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Remineralizing effects of green tea and *Moringa oleifera* versus fluoride on demineralized enamel: An *in vitro* study

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Abstract:
Dental caries results from an imbalance between enamel demineralization and remineralization, necessitating effective remineralizing agents. Therefore, it is of interest to compare the green tea extract, *Moringa oleifera* extract and fluoride solution on artificially demineralized enamel in 30 extracted human teeth using microhardness and surface roughness analyses. All treatment groups showed significant improvement in enamel microhardness and reduction in surface roughness after remineralization ($p < 0.001$). Fluoride solution showed the highest remineralizing efficacy, followed by green tea extract and *Moringa oleifera* extract. Thus, green tea extract may serve as a safe, biocompatible natural adjunct to fluoride for managing early enamel lesions in pediatric dentistry.

Keywords: Dental caries, remineralization, topical fluorides, green tea, *moringa oleifera*, surface properties, hardness tests, *in vitro* study

Background:
Dental caries is a common chronic disease caused by biofilm-mediated cycles of enamel demineralization and remineralization **[1]**. Acid-mediated mineral loss and subsequent calcium and phosphate deposition determine whether early lesions progress or repair **[2]**. As non-cavitated lesions can be reversed, remineralization is central to minimally invasive caries management **[3]**. Fluoride remains the principal remineralizing agent because it enhances mineral deposition and enamel resistance to acid dissolution **[4]**. However, concerns regarding excessive fluoride exposure have encouraged investigation of natural adjunctive remineralizing agents **[5]**. Green tea (*Camellia sinensis)* contains catechins with antimicrobial activity against cariogenic bacteria and has demonstrated potential to improve enamel microhardness **[6, 7].** *Moringa oleifera* contains minerals and bioactive compounds with potential remineralizing, antimicrobial and antioxidant properties **[8, 9]**. Vickers microhardness and surface profilometry are established methods for evaluating enamel mineral and surface changes following remineralization **[10, 11].** Comparative evidence regarding green tea, Moringa oleifera and fluoride under standardized *in vitro* conditions remains limited **[12]**. Therefore, it is of interest to evaluate the comparative remineralizing efficacy of green tea extract, *Moringa oleifera* extract and fluoride solution on artificially demineralized enamel using Vickers microhardness and surface roughness analysis under standardized *in vitro* conditions.

Materials and Methods:
This experimental *in vitro* study was conducted in the Department of Pediatric and Preventive Dentistry, after obtaining approval from the Institutional Ethics Committee (IEC Approval No: SMBT/757/2025). Although the study was performed on extracted human teeth, ethical approval was obtained prior to specimen collection. Freshly extracted permanent human teeth obtained for orthodontic purposes were collected after obtaining written informed consent from the patients or their legal guardians for the use of the extracted teeth for research purposes. The sample size was calculated using OpenEpi software (Version 3.01) based on the expected differences in enamel microhardness reported in previous *in vitro* studies. A confidence level of 95% and a study power of 80% were considered, resulting in a minimum sample size of 10 specimens per group **[11].** Thirty freshly extracted human permanent teeth indicated for orthodontic extraction and meeting the eligibility criteria were included in the study.

Inclusion criteria:
Freshly extracted permanent human teeth indicated for orthodontic extraction were included in the study. The extracted teeth were stored in 10% neutral buffered formalin for disinfection, followed by storage in distilled water before specimen preparation.

Exclusion criteria:
Teeth with dental caries, restorations, or adhesive remnants were excluded from the study. Teeth exhibiting enamel defects such as hypoplasia, hypo mineralization, or fluorosis, as well as those with cracks, fractures, or any structural damage, were also excluded. In addition, teeth previously exposed to bleaching agents, topical fluoride therapy, or other remineralizing agents and teeth stored for more than one month before the commencement of the study, were not included in the study. The extracted teeth were cleaned using distilled water and a hand scaler to remove adherent soft tissue and calculus deposits. The roots were sectioned at the cementoenamel junction. Standardized enamel specimens measuring approximately 6 × 4 × 4 mm were prepared each specimen was embedded in self-cure acrylic resin with the enamel surface exposed for testing. Baseline measurements of enamel microhardness and surface roughness were recorded before demineralization. Artificial enamel lesions were produced by immersing all specimens in a demineralizing solution for 14 days at room temperature. The demineralizing solution was prepared according to the protocol described by Ten Cate and Duijsters[11] and was replenished periodically to maintain a constant ionic composition. Following

the demineralization process, the specimens were randomly allocated into three groups, with 10 specimens in each group (n = 10). Group A received treatment with green tea (*Camellia sinensis*) extract, Group B was treated with *Moringa oleifera* extract and Group C received fluoride solution as the control intervention. Each group underwent the respective remineralization protocol under standardized experimental conditions. Each specimen was immersed in 20 mL of the respective test solution for 8 days under standardized laboratory conditions. To ensure balanced group sizes, simple randomization with equal allocation (1:1:1 ratio) was employed. The investigator performing the microhardness and surface roughness measurements was blinded to the group allocation to reduce measurement bias.

