

Original Article

Effect of dentifrices on their remineralizing potential in artificial carious lesions: An *in situ* study

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ABSTRACT

Background: The eventual sequel of dental caries is determined by the dynamic equilibrium between pathological factors which lead to demineralization and protective elements, which in turn leads to remineralization. Remineralization is the natural process for noncavitated demineralized lesions and relies on calcium and phosphate ions assisted by fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization. Hence, the present study was designed to evaluate the efficacy of fluoride dentifrices in remineralizing artificial caries-like lesions *in situ*.

Materials and Methods: A double-blind, randomized study with an initial washout period of 7 days was carried out for 3 weeks. Twenty volunteers were enrolled, who wore the intraoral cariogenicity test appliance having enamel slabs incorporated into them, for 3 weeks. 10 participants were instructed to use Group A dentifrice (fluoride) and the other 10 Group B dentifrice (nonfluoride) for brushing their teeth. The enamel slabs were analyzed by surface microhardness testing and scanning electron microscopy (SEM) at 3 intervals.

Results: No significant differences was seen in the microhardness values recorded for Group A and Group B at baseline and after demineralization ($P > 0.05$); however Group B exhibited lesser microhardness compared to Group A, after intra-oral exposure ($P < 0.05$). In the SEM analysis, the Group A enamel surfaces had more regular and longer crystallites to those of the Group B.

Conclusion: Fluoride dentifrices avert the decrease in enamel hardness and loss of minerals from the enamel surface to a large extent as compared to the nonfluoride dentifrices.

Key Words: Dental caries, dentifrices, fluoride, tooth remineralization

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INTRODUCTION

Dental caries is a universal oral disability present across the world and is a cause of great financial and social burden to both, the developed and the developing nations. Dental innovations have established and accorded that fluoride, a mineral found in rocks and soil, helps to prevent the tooth decay. Owing to the

dependence on refined carbohydrates, the incidence of caries has increased many folds in newly industrialized countries.^[1] Moreover, poor oral hygiene, insufficient dental care as well as the lacking preventive regimens have been blamed for this development.^[2] The ability of fluoride to inhibit or even reverse the initiation

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and progression of dental caries is well documented. The pioneer use of adjusted fluoride in water for caries control began in 1945 and 1946 in the United States and Canada when the fluoride concentration was adjusted in drinking water supply of four communities.^[3-6] Years after, the conclusion of the grand rapids fluoridation study, fluoride continues to be the main weapon in the battle against tooth decay. The success of water fluoridation in preventing and controlling dental caries has led to the development of fluoride-containing products, including dentifrice (i.e., toothpaste), mouth rinse, dietary supplements, and professionally applied or prescribed gel, foam, or varnish. The addition of fluoride to dentifrices began in the 1940's. Since then, its expanded use has led to a commendable reduction in the incidence of dental caries. Tooth brushing, using a fluoride dentifrice, is close to an ideal public health method in that its use is convenient, inexpensive, culturally approved and widespread in countries, where no national preventive system is scheduled or planned, or which have relatively high caries prevalence.

Fluoride works primarily via topical mechanisms which include inhibition of demineralization at the crystal surfaces inside the tooth,^[1] enhancement of remineralization at the crystal surfaces (the resultant remineralized layer is resistant to acid attack), and inhibition of bacterial enzymes.

Recently, fluoride dentifrices have been explored extensively for evaluation of its anticariogenic potential and the researchers around the globe unanimously vouch the beneficial effects of low concentration of fluoride in reducing tooth decay. However, a very few have dwelled into the aspect of remineralization of initial carious lesions in children, and no such study has been conducted in India. Therefore for the scrutiny of the ever growing debate on the use of fluoride dentifrices over the nonfluoride ones, this investigation was devised to ascertain, in a systemic fashion, through an *in situ* model, whether the microhardness and surface topography of enamel slabs treated with fluoride dentifrices would be similar to that of nonfluoride dentifrices.

MATERIALS AND METHODS

Experimental design

A double-blind 3 weeks randomized *in situ* study, with 1 week initial washout period, was carried out at Arya Kanya Gurukul, Ludhiana, Punjab, India. The various parameters analyzed were:

1. Microhardness of enamel slabs of two groups (fluoride and nonfluoride) and
2. Changes at ultrastructural level in enamel slabs of the two groups examined by scanning electron microscopy (SEM).

