ORIGINAL RESEARCH

Comparative Evaluation of Remineralizing Potential of Two Nonfluoridated Dentifrices to a Fluoridated Dentifrice on Artificial Enamel Caries Lesion: An *In-vitro* Study

Surabhi Raji[1], Praveena G[2], Veena Chandrababu[3], Drisya Soman[4], Vijitha Saleem[5]

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Abstract

Background: Dental caries is preventable and reversible with remineralizing agents. Due to fluorosis risks, nonfluoridated alternatives are gaining interest. This study compares the efficacy of fluoridated and nonfluoridated dentifrices on artificial enamel lesions.

Aim: To compare the remineralizing potential of nonfluoridated dentifrices containing theobromine and strontium chloride hexahydrate to a fluoridated dentifrice containing arginine and sodium monofluorophosphate on artificial enamel carious lesions.

Materials and methods: Fifty-one human premolars were sectioned into enamel blocks (10 × 10 × 2 mm) and embedded in acrylic resin. Artificial caries lesions were created using a demineralization solution at 37°C for 96 hours. After initial scanning electron microscopy (SEM) analysis, samples were divided into three groups: A (theobromine), B (strontium chloride hexahydrate), and C (arginine + sodium monofluorophosphate). Agents were applied twice daily for 2 minutes over 2 weeks, with storage in artificial saliva. Final SEM evaluation and scoring followed Gupta et al.'s criteria.

Result: Group C (fluoridated: arginine + sodium monofluorophosphate) had the highest remineralizing efficiency (4.76 ± 0.43), followed by group B (strontium chloride, 3.76 ± 0.56) and group A (theobromine, 1.64 ± 0.49). All differences were statistically significant with the largest between groups A and C (3.12). Strontium chloride showed superior performance among nonfluoridated agents, with results closer to the fluoridated dentifrice.

Conclusion: All dentifrices promoted enamel remineralization, with the fluoridated dentifrice being most effective. Strontium chloride hexahydrate outperformed theobromine among non-fluoridated agents. Despite lower efficacy, theobromine warrants further research.

Clinical significance: This study highlights the potential of nonfluoridated dentifrices as effective alternatives to fluoridated ones for enamel remineralization, offering safer options for individuals seeking fluoride-free oral care.

Keywords: Arginine and sodium monofluorophosphate, Enamel caries lesion theobromine, Fluoridated and nonfluoridated dentifrices, Fluoride, Remineralization, Strontium chloride hexahydrate.

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Introduction

Dental caries is a slow-progressing disease of the tooth's hard tissues caused by fermentable carbohydrates (mainly sugars), cariogenic bacteria in dental plaque, and an imbalance between demineralization and remineralization.[1] Fluoride reduces dental caries by replacing hydroxyl in hydroxyapatite to form fluorapatite, which is more acid-resistant. While fluoride has long been central to non-invasive caries management, concerns about overuse—especially in children—have arisen due to risks of dental and skeletal fluorosis.[2] As a result, attention has turned to alternative remineralizing agents. One such agent is theobromine, a natural compound from cocoa beans. It forms larger, more acid-resistant enamel crystallites and has been shown to increase enamel hardness. *In-vitro* studies also suggest that theobromine may offer superior antimicrobial activity against *Streptococcus* mutans compared to some fluoridated toothpastes.[3]

Theobromine, a natural alkaloid from the cacao plant, is a promising fluoride alternative in toothpaste. As a member of the methylxanthine family, it promotes enamel remineralization by enhancing apatite crystal growth and increasing enamel hardness. It facilitates the formation of larger, more acid-resistant enamel crystals, making it an effective, non-toxic remineralizing agent.[4] Strontium, chemically similar to calcium, has been widely used since the 1960s for dentin hypersensitivity relief. It adsorbs to calcified tissues and can occlude dentinal tubules, mimicking natural remineralization. While its direct role in caries prevention

[1]Department of Conservative Dentistry & Endodontics, Braces and Roots Dental Clinic, Kerala, India

[2–4]Department of Conservative Dentistry & Endodontics, Azeezia College of Dental Sciences & Research, Kollam, Kerala, India

[5]Department of Oral Healthcare, Dubai Health Authority, Dubai, United Arab Emirates

Corresponding Author: Veena Chandrababu, Department of Conservative Dentistry & Endodontics, Azeezia College of Dental Sciences & Research, Kollam, Kerala, India, Phone: +91 8921889759, e-mail: veenazgp@gmail.com

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remains unclear, strontium can substitute calcium in hydroxyapatite and enhance enamel crystallinity, especially when combined with fluoride.[5]

This study was conducted to compare the remineralizing effectiveness of fluoridated (arginine and sodium monofluorophosphate) and non-fluoridated (theobromine and strontium chloride hexahydrate) dentifrices on early enamel caries lesions.

