

A Comparative Evaluation of Remineralizing Effect of Different Herbal Dentifrices Available in Market: An *In Vitro* Study

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Abstract

Background: Dental caries, a dynamic process occurs when demineralization exceeds remineralization. With the advent of herbal dentifrices in the current world, it becomes necessary to evaluate the potential of remineralizing efficacy of herbal dentifrices specifically those available in the motherland of herbal medicine, India.

Aim: To evaluate the remineralizing potential of different herbal dentifrices available in market.

Objective: To compare and evaluate the efficacy of three different herbal dentifrices on remineralization of teeth.

Materials and Methods: Sixty non-carious premolars were included in this comparative study. The specimens were immersed in a demineralizing solution for 96 hours and depth of initial carious lesions formed was evaluated. The specimens were then equally divided into three groups of twenty teeth each (n=20) and exposed to pH cycle of 7 days where three herbal fluoride free dentifrices were used as remineralizing agents and further the remineralization depths were viewed under polarized light microscope. Data obtained was analyzed using ANOVA at (p<0.0001).

Results: Polarized light microscope image analysis revealed that all the three herbal dentifrices had a remineralizing potential. A statistically significant difference was seen in Group III (Dabur Meswak) followed by Groups II (Colgate Herbal) and I (Hiora-K) at (p<0.0001).

Conclusion: All three non-fluoridated herbal dentifrices evaluated under this study had a considerable remineralizing potential. It can be considered for use as a valuable therapeutic aid.

Keywords: Dentifrice; Non-fluoridated; De/re-mineralization; Polarized light microscope

Introduction

Dental caries is an infectious disease affecting human population [1]. The disease is recognized to require a host, the tooth in the oral environment, a dietary substrate, and aciduric bacteria. The saliva (also considered a host component); the substrate and the bacteria form a biofilm (plaque) that adheres to the tooth surface. Over time the presence of the substrate serves as a nutrient for the bacteria and the bacteria produce acids that can demineralize the tooth [2]. Dental caries cannot be treated using restorative method alone [1].

There is a delicate balance of demineralization and remineralization of enamel surface. Studies have shown that early carious lesions can be remineralized with fluoride being one of the most significant agents for promoting remineralization [3]. The most common source of topical fluoride for majority of children is dentifrices [4].

Dentifrices include toothpastes, gels and powders and provide three important functions. First, removal of debris, plaque and stained pellicle compared with use of a toothbrush only. Second, they polish teeth. Finally dentifrices act as vehicles for the delivery of therapeutic agents with benefits like fluorides, tartar control agents, desensitizing agents and remineralizing agents [5].

According to World Health Organization (1994), the decline of dental caries prevalence observed in many countries can be attributed to the widespread use of toothpaste that contains fluoride [6].

Evidence shows that carious lesions can be mineralized *in vitro* and *in vivo* if fluoride is taken in optimal quantity, as fluoride is one of the most significant agent for promoting remineralization. Fluorides are added to community water supplies and are available in the form of dietary supplements, topical applications, mouthwashes and dentifrices

[3]. Furthermore it is also generally accepted that the use of fluoride containing dentifrices has been one of the most important factors in the decline of dental caries [7].

Many investigators have studied the demineralization and remineralization of enamel lesions in permanent teeth by using conventional fluoridated and non-fluoridated dentifrices. Consequently the mineralization effect of low fluoride dentifrices available in Indian market on teeth remains unclear [7].

Hence, this study would evaluate and compare the remineralizing effect of different herbal dentifrices available in India with and without fluoride concentration used on artificial enamel lesion of teeth through polarised light microscope.

Materials and Methods

Sample selection

All sound caries free premolars extracted for orthodontic treatment were included in this study and all the samples were collected from department of oral and maxillofacial surgery, Darshan dental college and hospital, Udaipur.

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Dentifrices used

All the dentifrices used were herbal and non-fluoridated. The composition of Himalaya Hiora-K toothpaste (Group I) is: Twak (*Cinnamomum zeylanicum*), Lavanga (*Syzygium aromaticum*), Palakya (*Spinacia oleracea*), Triphala, Trikatu, Suryakshara, Yashada bhasma, (Potassium nitrate, Zinc oxide)

The composition of Colgate Herbal toothpaste (Group II) includes natural herbs like calcium carbonate, Chamomile, Eucalyptus, Myrrh and Sage which are known for their Oral care benefits.