Inclusion criteria were: Good general and dental health, normal salivary flow (≥ 0.7 mL/min), no antibiotic treatments in the 6 months preceding the study and full complement of permanent dentition with no carious lesions.

Volunteers and ethical aspects

The total population of Arya Kanya Gurukul, Ludhiana, Punjab, India, was screened and finally 20 girls, in the age group of 12-15 years, who had the full complement of permanent dentition without dental caries and meeting the above-stated inclusion criteria, were recruited to participate in the study. Participants were enrolled after parents provided the written informed consent, and the protocol was reviewed and approved by the Ethics Committee of Maharishi Markandeshwar (M.M.) Dental College and Hospital, M.M. University, Mullana, Ambala, Haryana, India. Volunteers were eligible if they exhibited the clinical absence of carious lesions and/or periodontal disease, and there was no history of intake of antibiotics and fluoride for the last 6 months. Children undergoing fixed or removable orthodontic treatment were excluded. As the volunteers were from a boarding school, their diet regimen was similar. The participants were educated and instructed to record their daily food consumption, including sugar intake in a diet diary, for 7 days.

Preparation of specimens

Premolars extracted due to orthodontic reasons were stored in 1% thymol. Using water cooled diamond disc, an enamel slab of dimensions 4 mm \times 3 mm \times 2 mm was incised from the buccal surface of each tooth. The dimensions of the enamel slabs were standardized with the use of a digital Vernier caliper, and all precautions were taken to maintain the uniformity of the measurements. The enamel slabs thus obtained were examined under a light microscope to exclude the ones that did not reveal an intact surface. Specimens were then embedded in individual acrylic resin blocks and polished in a polishing machine (Buehler Phoenix Beta) using 320, 420, 600 grades of silicon carbide abrasive papers to remove the variable 50-100 μ m approximately of surface layer. The final polishing was completed using 1 μ m

alumina paste. Slabs were then washed with deionized water to remove any residue of polishing paste. Any enamel cores found to have surface imperfections, or white spots were rejected. The specimen were sterilized with ethylene oxide at 55°C and thoroughly aerated to remove the remaining traces of the sterilant. Baseline microhardness values were recorded for all the slabs using an HMV Vickers microhardness apparatus. A load of 500 g was applied, and the indenter was dropped perpendicular to the polished enamel surface of the slab that was mounted on the acrylic block. The loading time for each block was 15 s. Three indentations were measured and averaged for each slab. The indentations were measured at ×40 magnification. The enamel slabs were removed from the acrylic blocks with burs, washed with deionized water and stored in containers duly marked (at room temperature) till use.

Creation of artificial caries-like lesions

Specimen were placed in plastic vials containing demineralizing solution in 3.1 mmol/L calcium chloride, 3.1 mmol/L sodium dihydrogen orthophosphate, and 50 mmol/L glacial acetic acid. The pH of the solution was adjusted at 4.5 by the use of 1 mol/L potassium hydroxide and each slab was immersed in 3 ml of this demineralizing solution and incubated at 37°C for 2 days. Thereafter, the solution was changed and kept for another 3 days at same temperature and pH. Each specimen was then rinsed with 15 ml deionized water and stored in plastic vials.

Intraoral procedures

In the screening visit, oral examination was performed by a calibrated examiner. The intraoral cariogenicity test appliances were prepared according to the guidelines of Pearce and Gallagher.^[6] Mandibular arch impressions of the subjects were made using alginate. Casts were poured, and on the working models, appliances were fabricated in self-cure acrylic resin so as to fit the lower dental arches of the subjects. The enamel slabs were embedded in the windows made on the buccal flanges of the appliances in the permanent first molar region. The surface of each slab was kept at the same level as that of the acrylic resin surface of the appliance. Enrolled children were given a nonfluoride dentifrice during the initial 1 week washout period. They were given standard uniform toothbrushes and were demonstrated to use a pea-size dentifrice and horizontal scrub technique. At the end of the washout period, the appliances were delivered to the volunteers, and they were instructed to wear

them 1 day prior to the use of a randomly assigned dentifrice for 21 days. The dentifrices were coded and blinded for both, the investigator and the volunteers. The allocation method was based upon the chit system, wherein, 20 slips of equal size and shape; 10 marked as Group A and 10 as Group B, were folded and pooled in a bowl and shuffled. Children were asked to pick a slip and were allotted the respective group as was written on the slip.

