RESEARCH ARTICLE

Remineralizing Potential of Low-fluoridated, Nonfluoridated and Herbal Nonfluoridated Dentifrices on Demineralized Surface of Primary Teeth: An *In Vitro* Study

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Abstract

Aim: The aim of this *in vitro* study is to evaluate the remineralizing potential of commercially available low-fluoridated, nonfluoridated, and herbal nonfluoridated child formula dentifrices on primary teeth.

Materials and methods: Total of 36 primary teeth were placed in the demineralizing solution for 96 hours to produce artificial carious lesions of approximately 100 μm depth, and then cut longitudinally into 30 sections of 100–150 μm thickness and randomly assigned to three groups. Sections were treated with low-fluoridated, nonfluoridated, and herbal nonfluoridated dentifrice. Lesions were evaluated using polarized light microscopy.

Results: Intragroup comparison of mean lesion depth from pretreatment to posttreatment among the three study groups revealed that maximum reduction in lesion depth was found to be in group I (low-fluoridated) followed in descending order by group III (herbal nonfluoridated) and group II (nonfluoridated), respectively.

Conclusion: Group I (low-fluoridated) and group III (herbal nonfluoridated) demonstrated remineralization of carious lesions by virtue of a decrease in lesion depth, whereas group II (nonfluoridated) showed an increase in lesion depth.

Clinical significance: Fluoride dentifrices are the most widely used products that deliver topical fluoride to the oral environment. The major drawback is the risk of dental fluorosis, which occurs because of ingestion of dentifrices, in preschool children. This necessitates use of preventive measures which include (1) reducing the amount of toothpaste used, (2) supervised brushing in preschool children and (3) developing low-fluoride toothpastes for minimizing risk of dental fluorosis. Further dental professionals must investigate effectiveness of increasingly popular "Herbal" products.

Keywords: Demineralization, Dentifrice, Fluoride, Herbal, Nonfluoride.

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Introduction

Dental caries, being one of the most easily avertible disease, is identified as the primary cause of oral pain and tooth loss[1] and is a progression resulting from an imbalance between multiple cycles of remineralization and demineralization.[2]

Many fluoridated toothpastes are currently available in market which when used on the regular basis provide maximum remineralization. However, the major drawback is the possibility of dental fluorosis in children, which occur because of less control over swallowing.[3] Low-fluoridated toothpaste have been promoted to reduce fluoride ingestion which minimizes the risk of dental fluorosis, but effectiveness of this toothpaste is controversial.[4]

Hence, other remineralizing agents were investigated, but their effectiveness is still questionable.[5]

Rising popularity of herbal toothpaste in the market, has pushed the dental professionals to study and infer the effectiveness of these products.[6,7] Many studies have been conducted to evaluate remineralizing potential of fluoride dentifrice but there are very few studies on comparison of low-fluoridated, nonfluoridated and herbal dentifrices. Hence, the present study was undertaken to assess the remineralizing potential of common dentifrices marketed as low-fluoridated, nonfluoridated, and herbal nonfluoridated on primary teeth.

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Materials and Methods

Study was conducted on 36 exfoliated or extracted primary anterior teeth. Criteria for inclusion were noncarious primary teeth (either extracted or exfoliated). Teeth with white spots and cracked areas were excluded. Teeth were cleaned and stored in distilled water until required.

The following dentifrices were used for comparison:

- Group I: Low-fluoridated Dentifrice (Kidodent)—Sodium Monofluorophosphate, Calcium Carbonate, Sodium carboxy-Methylcellulose, Glycerine, Calcium Peroxide, Xylitol, Sorbitol Solution, Polyethylene Glycol 400, Zedodent 165, Sodium Lauryl Sulfate, Titanium Dioxide, Polysorbate, Saccharin Sodium Menthol, Toothpaste Flavor, Sodium Benzoate, Purified water.
- Group II: Nonfluoridated Dentifrice (Pigeon)—Dicalcium phosphate Dihydrate, Purified water, Glycerine, Maltitol, Trimagnesium Phosphate, Carrageenan, Flavor, Alcohol, Denat, Sodium Lauryl Sulfate, Methylparaben, Propylparaben, CI 14,720 (Carmoisine).
- Group III: Herbal Nonfluoridated Dentifrice (Patanjali Dant Kanti Junior)—Foeniculum Vulgare, Glycyrrhiza Salvadora, Saladora Persica, Azadirachta Indica, Acacia Arabica, Mentha Spicata, Xanthoxylum Alatum, Syzygium Aromaticum.