The ingredients listed for Dabur Meswak Tooth Paste (Group III) include Calcium Carbonate, Sorbitol, Purified Water, Silica, Sodium Lauryl Sulphate, Flavor Sodium Silicate, Sodium Carrageenate, Sodium Carboxy Methyl Cellulose, Meswak (*Salvadora persica*) extract and Sodium Saccharin.

Demineralising and remineralising solutions

Demineralizing solution contains: 2.2 mM of calcium chloride, 2.2 mM of sodium hydrogen phosphate, 0.05 M acetic acid, 1 M potassium hydroxide will be mixed and its pH would be adjusted to 4.5. Remineralizing solution contain 1.5 mM calcium chloride, 0.9 mM sodium hydrogen phosphate, 0.15 potassium chloride will be mixed and its pH would be adjusted to 7. Demineralizing and remineralizing solutions were freshly prepared for each cycle and kept in separate plastic containers.

Dentifrice slurry preparation

Deionized water and toothpaste will be taken in the ratio of 3:1. To prepare this, 17 g of a toothpaste sample will be combined with 51 mL of deionized water and this slurry would be transferred in three tubes, stirring it well until a homogenous mix is obtained. A fresh dentifrice slurry preparation would be made before commencing every pH cycle.

Specimen preparation

All the specimens were kept in 10% formalin solution until used for study. They were coated with acid resistant nail varnish leaving 1 mm-narrow window on sound intact buccal or lingual surface.

Lesion formation

All specimens were placed in demineralizing solution for 96 hours to produce artificial carious lesions of approximately 100 μ m depth and then cut longitudinally into sixty sections and randomly assigned to three groups. Group I: Hiora-K, Group II: Colgate Herbal and Group III: Dabur Meswak.

pH cycle

All sections were subjected to the pH cycling procedure. Each cycle involved three hours of demineralization twice daily with two hours of remineralization in between. One-minute dentifrice slurry treatments were given before the first demineralizing cycle and both before and after the second demineralizing cycle and all specimens were placed in remineralising solution for overnight. This pH cycle continued for 7 days.

Fresh demineralizing and remineralizing solutions were prepared before each pH cycle, and pH of the both solutions was checked with a pH meter before use.

Post pH cycle lesion depth was measured under polarized light microscope.

Results

The present comparative *in vitro* study was carried out to evaluate the remineralizing potential of three herbal non fluoridated dentifrices on artificially induced white spot lesions.

This study was conducted at the Department of Pedodontics and Preventive Dentistry, Darshan Dental College and hospital, Udaipur in collaboration with Department of Oral Pathology, Pacific Dental College, Udaipur.

In this study, sixty extracted sound premolar teeth, selected depending on inclusion criteria were demineralized to create artificial white spot lesions. Further they were divided into the following experimental groups comprising of 20 samples each:

Group I: Hiora-K

Group II: Colgate Herbal

Group III: Dabur Meswak

The non-fluoridated herbal dentifrices in the groups mentioned above were used as remineralizing agents in a pH cycling model for a period of seven days. A polarized light microscope was used to assess the extent of remineralization observed in each sample of all the three study groups.

The results obtained were as follows:

Table 1 shows pre and post pH cycling comparison of mean values for group I. the mean of pre pH cycling was found to higher i.e. 93.95 ± 9.28 when compared with post pH cycling value which was 89.35 ± 9.01 . The intra group pre and post pH cycling comparison of mean value was found to be statistically significant with p-value (<0.0001).

Table 2 shows pre and post pH cycling comparison of mean values for group II.

The mean of pre pH cycling was found to higher i.e. 96.30 ± 6.44 when compared with post pH cycling value which was 91.90 ± 6.40 . The intra group pre and post pH cycling comparison of mean value was found to be statistically significant with p-value (<0.0001).

Table 3 shows pre and post pH cycling comparison of mean values for group III.

The mean of pre pH cycling was found to higher i.e. 93.21 ± 8.08 when compared with post pH cycling value which was 88.42 ± 7.77 . The intra group pre and post pH cycling comparison was found to be statistically significant with p-value (<0.0001).

Table 4 shows intergroup comparison of mean value of three study groups.

The intergroup comparison statistically non-significant for both pre pH cycling (0.38) and post pH cycling (0.385)

Table 5 shows the pre and post pH cycling comparison of mean values for Group I, Group II and Group III. The mean value of pre pH cycling in Group I, Group II and Group III is 93.95, 96.30 and 92.45 when compared with mean of post pH cycling value is 89.35, 91.90 and 87.75 respectively. Using paired T Test, the inter group comparison showed statistically significant result for all three groups.