The children, in both the groups, were instructed to brush their teeth, twice per day, once in the morning after breakfast, and once at night before going to bed, under the guidance and supervision of the school authorities. Subjects were refrained from using any other dentifrice other than their allocated product. During the study period of 3 weeks, the children brushed their teeth along with the appliances delivered to them, with the allocated toothbrushes. The amount of dentifrice taken per brushing was half the length of the toothbrush head, and the duration for each brushing was 2 min. A stopwatch was used to record the same. Immediately after brushing, the children were instructed to swish their mouth with 5 ml of water, so that carry over effect of fluoride dentifrice remains for a longer duration. The children were instructed to wear the appliances for 24 h, including during sleeping and eating, except for when they removed it for cleansing. None of these children used any other oral hygiene aid (i.e., flossing, mouth rinses, etc.).

At the end of the study period (i.e., after 21 days), the appliances were taken back from the children. The enamel slabs were removed from the appliances with acrylic burs, washed with deionized water, and stored in moist conditions in duly labelled plastic vials, until further analysis. Microhardness assessment and SEM analysis was performed as outlined previously.

Preparation of the sample for scanning electron microscopic analysis

The samples were vacuum dried for 1 h and then mounted on a SEM stub (aluminum discs), fixed with double sided adhesive carbon tape, coded and then put into sputter coater for coating for 4 min under Argon atmosphere. The samples were coated with a 100 Å thick film of Gold-Palladium and then placed in the vacuum chamber of the scanning electron microscope (ZEISS EVO 50). The acceleration voltage was standardized to 20 kV. The tilt angle and aperture were adjusted to optimize the quality

of photomicrograph. Samples were recorded at 1 K ($\times 1000$) and 5 K ($\times 5000$) magnifications at baseline and after demineralization.

Statistical analysis

The data collected was evaluated using SPSS Software, version 11.5 (IBM Corporation, N.Y., USA) for unpaired *t*-test to determine the significant difference if any between the groups. The statistical significant difference was considered if $P < 0.05$.

RESULTS

At baseline, there was no statistical significant difference in the average microhardness value of untreated, polished enamel slabs between the Group A (326.04 VHN) and Group B (324.54 VHN) ($P = 0.8700$) [Table 1]. After demineralization, the mean microhardness value for Group A was 138.8 VHN and for Group B was 142.83 VHN and the difference between the two was not statistically significant ($P = 0.5252$) [Table 1]. After 3 weeks of intra-oral exposure (IOE), there was statistically significant difference in terms of microhardness values, between the Groups A (238.71 VHN) and B (206.51 VHN) ($P = 0.0072$) [Table 1].

SEM analysis revealed smooth and homogenous surface topography in case of sound enamel at both 1 K and 5 K magnification [Figure 1a and b], whereas the demineralised enamel slabs showed irregularities and stepped lamination appearance [Figure 1c and d]. Group A's [Figure 1e and f] surfaces revealed longer crystallite formation compared to Group B's [Figure 1g and h].

Being a double-blind study, the dentifrices were decoded by the chief supervisor only after statistical evaluation of all parameters. The decoding revealed:

- Dentifrice A: Composed of standard chalk base-40% (approximately) with 1000 ppm sodium monofluorophosphate (MFP) and 0.13% calcium glycerophosphate.
- Dentifrice B: Composed of standard chalk base-40% (approximately) with no fluoride and no calcium glycerophosphate.