Dentifrice Slurry Preparation

For preparation of dentifrice slurry deionized water (F.M. Chemical Industries) and the dentifrice slurry were mixed in 3:1 proportion. To achieve this 17 gm of dentifrice [measured using Wensar high precision balance weighing machine, (MAD 200)] was dispensed from the respective tube and transferred into three tubes containing deionized water and stirred with stirring rod until it was mixed. Before each pH cycle, fresh slurry for all three toothpastes was prepared and bottled in separate containers.

Demineralizing and Remineralizing Solution Preparation

The demineralizing solution comprised 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05 M acetic acid, with1 M KOH added to maintain pH.

The remineralizing solution comprised 1.5 mM$CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCL added to adjust the pH.

Before each pH cycle, fresh demineralizing and remineralizing solutions were prepared and portable standard digital pH meter [MW 101 pH meter (Milwaukee)] was used to check pH of both the solutions.

Lesion Formation

Buccal surface of teeth were coated with nail varnish leaving a window of 1 mm and then teeth were subjected to demineralizing solution for 96 hours. Longitudinal enamel sections of 100–150 µm were prepared by using Isomet low speed saw. Ten sections were randomly divided into three equal groups.

pH Cycling Model

The sections were then placed in pH cycling system on a Flask shaker (Biosafety cabinet manufactures). Each cycle involved three hours demineralization twice daily with 2 hours of remineralization in between demineralization. All the groups were treated for 1 minute with dental slurry before 1st demineralization and before and after 2nd demineralization. Sections were placed in remineralizing solution for 24 hours. After 7 days, removed the nail varnish with acetone.

Polarized Light Microscope Measurement

To evaluate the lesion depth in the enamel, samples were photographed using polarized light microscope (Menzel vision and robotics), at both stages of pH cycling (before and after). This was achieved by immersing the section in water, which generally shows distinct demarcation between the initial lesion and sound enamel. Any variation in the lesion (pre- and posttreatment) can be ascertained from the same magnification photomicrographs taken during pH cycling, that is, with 4x camera lens under the polarized light microscope. The lesion depth was measured by using Image J software by an independent, blinded observer. The data thus obtained was subjected to statistical analysis.

Results

The intergroup comparison of mean pretreatment lesion depth was done using Kruskal Wallis test and it showed, a statistically significant difference in mean pretreatment lesion depth of samples among the three groups. *Post hoc* pairwise comparison was done by using Mann–Whitney *U* test and it revealed that the pretreatment mean lesion depth of samples in group I (low-fluoridated) (Fig. 1) was maximum followed in a descending order by group II (nonfluoridated) (Fig. 3) and group III (herbal nonfluoridated) (Fig. 5), respectively (Table 1).

Intergroup comparison of mean posttreatment lesion depth was done using Kruskal Wallis test which showed that there was a statistically significant difference in mean posttreatment lesion depth of samples among the three groups. *Post hoc* pairwise comparison was done by using Mann–Whitney *U* test and it revealed that the posttreatment mean lesion depth in group II (nonfluoridated) (Fig. 4) was found to be maximum followed in a descending order by group I (low-fluoridated) (Fig. 2) and group III (herbal nonfluoridated) (Fig. 6), respectively (Table 2).