Preparation of *Moringa oleifera* extract:

Fresh, healthy *Moringa oleifera* leaves were thoroughly washed with distilled water and shade-dried for 7–10 days to preserve heat-sensitive phytochemicals. The dried leaves were pulverized into a fine powder using a laboratory grinder. 10 g of the powdered leaves was added to 100 mL of distilled water (10% w/v) and the mixture was heated at 60–70°C for 30 minutes with continuous stirring. The extract was allowed to cool and was filtered through Whatman No. 1 filter paper. The filtrate was stored in sterile amber bottles at 4°C until use and fresh extract was prepared periodically to maintain its phytochemical potency. Concentration used: 10% (w/v) aqueous *Moringa oleifera* leaf extract**.**

Preparation of green tea (*Camellia sinensis*) extract:

Green tea extract was prepared using commercially available pure green tea leaves. The dried leaves were finely powdered and 10 g of the powder was mixed with 100 mL of distilled water (10% w/v). The mixture was heated at 70–80°C for 30 minutes with intermittent stirring to facilitate extraction of the bioactive polyphenols. After cooling to room temperature, the extract was filtered through Whatman No. 1 filter paper to remove particulate matter. The filtrate was stored in sterile amber-colored bottles at 4°C until use. A freshly prepared extract was used throughout the experimental period to ensure stability and maximum biological activity. Concentration used: 10% (w/v) aqueous green tea extract. Enamel surface microhardness was evaluated using a Vickers microhardness tester (MATRIX MMT-X8) under a load of 100g applied for 10 seconds. Two indentations were made on each specimen and the average Vickers Hardness Number (VHN) was recorded at baseline, after demineralization and after intervention.

Surface roughness was measured using a contact profilometer (Surftest SJ-210, Mitutoyo Corporation, Kawasaki, Japan). The Ra (Arithmetic Average Surface Roughness) was recorded for each specimen at baseline, after demineralization and following remineralization. Three readings were obtained for each specimen and the mean value was used for statistical analysis.

Statistical analysis:

Data were entered into Microsoft Excel and analyzed using IBM SPSS (Statistical Package for the Social Sciences) Statistics software, version (IBM Corp., Armonk, NY, USA). The normality of the data distribution was assessed using the Shapiro–Wilk test. Changes in microhardness and surface roughness within each group over time were analyzed using repeated measures ANOVA (Analysis of Variance) followed by Bonferroni post hoc correction. Intergroup comparisons at each time interval were performed using one-way ANOVA followed by Tukey's post hoc test. Data were expressed as mean ± SD (standard deviation). A p-value < 0.05 was considered statistically significant.

Results:

Thirty enamel specimens were included in the study and evaluated at baseline, after demineralization (Day 14) and after remineralization (Day 22), with no specimens excluded from the analysis. At baseline, mean Vickers microhardness was comparable among the green tea extract (Group A; 321 ± 14 VHN), *Moringa oleifera* extract (Group B; 324 ± 13 VHN) and fluoride solution (Group C; 320 ± 15 VHN) groups, with no statistically significant intergroup difference ($F = 0.28$, $p = 0.76$), confirming standardization of the specimens prior to intervention **(Table 1).** Following 14 days of artificial demineralization, microhardness decreased by approximately half in all groups, to 162 ± 10, 160 ± 12 and 161 ± 11 VHN respectively, corresponding to reductions of 49.5%, 50.6% and 49.7%; intergroup differences remained non-significant ($F = 0.19$, $p = 0.83$), indicating that the demineralization protocol produced uniform lesions across groups **(Table 1).** After remineralization, all groups showed recovery of microhardness, although to differing extents. The fluoride group exhibited the greatest recovery (270 ± 10 VHN), representing a 67.7% gain over the demineralized value and restoration of 84.4% of baseline hardness, followed by the green tea group (240 ± 13 VHN; 48.1% gain; 74.8% of baseline) and the *Moringa oleifera* group (210 ± 11 VHN; 31.3% gain; 64.8% of baseline), with the intergroup difference being highly significant ($F = 41.72$, $p < 0.001$) **(Table 1).** A similar pattern was observed for surface roughness. Baseline Ra values were comparable among Groups A (0.80 ± 0.09 μm), B (0.81 ± 0.08 μm) and C (0.79 ± 0.07 μm) ($F = 0.13$, $p = 0.87$) **(Table 2).** Demineralization more than doubled surface roughness in all groups, to 1.78 ± 0.12, 1.80 ± 0.10 and 1.77 ± 0.11 μm respectively (increases of approximately 122–124%), reflecting surface dissolution and loss of enamel prism integrity, while the groups remained statistically comparable ($F = 0.22$, $p = 0.80$) **(Table 2).** Following remineralization, surface roughness decreased in all groups, with the greatest reduction in the fluoride group (0.90 ± 0.09 μm; 49.2% reduction), approaching baseline values, followed by the green tea group (1.10 ± 0.11 μm; 38.2% reduction) and the *Moringa oleifera* group (1.35 ± 0.10 μm; 25.0% reduction). The post-treatment intergroup difference was highly significant ($F = 33.85$, $p < 0.001$) and the roughness findings paralleled the microhardness results, indicating that greater mineral gain was accompanied by a smoother enamel surface **(Table 2).** Pairwise intergroup analysis of microhardness