Materials and Methods

Study Population and Study Design

This comparative *in-vitro* study was conducted on randomly selected 51 freshly extracted premolar teeth collected from the Department of Oral and Maxillofacial Surgery, from unknown patients. A comparative *in-vitro* study was conducted to compare the remineralizing potential of nonfluoridated dentifrices to a fluoridated dentifrice on artificial enamel caries lesions.

Selection Criteria

Inclusion Criteria

Intact human premolar teeth.

Exclusion Criteria

Premolar teeth with developmental defects, caries, cracks, fractures, and restoration.

Procedure

Fifty-one freshly extracted premolar teeth meeting the inclusion criteria (intact crowns, no caries, cracks, restorations, or developmental defects) were collected from the Department of Oral and Maxillofacial Surgery from unknown patients. The total sample size of 51 was determined based on previous *in-vitro* remineralization studies and calculated to achieve 80% statistical power and a 5% significance level (Lwanga & Lemeshow).[6] Extracted teeth were stored in a 0.1% thymol solution at room temperature and used within 1 month. Crowns were decoronated using a diamond disc under water cooling, and enamel blocks approximately 10 × 10 × 2 mm were prepared from the labial surface and embedded in self-curing acrylic resin blocks (11 × 11 × 4 mm) for ease of handling. Artificial caries lesions were induced following Ten Cate's protocol by incubating specimens at 37°C for 96 hours in 10 mL of demineralization solution per sample, prepared using 2.2 mM calcium chloride, 2.2 mM sodium dihydrogen phosphate, and 0.05 M acetic acid, with pH adjusted to 4.4 using 1.0 M potassium hydroxide; the solution was refreshed every 48 hours to maintain consistent conditions.

After demineralization, specimens were rinsed with distilled water and subjected to baseline scanning electron microscopy (SEM) analysis to confirm uniform lesion formation. Baseline SEM analysis was performed after demineralization to confirm uniform lesion formation. Due to the high cost and time requirements of SEM imaging, pre-SEM was carried out on a subset of samples ($n = 3$) randomly selected from each group. This approach is consistent with previous *in-vitro* studies, such as Prabhakar and Arali.[7] The 51 samples were then randomly divided into three groups of 17 samples each: Group A (theobromine), group B (strontium chloride hexahydrate), and group C (arginine with sodium monofluorophosphate). Dentifrices were applied for 2 minutes using a cotton tip applicator, followed by rinsing with distilled water, and application was repeated twice daily for 2 weeks, with specimens stored in artificial saliva between applications to simulate oral conditions. After the treatment period, samples were dehydrated, mounted on aluminum stubs, sputter-coated with gold, and examined under SEM at 15 kV and 1000× magnification; surface morphology was evaluated and scored using the remineralization criteria described by Gupta et al.[8]

Results

Group C (fluoridated: arginine + sodium monofluorophosphate) had the highest remineralizing efficiency (4.76 ± 0.43), followed by group B (strontium chloride, 3.76 ± 0.56) and group A (theobromine, 1.64 ± 0.49). All differences were statistically significant, with the largest between groups A and C (3.12). Strontium chloride showed superior performance among nonfluoridated agents, with results closer to the fluoridated dentifrice.

Scanning electron microscopy imaging confirmed complete demineralization of tooth samples, revealing deep prismatic holes and a characteristic honeycomb pattern (Fig. 1). Upon remineralization, group A samples treated with theobromine (nonfluoridated dentifrice) showed reduced prismatic hole depth and retained the honeycomb appearance (Fig. 2), corresponding to remineralization scores of 1 and 2 as per Gupta et al.'s criteria. Group B samples treated with strontium chloride hexahydrate exhibited amorphous or globular mineral deposits on enamel surfaces, with partial prism core filling (Fig. 3), achieving higher remineralization scores of 3 and 4.