Intragroup comparison of mean lesion depth from pretreatment to posttreatment among the three study groups was done using Wilcoxon signed rank sum test which revealed that there was depletion in lesion depth of samples in group I (low-fluoridated) was found to be the most followed in descending order by group III (herbal) and group II (Nonfluoridated), respectively (Table 3).

Intergroup comparison of mean percentage depletion in lesion depth was done using Kruskal–Wallis test which showed that there was a statistically significant difference in mean posttreatment lesion depth of samples among the three groups. *Post hoc* pairwise comparison was done by using Mann–Whitney *U* test and it was showed that the posttreatment mean percentage depletion in lesion depth of samples in group I (low-fluoridated) was found to be the most followed in descending order by samples

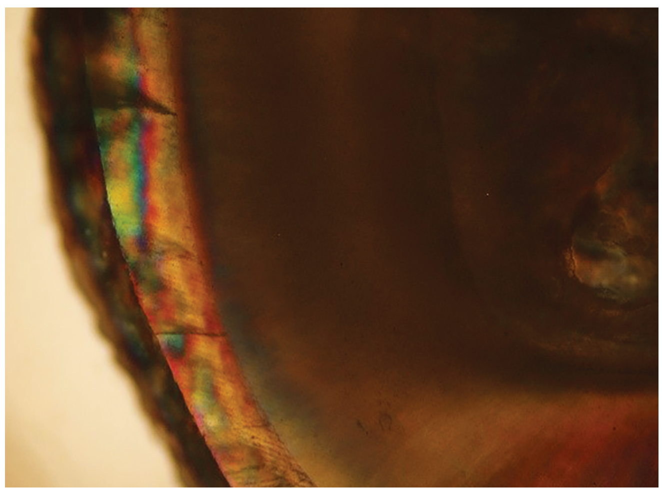

Fig. 1: Pretreatment photomicrograph (group I)

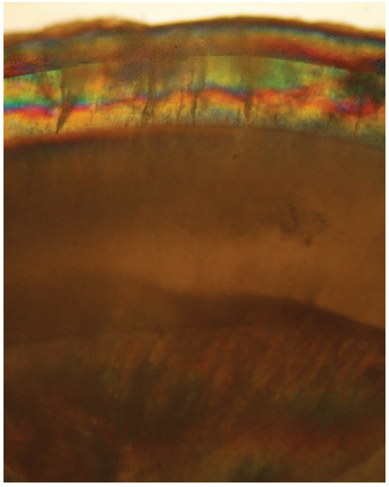

Fig. 2: Posttreatment photomicrograph (group I)

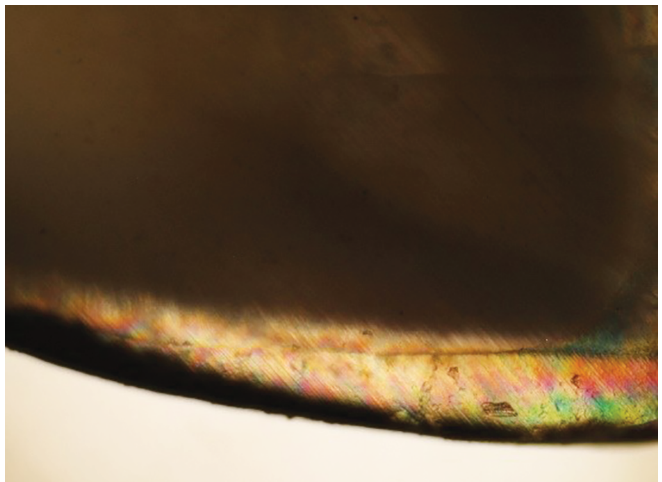

Fig. 4: Posttreatment photomicrograph (group II)