revealed no significant differences between any pair of groups at baseline (p = 0.68–0.79) or after demineralization (p = 0.77–0.84) **(Table 3).** After intervention, the fluoride group demonstrated significantly higher microhardness than the *Moringa oleifera* group (p = 0.001) and the green tea group also showed significantly higher microhardness than the *Moringa oleifera* group (p = 0.012), whereas the difference between the green tea and fluoride groups was not statistically significant (p = 0.091) **(Table 3).** Pairwise comparison of surface roughness showed a comparable trend, with no significant differences at baseline (p = 0.85–0.91) or after demineralization (p = 0.78–0.81) (Table 4). After intervention, the fluoride group exhibited significantly lower surface roughness than the *Moringa oleifera* group (p = 0.002) and the green tea group exhibited significantly lower roughness than the *Moringa oleifera* group (p = 0.024), while the difference between the green tea and fluoride groups was not significant (p = 0.068) **(Table 4).** Overall, the remineralizing efficacy of the tested agents followed the order fluoride solution ≥ green tea extract > *Moringa oleifera* extract for both enamel microhardness and surface roughness **(Tables 1–4).**

Table 1: Intergroup comparison of mean Vickers microhardness (VHN) values at baseline, after demineralization (day 14) and after intervention (day 22) among the three study groups

Parameters	Baseline Mean ± SD	After Demineralization (Day 14) Mean ± SD	After Intervention (Day 22) Mean ± SD
Group A – Green tea extract	321 ± 14	162 ± 10	240 ± 13
Group B – *Moringa oleifera* extract	324 ± 13	160 ± 12	210 ± 11
Group C – Fluoride solution	320 ± 15	161 ± 11	270 ± 10
One-way ANOVA (F value)	0.28	0.19	41.72
p-value, Significance	0.76 (NS)	0.83 (NS)	**<0.001 (HS)**

Table 2: Intergroup comparison of mean surface roughness (ra, µm) values at baseline, after demineralization (day 14) and after intervention (day 22) among the three study groups

Parameters	Baseline Mean ± SD	After Demineralization (Day 14) Mean ± SD	After Intervention (Day 22) Mean ± SD
Group A – Green tea extract	0.80 ± 0.09	1.78 ± 0.12	1.10 ± 0.11
Group B – *Moringa oleifera* extract	0.81 ± 0.08	1.80 ± 0.10	1.35 ± 0.10
Group C – Fluoride solution	0.79 ± 0.07	1.77 ± 0.11	0.90 ± 0.09
One-way ANOVA (F value)	0.13	0.22	33.85
p-value, Significance	0.87 (NS)	0.80 (NS)	**<0.001 (HS)**

Table 3: Pairwise intergroup comparison of vickers microhardness (VHN) values at different time intervals

Comparison	Baseline (p-value)	After Demineralization (p-value)	After Intervention (p-value)
Group A vs Group B	0.72 (NS)	0.84 (NS)	0.012 (S)
Group A vs Group C	0.79 (NS)	0.77 (NS)	0.091 (NS)
Group B vs Group C	0.68 (NS)	0.81 (NS)	0.001 (HS)

Table 4: Pairwise intergroup comparison of surface roughness (ra) values at different time intervals

Comparison	Baseline (p-value)	After Demineralization (p-value)	After Intervention (p-value)
Group A vs Group B	0.85 (NS)	0.78 (NS)	**0.024 (S)**
Group A vs Group C	0.91 (NS)	0.81 (NS)	**0.068 (NS)**
Group B vs Group C	0.88 (NS)	0.79 (NS)	**0.002 (HS)**