While micro-computed tomography combined with vibrational spectroscopies or X-ray diffraction is ideal, SEM and microhardness testing serve as effective alternatives. Scanning electron microscopy analysis showed that group C samples treated with fluoridated dentifrice (Arginine and sodium monofluorophosphate) exhibited smooth, homogeneous surfaces with faint prism shadows (Fig. 4), achieving remineralization scores of 4 and 5 per Gupta et al.'s criteria. Group B (Strontium chloride hexahydrate) showed moderate remineralization with scores of 3 and 4, while group A

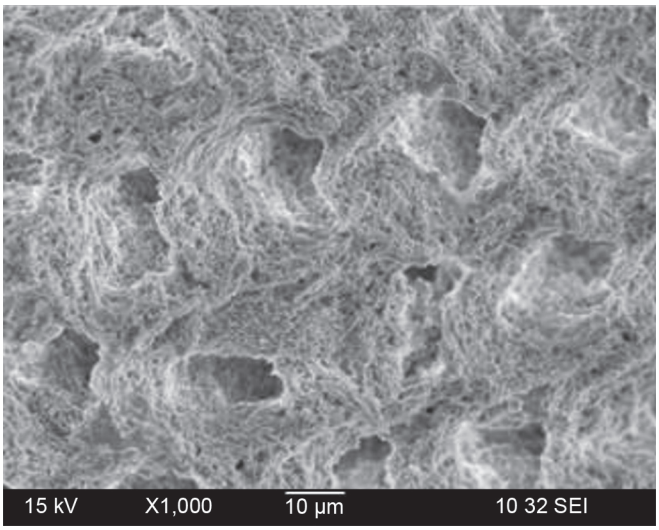


Fig. 1: Scanning electron microscopy image of demineralized sample

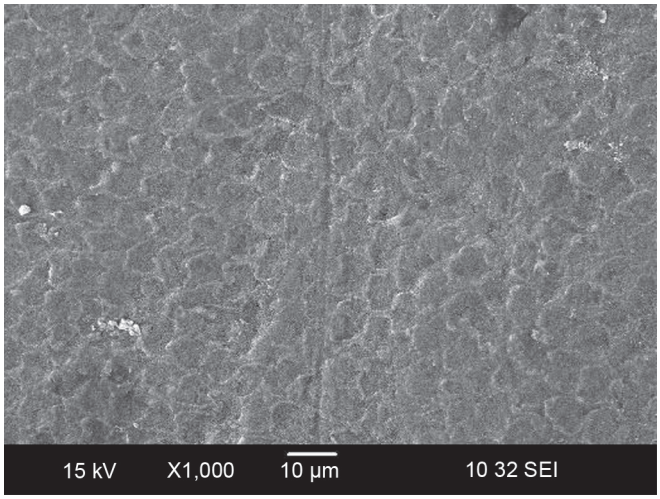


Fig. 2: SEM image of samples remineralized with nonfluoridated dentifrice group A: Theobromine

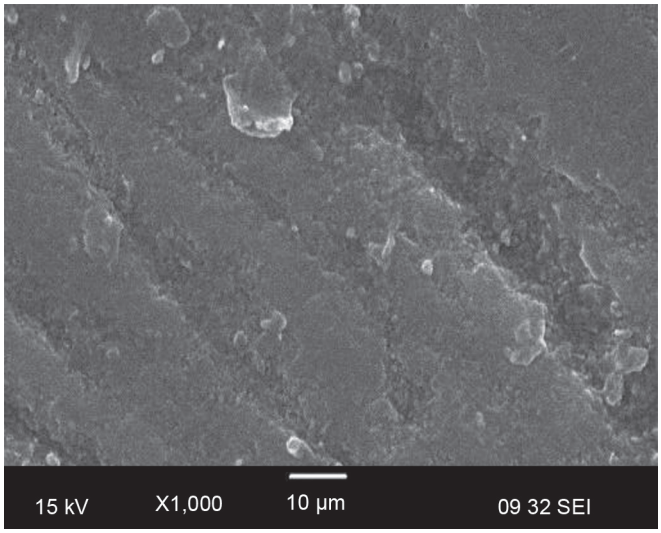


Fig. 3: SEM image of samples remineralized with nonfluoridated dentifrice group B: Strontium chloride hexahydrate

(Theobromine) showed the least, with scores of 1 and 2. Scoring was done by a blinded evaluator to avoid bias (Fig. 5; Tables 1 and 2).