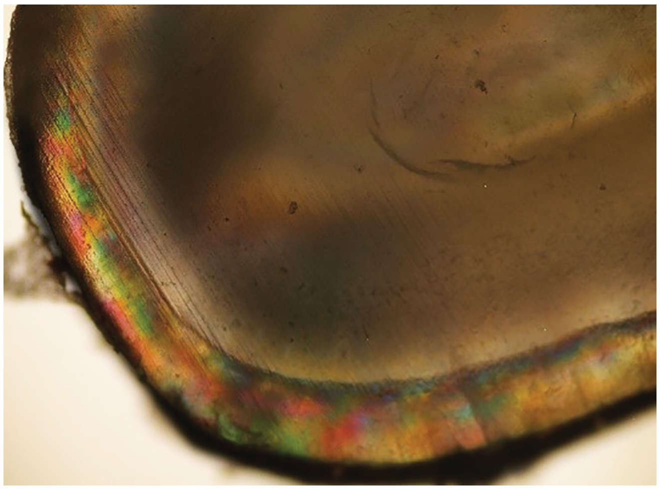

Fig. 3: Pretreatment photomicrograph (group II)

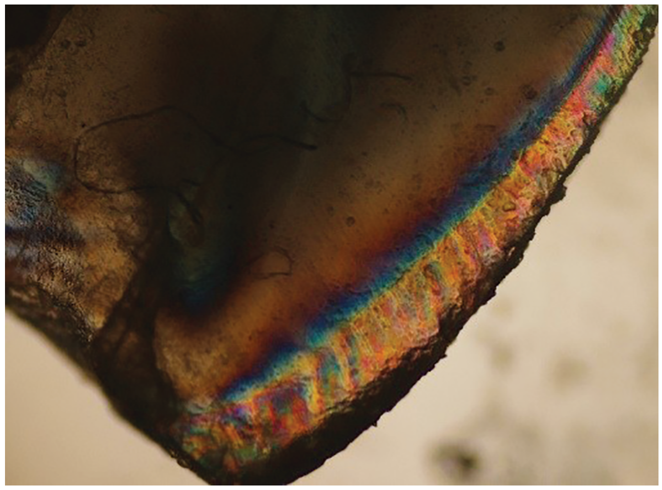

Fig. 5: Pretreatment photomicrograph (group III)

in group III (herbal nonfluoridated) and group II (nonfluoridated), respectively (Table 4).

Discussion

Dental caries is a contagious disease of teeth identified by a multifactorial etiology that results in destruction of hard tissues (enamel and dentine).[8] In the oral cavity is remineralization and demineralization cycle are in equilibrium, any interference in the balance of the cycle causes dental caries.

Remineralization is defined as the normal repair process of restoring minerals ion in the hydroxyapatite's lattice structure. Demineralization happen at a low pH when mineral ions are under saturated in oral cavity, with respect to mineral content of tooth.[4]

Nowadays, fluoride due to its remineralizing potential, recognized as the active ingredient in the oral hygiene products which is responsible for decrease in prevalence of dental caries worldwide.[9]

Fluoride toothpastes are among the most effective cariostatic agents. Hence, they are frequently used vehicle for fluoride delivery to the oral cavity in majority of the children.[10] However, study of Mitropoulos's and Milsom in 1990 identified fluoridated toothpaste as the only major cause of fluorosis.[11] Further, Burt and Mascarenhas in 1998 stated evidence of fluorosis in ages below six caused from swallowing fluoridated toothpaste.[12] Additionally Hong *et al.*, in 2006 advocated against use of fluoridated toothpaste in children who unable to expectorate toothpaste based on clear notable and positive association in prevalence of fluorosis and level of fluoride intake in early childhood years (0–3).[13]

Thus, for children identified at risk for developing mottled teeth idea of low-fluoride toothpastes were promoted. However, the fluoridated concentration in these toothpastes (400–550 ppm) was not different from fluoridated toothpaste (1055 ppm F) for prevention of caries and they did not significantly reduce risk of fluorosis.

Hence, other remineralizing agents were investigated including calcium phosphate-based remineralization system. Some of the currently available nonfluoride topical remineralizing agents are CPP-ACP, Sodium Calcium Phosphosilicate, Polyols, Nano-hydroxyapatite, Tricalcium phosphate, Theobromine, and Dicalcium phosphate dihydrate (DCPD). One of the most commonly used calcium phosphate based remineralizing system, dicalcium phosphate dihydrate, and is been used in fluoridated toothpastes to enhance the remineralizing effect of fluoride.