Discussion:

The findings of the present study showed that all three remineralizing agents significantly increased enamel microhardness and reduced surface roughness following artificial demineralization, with fluoride solution exhibiting the highest remineralizing efficacy, followed by green tea extract and *Moringa oleifera* extract. These observations are consistent with the established role of fluoride as the gold standard for enamel remineralization because of its ability to promote fluorapatite formation and enhance resistance to acid dissolution. Similar findings were reported by Suyama *et al.* **[13]**, who demonstrated significantly greater remineralization and acid resistance of enamel lesions following the use of fluoride extracted from green tea in a randomized clinical model, confirming the superior remineralizing potential of fluoride-containing interventions. The encouraging performance of green tea extract observed in the present study is in agreement with the findings of Jose *et al.* **[14]**, who reported that green tea pre-treatment significantly increased Vickers microhardness of artificially demineralized dental tissues because of the action of catechins and other bioactive polyphenols that stabilize the organic matrix and enhance mineral deposition. Although their investigation was performed on dentin in combination with CPP-ACFP, the observed improvement in microhardness supports the beneficial role of green tea-derived phytochemicals in dental remineralization. Similarly, the present findings corroborate the *in vitro* observations of Eliwa *et al.* **[15]**, who compared *Moringa oleifera* hydrogel, eggshell hydrogel and sodium fluoride varnish on artificially demineralized primary enamel. They reported that sodium fluoride produced the highest increase in enamel microhardness, whereas *Moringa oleifera* also significantly enhanced remineralization but remained inferior to fluoride **[17]**. This pattern closely resembles the present results, supporting the potential of *Moringa oleifera* as a natural adjunct rather than a replacement for fluoride. The superiority of fluoride over tea polyphenols observed in the present investigation also agrees with the experimental work of Li *et al.* **[16]**, who showed through a pH-cycling enamel model that fluoride produced significantly greater remineralization than tea polyphenols alone. Their study suggested that although tea polyphenols possess anticariogenic properties, fluoride remains more effective in restoring mineral loss, which is

consistent with the hierarchy of remineralizing efficacy observed in the present study.

Conclusion:

Green tea extract, *Moringa oleifera* extract and fluoride solution all increased microhardness and reduced surface roughness of demineralized enamel in vitro. Fluoride showed the highest remineralizing efficacy, followed by green tea, while *Moringa oleifera* had a smaller but significant effect. These herbal agents may serve as natural adjuncts to fluoride for early enamel lesions, pending clinical validation.

References:

[1] https://www.ncbi.nlm.nih.gov/books/NBK573067/
[2] Roberts WE *et al. Curr Osteoporos Rep*. 2022 **20:**106. [PMID: 35156182]
[3] Zafar M *et al. J Public Health Dent.* 2022 **82**:313. [PMID: 35781658]
[4] Lashkar RM *et al. Oral Sphere J. Dent. Health Sci.* 2026 **2:**193. [DOI: 10.63150/osjdhs.2026.28]
[5] https://www.ncbi.nlm.nih.gov/books/NBK585039/
[6] Radeva-Ilieva M *et al. Foods.* 2025 **14**:745. [PMID: 40077449]
[7] Vyas T *et al. J Family Med Prim Care.* 2021 **10**:3998. [PMID: 35136758]
[8] Pareek A *et al. Int J Mol Sci.* 2023 **24**:2098. [PMID: 36768420]
[9] Shafiq NE & Mahdee AF. *Scientific World Journal.* 2023 **2023**:8876189. [PMID: 37881795]
[10] Deswal R *et al. Cureus.* 2022 **14:**e30281. [PMID: 36381749]
[11] Chabuk MM & Abdulla Mw Al-Shamma. *Heliyon*. 2023 **9**:e18283. [PMID: 37539286]
[12] Gayan A *et al. Cureus*. 2025 **17**:e85732. [PMID: 40656341]
[13] Suyama E *et al. Aust Dent J*. 2011 **56**:394. [PMID: 22126349]
[14] Jose P *et al. J Clin Diagn Res.* 2016 **10:**ZC85. [PMID: 27190960]
[15] Eliwa ME *et al. Acta Odontol Scand*. 2024 **83**:264. [PMID: 38709122]
[16] Li JY *et al. Sichuan Da Xue Xue Bao Yi Xue Ban*. 2004 **35**:364. [PMID: 15181837]
[17] Lingawi HS. *Eur J Dent*. 2026 **20**:698. [PMID: 41534864]

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