Discussion

This study compared the remineralizing efficiency of two non-fluoridated dentifrices—Theobromine (group A) and Strontium chloride hexahydrate (group B)—with a fluoridated dentifrice containing Arginine and sodium monofluorophosphate (group C) on artificially induced enamel caries.

Demineralization was achieved using Ten Cate's protocol, where samples were immersed in solution for 96 hours to produce lesions 60–100 µm deep, simulating *in-vivo* conditions.[9] Remineralizing agents were applied twice daily for 14 days, following the methods by Shirahatti et al.[10] with specimens stored in artificial saliva to mimic the oral environment. The application was done with a cotton tip for 2 minutes, aligning with American Dental Association's (ADA) recommendation findings that 2-minute brushing improves plaque removal and oral health. Scanning electron microscopy, a sensitive

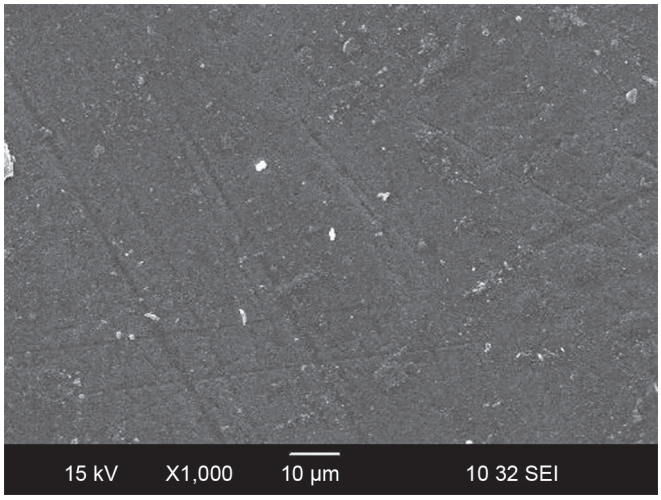


Fig. 4: SEM image of samples remineralized with fluoridated dentifrice group C: Arginine and sodium monofluorophosphate