DCPD not only increases the level of calcium ion in plaque fluid but also acts as a fluoride carrier leading to enhanced uptake of fluoride in artificial enamel caries.[5] But However, the DCPD system drawback lies in increased cost and complexity in oral hygiene procedure.

Therefore, lately people's interest has been shifted to herbal toothpaste. These toothpaste do not contain any synthetic ingredients, which at times may cause diseases or other health tissues.[14] Their effectiveness can be accounted to various properties including antimicrobial, anti-inflammatory, antifungal, antidiabetic, astringent action, antidiabetic, antifungal, analgesic, and antiseptic properties. However, there is lack of clinical research and literature on efficacy of herbal based toothpaste due to which there no professional consensus with respect to the use of natural products.[15] Therefore this study was conducted to compare the efficacy of low-fluoridated, nonfluoridated, and herbal nonfluoridated dentifrices on primary teeth.

Ten Cate and Duijsters were first to propose the concept of *in vitro* pH cycling model. Two different types of pH-cycling models, 7 days and the 10 days pH-cycling are used.[16] In the present study, 7 days pH cycling is used. Based on the report of Thaveesangpanich

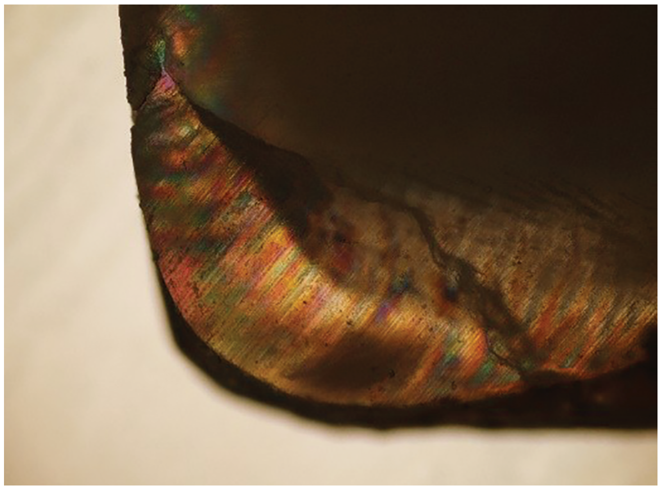

Fig. 6: Posttreatment photomicrograph (group III)

Table 1: Intergroup comparison of mean pretreatment lesion depth

	Lesion depth	*N*	*Mean*	*Std. deviation*	*95% confidence interval for mean*		*Minimum*	*Maximum*
					Lower bound	*Upper bound*		
Pretreatment	• Low-fluoridated	12	148.32	1.12	147.61	149.03	146.15	149.16
	• Nonfluoridated	12	128.65	1.71	127.56	129.74	126.40	130.40
	• Herbal nonfluoridated	12	125.4	1.81	124.31	126.61	122.46	128.46
	p^a value		<0.0001, S					
	Post hoc pairwise comparison[b]		2<3<1					

Table 2: Intergroup comparison of mean posttreatment lesion depth

	Lesion depth	*N*	*Mean*	*Std. deviation*	*95% confidence interval for mean*		*Minimum*	*Maximum*
					Lower bound	*Upper bound*		
Posttreatment	• Low-fluoridated	12	116.39	2.04	115.09	117.68	111.80	118.89
	• Nonfluoridated	12	122.13	4.78	119.10	125.17	115.30	130.36
	• Herbal nonfluoridated	12	114.37	2.11	113.03	115.71	110.45	118.45
	p^a value		<0.0001, S					
	Post hoc pairwise comparison[b]		2<1<3					

Table 3: Intragroup comparison of mean lesion depth from pretreatment to posttreatment