DISCUSSION

In the present study, the microhardness values of normal enamel slabs ranged from 296.6 to 350 VHN for Group A with a mean of 326.0 and 277 to 353.3 VHN with an average of 324.5 for Group B, which was in accordance with the results of Ryges and Foley^[4] who reported the hardness of enamel to be in the range of 270-350 KHN and 250-360 VHN. After IOE,

Table 1: Comparison of micro hardness changes between Group A and Group B

Group	Mean \pm SD	<i>t</i>	<i>P</i>
Baseline			
A	326.04 \pm 17.59	0.166	0.8700 (>0.05)
B	324.51 \pm 22.41		
Demineralisation			
A	138.8 \pm 16.15	0.648	0.5252 (>0.05)
B	142.83 \pm 11.20		
Remineralisation			
A	238.71 \pm 23.41	3.027	0.0072** (<0.05)
B	206.51 \pm 24.15		

**Statistically significant. SD: Standard deviation.

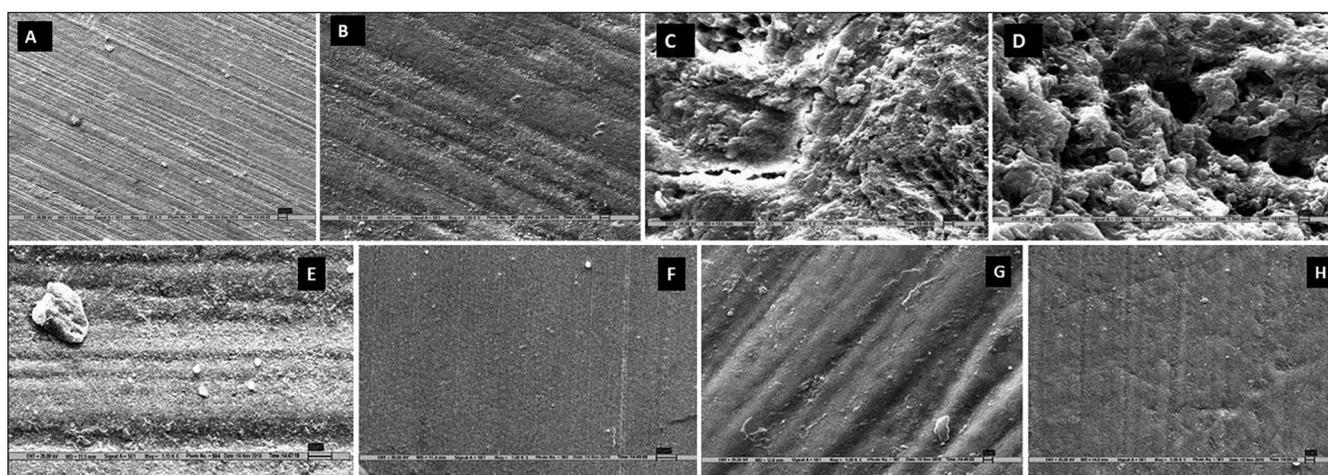


Figure 1: (a) Sound enamel slab at 1 K, (b) Sound enamel slab at 5 K, (c) Demineralised enamel slab at 1 K, (d) Demineralised enamel slab at 5 K, (e) Remineralised enamel slab (Group A) at 1 K, (f) Remineralised enamel slab (Group A) at 5 K, (g) Remineralised enamel slab (Group B) at 1 K, (h) Remineralised enamel slab (Group B) at 5 K.

the average percentage recovery in the microhardness was 53.37% for Group A and 35.05% for Group B. The present findings are consistent with the results of the studies carried out by Marines nobre dos santos.^[7] Furthermore, Featherstone *et al.*^[8] reported a 40-50% increase in the surface microhardness by the use of MFP dentifrices for 14 days. The increase in surface microhardness can be attributed to the structural repair due to remineralization. The cariostatic effects of dentifrices are ascribed to their ability to increase the rate of repair of carious enamel lesions through remineralization and increasing the resistance of remineralized areas to secondary acid attack.

Rehardening of lesions in Group B explains the remineralizing ability of saliva.^[9] Similar results were reported by Marines nobre dos santos.^[7] The significant increase in hardness observed in Group A explains increased remineralisation due to MFP in the presence of calcium glycerophosphate. The studies by Duke *et al.*^[10] and Lynch and ten Cate^[11] have also proved increased delivery of calcium to the plaque with enhanced protection from enamel demineralization when calcium glycerophosphate was added to sodium MFP dentifrice. The anti-caries efficacy of fluoride dentifrices have been established in numerous clinical trials over the last four decades.^[12] Researchers have suggested that the widespread use of fluoride dentifrices may have contributed significantly to the current decline in dental caries in the developed and developing countries.^[13,14] Fluoride concentration of dentifrices ranges between 850 and 1200 ppm, hence dentifrices containing 1000 ppm fluoride were used in the study. Sodium MFP is known to be a superior cariostatic agent as it maintains the apatite structure of enamel. It is also less susceptible to inactivation by dentifrice abrasive systems.^[15] Caries inhibition has been attributed either to the specific MFP anion or to the fluoride arising from MFP in the oral environment due to hydrolysis. Some authors report that phosphates and fluorides act independently in reducing caries.^[9]