Table 6 shows intergroup comparison of mean value of three study groups.

The intergroup comparison of mean values was found to be

Descriptive Statistics					
	N	Mean	Std. Deviation	Minimum	Maximum
Pre	20	93.95	9.288	75	112
Post	20	89.35	9.016	71	105

p-value <0.0001 S
Wilcoxon sign ranked test: Indicates statistical significance

Table 1: Pre and Post pH cycling comparison of mean values for Group I (Hiora-K),

Descriptive Statistics					
	N	Mean	Std. Deviation	Minimum	Maximum
Pre	20	96.3	6.441	86	107
Post	20	91.9	6.406	81	102

p-value <0.0001 S

Table 2: Pre and Post pH cycling comparison of mean values for Group II (Colgate Herbal), Wilcoxon sign ranked test: Indicates statistical significance.

Descriptive Statistics					
	N	Mean	Std. Deviation	Minimum	Maximum
Pre	19	93.21	8.08	78	110
Post	19	88.42	7.77	75	103

p-value <0.0001 S
Wilcoxon sign ranked test: Indicates statistical significance.

Table 3: Pre and Post pH cycling comparison of mean values for Group III (Dabur Meswak).

Groups		Pre	Post
Group I	Mean	93.95	89.35
	N	20	20
	Std. Deviation	9.288	9.016
Group II	Mean	96.3	91.9
	N	20	20
	Std. Deviation	6.441	6.406
Group III	Mean	93.21	88.42
	N	19	19
	Std. Deviation	8.08	7.77
Total	Mean	94.51	89.92
	N	59	59
	Std. Deviation	7.992	7.811
p-value		0.381 NS	0.385 NS

Note: Kruskalwalli's test: Indicates statistical non-significance

Table 4: Intergroup Comparison of Mean Values.

Group (Pre vs. Post)	Mean	N	Std. Deviation	p-value
Group I	Pre	93.95	20	0.000*
	Post	89.35	20	
Group II	Pre	96.3	20	0.000*
	Post	91.9tt	20	
Group III	Pre	92.45	20	0.000*
	Post	87.75	20	

Note: Test applied: paired t-test.
* indicates statistical significance.

Table 5: Pre and Post pH cycling comparison of mean values for Group I, Group II and Group III.

Post Group Comparison	N	Mean	Std. Deviation	p-value
Group I	20	89.35	9.01	0.256**
Group II	20	91.9	6.4	
Group III	20	87.75	8.13	
Total	60	89.66	7.98	

Note: Test applied: One-way ANOVA.
** indicates statistical non-significance.

Table 6: Intergroup comparison of mean values of post pH cycle.

statistically non-significant for post pH cycling (p-value 0.25) using one way ANOVA.

Discussion

“Oral health for a healthy life” was the theme declared by World Health Organization on World Health Day in 1994 [8]. Good oral health includes disease free teeth and supporting tissues and are important for overall physical health. Maintaining good oral hygiene is a must for having healthy teeth and gingiva [9]. Moreover, brushing associated with dentifrices continues to be the most used and efficient procedure of self-care in the practice of oral hygiene in most countries [10].

Modern allopathic toothpastes contain some ingredients that can be harmful and show allergic contact cheilitis [11] and tooth staining [12]. To overcome these, the use of herbal remedies has assumed a global dimension [13]. The earliest recorded evidence of herbal medicine use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years [14]. India is known for its traditional medicinal systems-Ayurveda, Siddha, and Unani. Medical systems are found mentioned even in the ancient Vedas and other scriptures. The Ayurvedic concept appeared and developed between 2500 and 500 BC in India [15]. According to the World Health Organization (WHO), up to 80% of the population in developing countries uses plants and its products as traditional medicine for primary health care [16].

In the present study, Gujarathi et al. [5] method for *in vitro* demineralization of teeth to produce artificial carious lesion and remineralization of teeth using pH cycle was followed.

pH Cycle

In the present study, according to the concept of *in vitro* pH cycle proposed by Ten Cate and Duijsters in 1982, samples were placed in pH cycle for 7 days to simulate *in vivo* high and low pH changes that occur periodically [17]

Demineralization (pre pH cycling): The demineralizing media are undersaturated with hydroxyapatite and its pH usually ranges from 4.0–5.0 since at this pH, initial carious lesions known as white spot lesions of varying depth are easily formed [18].