Group		*Mean*	*N*	*Std. deviation*	*p-value*
• Low-fluoridated	Pre	148.3209	12	1. 11,698	0.002, S
	Post	116.3863	12	2. 04,113	
• Nonfluoridated	Pre	128.6523	12	1. 71,229	0.01,S
	Post	122.13	12	4.78	
• Herbal nonfluoridated	Pre	125.4579	12	1. 80,908	0.002,S
	Post	114.3695	12	2. 10,891	

Table 4: Percentage reduction in lesion depth

		N	*Mean*	*Std. deviation*	*95% confidence interval for mean*		*Minimum*	*Maximum*
					Lower bound	*Upper bound*		
Percent reduction	I Low-fluoridated	12	21.53	1.00	20.90	22.17	19.75	23.50
	II Nonfluoridated	12	5.06	3.75	2.67	7.44	-0.74	10.9
	III Herbal nonfluoridated	12	8.4	0.93	8.25	9.43	7.79	11.07
	p^a value		<0.0001, S					
	Post hoc pairwise comparison[b]		3<1<2					

et al., wherein, it is reported that by 8th day of experiment all the sections were eroded thus rendering them inappropriate for evaluation. However, shortening in duration of the pH cycling might induce results that are insufficient to produce normal de/remineralization process. In the present study, before each cycle, the remineralizing and demineralizing solution were prepared and the pH was checked to avoid the possibility of saturation in solution as recommended by Gujrathi et al., in 2015.[17]

In the present study, primary incisors were collected by reason of limited alterations in the morphology and the wide flat surface area of enamel of buccal surface[18] and stored in distilled water till further use.[19] The nail varnish was applied, leaving 1 mm narrow window on the intact and sound buccal surface.[20] Specimens were photographed by using polarized light microscope to quantitatively access the lesion depth of enamel.[21]

When the percentage reduction in lesion depth is compared among three groups the mean value of group I (low-fluoridated- 21.53) was maximum followed in descending order by group III (herbal nonfluoridated- 8.84) and group II (nonfluoridated- 5.06), respectively.

The maximum remineralizing potential of low-fluoridated dentifrice compared to nonfluoridated and herbal dentifrices, could be ascribed to the presence of small amount of fluoride in the low fluoride dentifrice. This result is in agreement with the earlier studies of by Thaveesangpanich et al., Itthagarun et al., and Ekambaram et al., who found that 500 ppm fluoridated toothpaste prohibit demineralization and promoted remineralization among primary teeth.[22–24]

Deshpande et al., in 2014 stated that Colgate total toothpaste remains the benchmark as far as antimicrobial effectiveness is concerned. However, it is a forgone conclusion that a 1000-ppm fluoridated toothpastes is not safe for children. Kidodent has shown low level of fluoride along with significant quantity of antimicrobial activity, which makes it a right choice for use in children.[25,26]

In present study, low-fluoridated toothpaste containing sodium monofluorophosphate was tested.

According to Ten Cate JM, Ekambaram M *et al.*, Monofluorophosphate is when applied topically to the tooth surface is an effective caries inhibitor. This activity has been linked by many investigators to interaction between hydroxyapatite and monofluorophosphate.[16,24]

On comparing the percentage reduction in lesion depth among different groups, group III (herbal nonfluoridated) showed more reduction in lesion depth than group II (nonfluoridated) but less than group I (low-fluoridated). The reason for better effectiveness of herbal toothpaste could be attributed to the various herbal extracts in the herbal dentifrice.