The demineralization of teeth was carried out as per the technique described by Stephen *et al.*^[16] In this method, a buffer solution system was used, and the lesions were created over 5 days at 37°C. The intra-oral appliances used in the study were similar to the designs of Pearce and Gallahager, which is a modification of the original intra-oral cariogenicity test model devised by Koulourides *et al.*^[14] It has been observed that relative value of critical pH is significantly higher for children than for adults in both

resting and stimulated saliva.^[17] It has been observed that children exhibit a greater thermodynamic driving force for demineralization at low oral pH, and a lower force for remineralization at normal oral pH. This has led to an increased risk of demineralization in the child population.^[18]

A key parameter to be considered for the remineralization potential is the extent of variations in calcium concentration between resting saliva (where it is low) and stimulated saliva (where it is higher). Salivary calcium concentrations are comparatively lower in children than adults, whereas there is no considerable difference in the levels of phosphate concentrations.^[19]

Unerupted or newly erupted teeth may behave differently from older teeth in intra-oral models. Besides the difference in porosities and mineral content, they exhibit certain histological changes too. Fluoride penetrates more readily in deeper layers of immature teeth.^[20,21] Typically, the newly erupted teeth have clearly demarcated and obviously visible perikymata lines, whereas the same are lacking in the old teeth, which can most likely be attributed to abrasion. SEM of initial lesions demonstrates that prism pits, along the perikymata lines, tend to be the initial sites of acid penetration during lesion formation. Furthermore, globules of calcium fluoride-like material are also preferentially formed along these lines. For mechanistic intra-oral studies, it may, therefore, be of significance, be it be with the use of immature enamel from impacted or newly erupted teeth or enamel from older individuals, due to the difference in reactivity.^[18,19] The photomicrographs revealed features which are similar to those made by Hubbard.^[22] Group A photomicrograph, after remineralization, revealed a regular, smooth and homogenous appearance of the surfaces as compared to the baseline photomicrograph, whereas the photomicrographs of Group B revealed the surface depressions due to the dissolution of enamel rods which have not improved considerably compared to study group at 1 K magnification. At higher magnification, (5K), the photomicrographs of Group B showed irregularities that were of a lesser amount as compared to the demineralisation photomicrograph but more than Group A. Some discrete zones of crystallite formation are also seen. These findings are in accordance with Moller and Schröder,^[23] who reported that *in vivo* remineralization results in a more regular, smoother and homogenous

surface. Hence, it can be concluded that the use of fluoride dentifrice results in a more homogenous surface with densely packed crystallites. Similar findings have been reported previously.^[24] Under clinical conditions, it can be questioned if this is a result of remineralization alone or a combination of remineralization and mechanical wear through brushing. The appearance of the surface after treatment supports this assumption.

CONCLUSION

The study has demonstrated that MFP dentifrices prevent the decrease in enamel microhardness and the loss of minerals from the enamel surface to a large extent, as compared to nonfluoride dentifrices. Thus fluoride dentifrices have the potential to remineralize previously demineralised enamel as compared to non fluoride dentifrices. The elevated plaque calcium levels have the potential to elevate the level of fluoride in the plaque, which is considered to be another parameter attributing to reduced dental caries experience.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