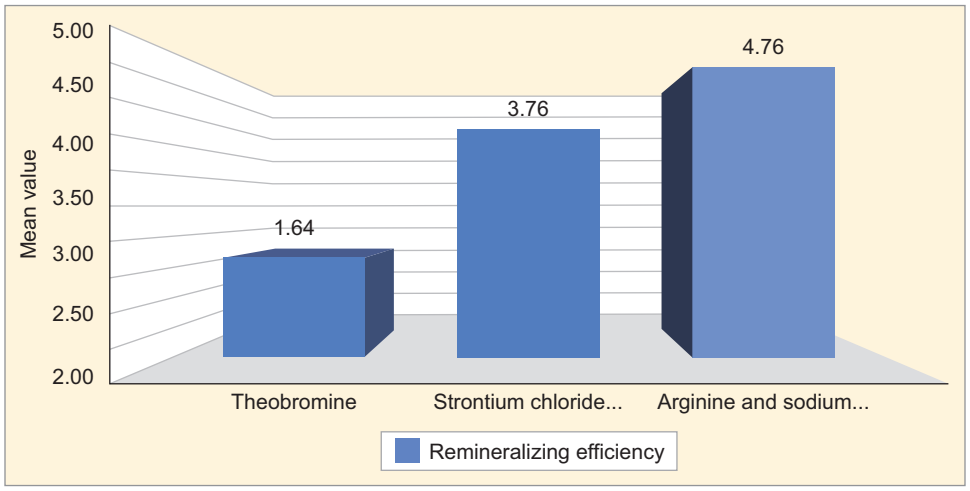


Fig. 5: Intergroup comparison based on remineralizing efficiency

Table 1: Evaluation parameters for remineralization

Pattern observed	*Scoring criteria*
Deep prismatic holes giving a typical honeycomb appearance and/or single or groups of focal holes.	0 (demineralized)
Fine particle-like granular Variable-sized deposits with uneven distribution.	1
Reduction in the depths of prismatic holes due to deposition of minerals within the prism cores. Honeycomb appearance is still present.	2
Enamel prism pits are visible with mineral deposit only partially filling up the prism cores. Honeycomb appearance not visible	3
Amorphous precipitate-like deposit scattered unevenly on the surface or globular particles deposited uniformly on the enamel surface.	4
Smooth homogeneous flat surface with slightly discernible prism shadows.	5 (sound)

Table 2: Intergroup comparison based on remineralizing efficiency

Groups		*N*	*Remineralizing efficiency*		*p-value*
			Mean	*SD*	
A	Theobromine	17	1.64	0.49	0.001*
B	Strontium chloride hexahydrate	17	3.76	0.56	
C	Arginine and sodium monofluorophosphate	17	4.76	0.43	

ANOVA test applied; SD, standard deviation; Level of significance: ≤0.05; *Statistically significant

and accurate technique, was used to assess surface changes, porosity, and crystal structure, with scoring based on Gupta et al.'s remineralization criteria.[8]

Remineralization scores were compared between two groups at a time using an Independent *t*-test. In our study, statistically significant differences were observed between all groups ($p \leq 0.05$). The fluoridated dentifrice showed the highest remineralization, followed by Strontium chloride, with Theobromine being the least effective. Strontium chloride hexahydrate demonstrated superior remineralization among the nonfluoridated agents, highlighting its potential beyond known desensitizing effects. The fluoridated dentifrice's superior performance is likely due to the combined action of sodium monofluorophosphate (promoting fluorapatite formation and antibacterial effects) and arginine (enhancing pH and mineral uptake).[11] The use of artificial saliva may have supported remineralization in all groups.

The antibacterial effect of sodium monofluorophosphate inhibits *Streptococcus* mutans by reducing its glycogen content, while fluoride reduces hydroxyapatite dissolution in acidic environments, which enhances remineralization.[12] Studies have also shown that sodium monofluorophosphate reduces dental caries and decayed, missing, and filled surfaces (DMFS), decayed, missing, and filled teeth (DMFT) values.[13,14] Arginine, a natural semi-essential amino acid in human saliva, helps neutralize acidic byproducts of sugar metabolism by promoting an alkaline environment. This reduces the cariogenic potential of oral biofilms.[15] Chandru et al. compared toothpaste containing arginine and sodium monofluorophosphate, calcium silicate, and zinc hydroxyapatite, concluding that the Arginine and sodium monofluorophosphate combination was a strong remineralizing agent.[16] The synergistic effect of arginine, fluoride, and calcium carbonate enhanced enamel hardness and resistance to acid attack, promoting maximum remineralization. Arginine, when combined with calcium and sodium monofluorophosphate in toothpaste, enhances remineralization by attracting phosphate and calcium ions to promote hydroxyapatite crystal growth.[17]

Remineralizing agents, such as fluoride, create a supersaturated environment that aids in forming a fluorapatite layer, preventing further mineral loss. Fluoride is particularly effective in remineralizing early lesions and preventing caries, though excessive fluoride use can cause dental fluorosis.[18] Fluoridated toothpaste is a widely used preventive agent but has been linked to fluorosis, especially in children. Burt and Mascarenhas linked fluorosis in children under six to swallowing fluoridated toothpaste, causing tooth discoloration. Skeletal fluorosis, resulting from long-term fluoride exposure, leads to stiffness and pain due to fluoride deposition in bones.[19] The International Academy of Oral Medicine and Toxicology (IAOMT) has raised concerns about fluoride consumption, citing potential risks such as high blood pressure, osteoarthritis, and neurological issues. Recommended fluoride levels are 1000 ppm for children up to 3 years and 1350–1500 ppm for those older.[20] Sodium fluoride, sodium monofluorophosphate, and amine fluoride are common fluoride forms in dentifrices.

