

In order to avoid any potential interference with the hypothesis under investigation, the artificial saliva composition employed in this study did not contain fluoride.

Demineralization is difficult to measure or observe clinically, but it is simpler to locate areas of demineralization when a white-spot lesion forms.[14] Samples were stored in demineralization solution for 96 hours, as samples in the caries solution corresponds to almost three months of actual time.

It has been stated by A Fabio et al in 2015 that examination by stereomicroscopy is reliable method and based on the technological developments measurement in millimetres is more accurate by digital method (software) when compared to visual.[15]

Remineralization procedure was carried out using rubber cup for 2 mins daily for 21 days.

When Group I dentifrice toothpaste was used, approximately 4 µm of remineralised enamel was formed while group 2 revealed approximately of 4.6 µm of remineralised enamel.

Furthermore, because Cpp-Acp incorporation inhibits the quick transformation of ions into calcium phosphate phases, the ions would be stabilised and kept in a condition that would promote diffusion into the subsurface lesion via activity gradients.

The decrease in the depth of demineralized areas served as a direct indicator of remineralization, as it cannot be measured directly.

This could be correlated to the findings of Uysal et al in 2011 which showed improved conditions against demineralisation when aloe and chitosan products were used.[16]

In this study, significant remineralization was seen in both the groups when comparison was made from the difference in depth of demineralisation to remineralisation as $p<0.05$ yet remineralisation tendency was seen to be more in Experimental Herbal pediatric dentifrice. This could be attributed to the presence of chitosan ,propolis and xylitol in the experimental herbal toothpaste .

This was illustrated in a study by Nimbeni et al. (2021) in a systemic review, On the surface of partially demineralized dentin, it was found that phosphorylated chitosan significantly increased the deposition of phosphate and calcium ions, promoting the nucleation of crystals.[17]

However, as there is paucity of data on the remineralization efficacy of herbal non fluoridated dentifrices, this parameter of our study could not be compared to other studies.

Thus concluding, we can justify herbal non fluoridated dentifrices to be preventive, protective, nutritive and curative. Nevertheless, due to limited literature regarding herbal non-fluoridated dental formulations, we propose more studies to be undertaken to authenticate the herbal products.

Summary and Conclusion

Based on the results obtained from the present study it can be concluded that

1. Both the Herbal Experimental and commercially available dentifrice incorporated with Cpp-Acp complex exhibited the property of remineralization.
2. Experimental herbal pediatric dentifrice when incorporated with Cpp-Acp, had better remineralising efficacy when compared to Himalaya herbal pediatric dentifrice incorporated with Cpp-Acp complex.
3. Considering the cost - benefit ratio, judicious use of herbal dentifrice with Cpp-Acp complex could serve as a valuable therapeutic aid.

Further specific experimental quantification and evaluations need to be carried out in future to develop this dentifrice as a product to be benefitted by the society. Additionally, more studies should be undertaken to authenticate the remineralising potential of herbal dentifrices considering The role of herbal dentifrices as a remineralising agent cannot be underestimated.

Limitation

The limitations in our study are the varying pH levels in the mouth that cannot be duplicated in vitro i.e. the lack of oral microflora in the artificial salivary solution, control over salivary flow rate, and harder acidogenic challenges used in the intermittent period.

References

1. Fernando JR, Shen P, Sim CPC, Chen YY, Walker GD, Yuan Y, Reynolds C, Stanton DP, MacRae CM, Reynolds EC. Self-assembly of dental surface nanofilaments and remineralisation by SnF2 and CPP-ACP nanocomplexes. Sci Rep. 2019 Feb 4;9(1):1285.
2. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evid Based Complement Altern Med. 2001;2011:680354
3. Singhal RK, Rai B. Remineralization Potential of Three Tooth Pastes on Enamel Caries. J Med Sci 2017;5(05):664–666
4. Alhussain AM, Alhaddad AA, Ghazwi MM, Farooq I. Remineralization of artificial carious lesions using

a novel fluoride incorporated bioactive glass dentifrice. Dent Med Probl. 2018 Oct-Dec;55(4):379-382.

5. Abou Neel EA, Aljabo A, Strange A, et al. Demineralization-remineralization dynamics in teeth and bone. Int J Nanomedicine. 2016;11:4743-4763.
6. Orisakwe OE, Okolo KO, Igweze ZN, et al. Potential hazards of toxic metals found in toothpastes commonly used in Nigeria. Rocz PanstwZaklHig 2016;67(2):197–204.
7. Lippert F. An introduction to toothpaste - its purpose, history and ingredients. Monogr Oral Sci. 2013;23:1-14.
8. M. Sahlan and I. Pramadewi. Int. J. Pharm. Bio. Sci. 2012, 3(3) : 472-478
9. E. C. Reynolds, "Calcium phosphopeptide complexes." Google Patents,B1 24- Aug-2004
10. Torwane AN, Gouraha A, Maheshwari A, et al. To evaluate the efficacy of herbal dentifrice in the reduction of dental caries compared against commercially available fluoride containing dentifrice. World J Pharm Pharm Sci. 2015;4(4):800–807.
11. Ruan J.-H., Li J., Adili G., Sun G.-Y., Abuduaini M., Abdulla R., Maiwulanjiang M., Aisa H.A. Phenolic Compounds and Bioactivities from Pomegranate (Punica granatum L.) Peels. J. Agric. Food Chem. 2022;70:3678– 3686
12. Bhatt N, Bapat S, Asawa K, Tak M, Chaturvedi P, Gupta VV, George PP. The antiplaque efficacy of propolis-based herbal toothpaste: A crossover clinical study. J Nat Sci Biol Med. 2015 Jul-Dec;6(2):364-8.
13. Rirattanapong P, Vongsavan K, Saengsirinavin C, Khumsub P. The efficiency of child formula dentifrices containing different calcium and phosphate Bibliography P a g e | 83 compounds on artificial enamel caries. J Int Soc Prev Community Dent. 2016 Nov-Dec;6(6):559-567
14. Silverstone LM, Wefel JS, Zimmerman BF, Clarkson BH, Featherstone MJ. Remineralization of natural and artificial lesions in human dental enamel in vitro. Effect of calcium concentration of the calcifying fluid. Caries Res. 1981;15(2):138-157
15. Alvarenga FAS, Pinelli C, Loffredo LCM. Reliability of marginal microleakage assessment by visual and digital methods. Eur J Dent. 2015;9(1):1-5
16. Uysal T, Akkurt MD, Amasyali M, Ozcan S, Yagci A, Basak F, Sagdic D. Does a chitosan-containing dentifrice prevent demineralization around orthodontic brackets? Angle Orthod. 2011 Mar;81(2):319-25
17. Nimbeni SB, Nimbeni BS, Divakar DD. Role of Chitosan in Remineralization of Enamel and Dentin: A Systematic Review. Int J Clin Pediatr Dent. 2021 JulAug;14(4):562-56