REFERENCES

- Brunelle JA, Carlos JP. Recent trends in dental caries in U.S. children and the effect of water fluoridation. *J Dent Res* 1990;69:723-7.
- Featherstone JD. Prevention and reversal of dental caries: Role of low level fluoride. *Community Dent Oral Epidemiol* 1999;27:31-40.
- Mohammadi TM, Hajizamani A, Hajizamani HR, Abolghasemi B. Fluoride varnish effect on preventing dental caries in a sample of 3-6 years old children. *J Int Oral Health* 2015;7:30-5.
- Ryge G, Foley DE, Fairhurst CW. The study of enamel microhardness with atomic force microscopy and microhardness. *J Dent Res* 1961;40:1116-21.
- Featherstone JD, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. *Caries Res* 1983;17:385-91.
- Pearce EI, Gallagher IH. The behaviour of sucrose and xylitol in an intra-oral caries test. *N Z Dent J* 1979;75:8-14.
- Nobre-dos-Santos M, Rodrigues LK, Del-Bel-Cury AA, Cury JA. *In situ* effect of a dentifrice with low fluoride concentration and low pH on enamel remineralization and fluoride uptake. *J Oral Sci* 2007;49:147-54.
- Featherstone JD, Cutress TW, Rodgers BE, Dennison PJ. Remineralization of artificial caries-like lesions *in vivo* by a self-administered mouthrinse or paste. *Caries Res* 1982;16:235-42.
- Rošin-Grget K, Peroš K, Sutej I, Bašić K. The cariostatic mechanisms of fluoride. *Acta Med Acad* 2013;42:179-88.
- Duke SA, Rees DA, Forward GC. Increased plaque calcium and phosphorus concentrations after using a calcium carbonate toothpaste containing calcium glycerophosphate and sodium monofluorophosphate. Pilot study. *Caries Res* 1979;13:57-9.
- Lynch RJ, ten Cate JM. Effect of calcium glycerophosphate on demineralization in an *in vitro* biofilm model. *Caries Res* 2006;40:142-7.
- Yin W, Hu DY, Li X, Fan X, Zhang YP, Pretty IA, *et al.* The anti-caries efficacy of a dentifrice containing 1.5% arginine and 1450 ppm fluoride as sodium monofluorophosphate assessed using quantitative light-induced fluorescence (QLF). *J Dent* 2013;41 Suppl 2:S22-8.
- Oliveira MJ, Martins CC, Paiva SM, Tenuta LM, Cury JA. Estimated fluoride doses from toothpastes should be based on total soluble fluoride. *Int J Environ Res Public Health* 2013;10:5726-36.
- Koulourides T, Phantumvanit P, Munksgaard EC, Housch T. An intraoral model used for studies of fluoride incorporation in enamel. *J Oral Pathol* 1974;3:185-96.
- Kiranmayi M, Nirmala SV, Nuvvula S. Appraisal of the remineralizing potential of child formula dentifrices on primary teeth: An *in vitro* pH cycling model. *Contemp Clin Dent* 2015;6 Suppl 1:S81-5.
- Stephen KW, Damato FA, Strang R. An *in situ* enamel section model for assessment of enamel re/demineralization potential. *J Dent Res* 1992;71:856-9.
- Cury JA, Tenuta LM. Evidence-based recommendation on toothpaste use. *Braz Oral Res* 2014;28:1-7.
- Carey CM. Focus on fluorides: Update on the use of fluoride for the prevention of dental caries. *J Evid Based Dent Pract* 2014;14 Suppl:95-102.
- Langhorst SE, O'Donnell JN, Skrtic D. *In vitro* remineralization of enamel by polymeric amorphous calcium phosphate composite: Quantitative microradiographic study. *Dent Mater* 2009;25:884-91.
- Ogaard B, Rølla G. The *in vivo* orthodontic banding model for vital teeth and the *in situ* orthodontic banding model for hard-tissue slabs. *J Dent Res* 1992;71:832-5.
- Girenes G, Ulusu T. An *in vitro* evaluation of the efficacy of a novel iontophoresis fluoride tray on remineralization. *J Clin Exp Dent* 2014;6:e327-34.
- Hubbard MJ. Correlated light and scanning electron microscopy of artificial carious lesions. *J Dent Res* 1982;61:14-9.
- Möller H, Schröder U. Early natural subsurface caries. A SEM study of the enamel surface before and after remineralization. *Caries Res* 1986;20:97-102.
- Bansal K, Gauba K, Tewari A, Chawla HS, Sahni A. *In vivo* remineralization of artificial enamel carious lesions using a mineral-enriched mouthrinse and a fluoride dentifrice: A polarized light microscopic comparative evaluation. *J Indian Soc Pedod Prev Dent* 2010;28:264-70.