Due to fluoride's limitations and systemic effects, nonfluoridated remineralizing agents like Theobromine and Strontium chloride hexahydrate were considered. Theobromine, a low-toxicity alkaloid, promotes remineralization by forming larger crystals than hydroxyapatite.[21] However, in this study, arginine and sodium monofluorophosphate showed higher remineralizing efficiency compared to Theobromine. Amaechi and colleagues demonstrated that theobromine significantly enhanced enamel remineralization—comparable to that of NaF—with significantly lower required concentration, indicating that small-molecule theobromine can promote hydroxyapatite crystal growth in an apatite-forming medium.

A recent systematic review further confirmed that theobromine increases enamel microhardness to levels similar to fluoride. However, under simulated plaque fluid conditions, theobromine alone did not affect remineralization, suggesting that its efficacy may depend on environmental factors.[22–24] Strontium chloride hexahydrate, used for over 50 years in dentifrices and as a desensitizing agent, seals dentinal tubules and reduces sensitivity. Previous studies have shown that the presence of strontium levels in water lowers caries prevalence by replacing the calcium ion (Ca^{2+}) in crystals [$Ca_{10}(PO_4)_6(OH)_2$], thus enhancing enamel remineralization. Fluoride and strontium are known to promote anti-caries effects, as a synergistic anticariogenic process, with the possibility that the strontium-fluoride combination decreases solubility and improves enamel crystallinity.[6,25] While its remineralizing effect on early enamel caries is less studied, it shows potential by precipitating on tooth surfaces, suppressing acid production, and promoting mineral calcification. Due to its similarity to calcium, strontium can strongly bind to calcified tissues and aid in simulating natural remineralization.[26]

In our study, strontium chloride hexahydrate (nonfluoridated) showed higher remineralizing efficiency than theobromine but lower than the fluoridated dentifrice containing arginine and sodium monofluorophosphate, supporting its use for reversing early enamel lesions among nonfluoridated agents. The study concluded that fluoridated dentifrice had the highest remineralizing potential, followed by nonfluoridated dentifrices, with theobromine being the least effective. A limitation of this study is that the study was performed *in-vitro* and that pre-SEM analysis was performed only on a subset of samples due to resource constraints, which may limit generalizability.

Conclusion

- All dentifrices showed remineralization in early enamel caries.
- The fluoridated dentifrice demonstrated significantly higher remineralizing efficiency than nonfluoridated options.
- Among nonfluoridated dentifrices, strontium chloride hexahydrate showed superior remineralizing potential compared to theobromine.

Ethics Approval Statement

After due consideration,the study titled "Comparative evaluation of remineralizing potential of two nonfluoridated dentrifices to a fluoridated dentrifice on artificial enamel caries lesion: An *in-vitro* study" has presented study related documents and were reviewed and the institutional Ethics committee has approved the study.

Data Availability Statement

The data supporting the findings of this study are available from The corresponding author upon request.

Artificial Intelligence (AI) Disclosure

No AI-assisted technologies were used.

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Authors' Contributions

Conceptualization – Dr Surabhi Raji, Dr Praveena G, Dr Veena Chandrababu.
Methodology – Dr Drisya Soman, Dr Surabhi, Dr Vijitha Saleem, Dr Veena Chandrababu.
Investigation – Dr Surabhi Raji, Dr Veena Chandrababu.
Data curation – Dr Praveena G, Dr Surabhi Raji, Dr Veena Chandrababu.
Formal analysis: Dr Praveena G, Dr Vijitha Saleem, Dr Drisya Soman.
Writing – original draft – Dr Surabhi Raji, Dr Veena Chandrababu.
Writing – review and editing – Dr Drisya Soman, Dr Vijitha Saleem, Dr Praveena G.
Supervision – Dr Praveena G, Dr Vijitha Saleem, Dr Drisya Soman.
Project administration – Dr Praveena G, Dr Surabhi Raji, Dr Drisya Soman.
Funding acquisition – Dr Surabhi Raji, Dr Veena Chandrababu.

Orcid

Surabhi Raji https://orcid.org/0009-0004-9455-4502
Praveena G https://orcid.org/0000-0001-8784-6081
Veena Chandrababu https://orcid.org/0009-0008-7156-3771
Drisya Soman https://orcid.org/0000-0002-5656-1195
Vijitha Saleem https://orcid.org/0009-0004-1881-6611

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