The role of herbal toothpaste in reducing caries was established by Torwane et al., in 2015, who found that both herbal and commercially available dentifrices showed reduction in development of dental caries.[7]

According to Al-Otaibi et al. and Palombo, the synthetic compounds in traditional toothpaste may be harmful to human and environment and which is in stark contrast to plant products which have stood the test of the time for their efficacy, cultural acceptability, and safety. Thus, herbal dentifrice containing plant extracts was selected in our study.[27,28]

Kumar et al. suggested that antibacterial activity is similar with slightly better result in herbal toothpastes when compared against conventional toothpastes. This activity may be attributed to the efficacy of various components of the toothpaste in inhibiting growth of various organisms. The present study agrees with the above result.[29]

Out of the various ingredients of herbal toothpaste fennel seeds and neem were used in major proportion. A study by Ajithkrishnan et al. found that consumption of fennel seeds causes considerable rise in salivary pH, which is attributed to anticariogenic effect by preventing demineralization.[30]

Patil et al, in 2009 concluded that both neem containing toothpaste as well as a fluoridated toothpaste are equally efficacious in combating dental caries.[31] The result of the above study is similar to the result of present study where both low-fluoridated and herbal nonfluoridated dentifrices gave better results than nonfluoridated dentifrice.

On comparing the percentage reduction in lesion depth among the three groups, group II (nonfluoridated) showed least reduction in lesion depth when compared with group I (low-fluoridated) and group III (herbal nonfluoridated).

In the present study, least remineralization was observed in nonfluoridated group whose major ingredient was dicalcium phosphate dihydrate.

The abrasive agent in toothpaste is usually DCPD. It also leads to enhancement of remineralization of fluoride.[5]

Dentifrices incorporate braised systems to control built up naturally occurring stains on the teeth, some common abrasives are dicalcium phosphate, sodium bicarbonate, silica or calcium carbonate. Kumar et al., in 2013 stated that substantivity or antimicrobial activity of active ingredient may be modified by abrasives and detergents.[6]

In the present study deionized water is used to formulate dentifrice slurry, and according to Rirattanapong et al., in 2016, DCPD is sparingly soluble in a neutral solution (deionized water) so they are not expected to remain inactive. Similar finding was observed in the present study where posttreatment with nonfluoridated dentifrice containing DCPD there was an increase in lesion depth after posttreatment.[5]

The mechanism of action of DCPD is similar to CPP-ACP and TCP, as and act as a calcium phosphate reservoir, buffering the free calcium and phosphate ion activities and increase the level of calcium phosphate in plaque thus, enhancing enamel remineralization and decreasing enamel demineralization.[32] Brochner et al., in 2011 showed that the fluoride toothpaste was similar to the remineralization of CPP-ACP. Thus, using the CPP–ACP may lead to complex and expensive oral hygiene procedure.[33] Further, anticariogenic ability of CPP-ACP was questioned in study by Güçlü et al., in 2016 which inferred that CPP-ACP combined with fluoride resulted in no clinical advantage over fluorides alone.[34] Rirattanapong et al., in 2015 concluded that increased cost with no additional benefit of tricalcium phosphate makes its addition to fluoridated toothpaste for use in primary tooth unnecessary.[35]

The long term *in vivo* performance of calcium phosphate compounds for their application against dental caries in literature is scarcely available.[31,36] Since, most studies were conducted *in vitro* and randomized clinical trial data is unavailable. Thus, there is a mandatory need for further studies.

Conclusion

The present study, remineralizing potential of low-fluoridated, nonfluoridated and herbal nonfluoridated dentifrices on demineralized surface of primary teeth indicates that all three dentifrices showed caries preventive effect *in vitro*, but low-fluoridated dentifrice had the highest and nonfluoridated dentifrice had the lowest efficacy. Fluoride toothpaste is an effective, economical and widely available product for prevention of dental caries in general population. Therefore, fluoride toothpaste should be advised to patients by dental health professionals and further advances in study of herbal product may help to make oral health care better.

Clinical Significance

Fluoride dentifrices are the extensively used products that deliver topical fluoride to the oral cavity. The major drawback being the risk of dental fluorosis, which occurs because of the ingestion of dentifrices in preschool children. Preventive measures that minimize the risk of dental fluorosis includes (1) developing low-fluoride toothpastes, (2) reduced the amount of toothpaste used, and (3) supervised brushing in preschool children. Further dental professionals must investigate effectiveness of increasing popular herbal products.

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