According to Nalbantgil et al. [19] the immersion of the samples in the caries solution for 96 hours represents approximately 3 months of real time.

Similar studies were performed by Zhi et al. [20], Advani et al. [21], Gujarathi et al. [5], Malekafzali et al. [7] and Rirattanapong et al. [22]. They immersed the study samples in a demineralizing solution and found that carious lesions were formed approximately 60 to 100 μ m depth after 96 hours.

In contrast, Yang et al. [23] used a demineralizing solution (having pH 4.3) and produced carious lesion of about 650 μ m depth after 48 hours of immersion.

Post pH Cycle (Remineralization): The focus in caries management has shifted in recent years to non-invasive treatment of early enamel lesions by means of remineralizing agents. In our study, white spot lesions were pH cycled for 7 days.

When Group I (Hiora-K) toothpaste was used, approximately 4 μ m of remineralised enamel was formed. The presence of yashada bhasma, a zinc-based ayurvedic metallic preparation in Hiora-K toothpaste can be a probable reason for its remineralizing potential. This can be correlated to the findings of Lynch [24] who stated that, “zinc is an essential trace element in the mouth and is present naturally in plaque,

saliva and enamel. Zinc in lower concentrations can reduce enamel demineralisation as well as modify remineralization”.

When Group II (Colgate Herbal toothpaste) was used approximately 4.4 μ m of remineralised enamel was formed. The Calcium present in Colgate Herbal toothpaste is the probable constituent that is known to promote remineralization. This inference can be drawn from an explanation stated by Amaechi et al. [25] in their paper. In the absence of fluoride, calcium and phosphorus are responsible for remineralizing enamel [25].

Similarly, when Group III (Dabur Meswak toothpaste) was used approximately 4.79 μ m of enamel was remineralised in accordance with the statement given by Amaechi et al. [25] that an ideal remineralization material diffuses or delivers calcium into the subsurface lesion.

All three dentifrices showed a remineralising potential, though statistical analysis showed a non-significant difference. Highest remineralization was seen in Group III followed by group II and least remineralization in Group I.

Our results were in accordance with Bilgin et al. [26] and Gocmen et al. [12]. Bilgin et al. [26] compared the efficacy of ginger, honey and rosemary herbal preparations with fluoride containing dentifrices and observed enhanced remineralization with herbal products followed by fluoridated dentifrices. Similarly, Gocmen et al. [12] determined the efficacy of herbal medicaments like ginger, rosemary and honey on remineralization of initial artificial subsurface enamel lesions and found enhanced remineralization with the use of herbal medicaments as compared to fluoridated toothpaste. They credited the remineralizing efficacy of ginger to its fluoride content (79 mg/kg of fluoride per 8 mg ginger) which made it a potent antimicrobial and remineralizing agent.

Rirattanapong et al. [27], Shashikala et al. [28], Jabbarifar et al. [29] and Agarwal et al. [30], performed studies using pH cycle and gave significantly greater remineralization results in fluoridated dentifrices than non-fluoridated dentifrices.

Comar et al. [31] concluded that fluoride containing pastes were the best option to reduce dental demineralization when compared to non-fluoridated paste.

Due to paucity of literature on the remineralization potential of herbal non fluoridated dentifrices, the following parameter of our study could not be compared to other studies.

The best oral treatment is the one that controls diseases like dental caries for the longest period with minimal adverse effects. Ayurvedic medicines are one of the most ancient systems of treatment in India and are now spreading globally. The use of herbs and herbal products have recently gained horizon as an alternative to allopathic medicine. The focus is being shifted from relief to prevention, better than cure.

As derived from the results obtained in our study, the herbal non-fluoridated dentifrices appear to have a considerable remineralizing potential.

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.

Conclusion

Following conclusions can be drawn from the study:

1. All the herbal fluoride free dentifrices used in the present study exhibited the property of remineralization.
2. Highest remineralization was seen in Group III (Dabor meswak) followed by group II (Colgate herbal) and least remineralization in Group I (Hiora- K).
3. Role of herbal non-fluoridated dentifrices as a remineralizing agent cannot be underestimated.
4. Considering the cost-benefit ratio, judicious use of herbal non fluoridated dentifrice could serve as a valuable therapeutic aid.

However, more studies should be undertaken to authenticate the remineralizing potential of herbal dentifrices.

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