Original Article

Effect of a Nano-hydroxyapatite Toothpaste on Enamel Erosive Lesions of Third Molars Induced by Exposure to Orange Juice

Marzieh Mehrjoo[1], Roza Haghgoo[2], Motahare Ahmadvand[2]

[1]Private Practitioner, Tehran, Iran, [2]Department of Pediatric Dentistry, Faculty of Dentistry, Shahed University, Tehran, Iran

Abstract

Objectives: This study aimed to assess the effect of a nano-hydroxyapatite (nano-HA) toothpaste on erosive enamel lesions of third molars induced by exposure to orange juice. **Materials and Methods:** In this *in vitro*, experimental study, the microhardness of 24 sound-extracted third molars was measured by a Vickers tester. The teeth were then randomly assigned to three groups (n = 8) of nano-HA toothpaste (Pharmed), 1.23% sodium fluoride gel, and artificial saliva. The teeth were exposed to orange juice for 5 min daily for 7 days and were then exposed to nano-HA toothpaste, fluoride gel, or artificial saliva (depending on their group allocation) for 10 min a day. The microhardness of the teeth was measured again after 7 days. Data were analyzed using paired t-test, analysis of variance, and Bonferroni test (alpha = 0.05). **Results:** Within-group comparisons showed a significant reduction in microhardness of the teeth after the intervention in artificial saliva (P = 0.000), and fluoride gel (P = 0.002) groups. However, no significant reduction occurred in the microhardness of the nano-HA group, compared with the baseline (P = 0.132). Between-group comparisons revealed no significant difference in the microhardness of the three groups at baseline ($P > 0.05$). However, after the intervention, the microhardness of the nano-HA group was significantly higher than that of other groups ($P < 0.05$). However, the difference in secondary microhardness between fluoride gel and artificial saliva groups was not significant (P = 1.00). **Conclusion:** Pharmed toothpaste containing nano-HA has optimal efficacy for remineralization of enamel erosive lesions induced by exposure to orange juice.

Keywords: *Artificial saliva, dental enamel, hydroxyapatites, sodium fluoride, tooth erosion, toothpastes*

Introduction

Chemical wear of teeth, also known as dental erosion, occurs as the result of frequent direct contact of teeth with acids, which results in dissolution and degradation of enamel and dentin.[1,2] In other words, dental erosion refers to the irreversible destruction of tooth structure through a chemical process irrespective of bacterial activity.[1] At present, dental erosion has become more common in the young population due to greater consumption of carbonated drinks and fruit juices.[3] The prevalence of erosion varies from 13% to 60% in the literature; however, a consensus has been reached on the increasing prevalence of erosion worldwide.[4]

Erosion mainly occurs due to exposure to acidic substances, and a correlation exists between the consumption of nonalcoholic beverages and dental erosion in adults.[5] A considerable increase in the consumption of nonalcoholic beverages, diet cokes, and fruit juices is among the main causes of the growing prevalence of dental erosion.[6]

Dental erosion has adverse consequences such as hypersensitivity to hot and cold foods and drinks, toothache, spontaneous hypersensitivity, dentin exposure, pulp involvement, compromised esthetics, and destruction of tooth structure.[7] Chemical tooth wear is irreversible, and if left untreated, it can eventually lead to tooth loss.[8] Thus, several treatment options have been proposed to increase enamel microhardness following the consumption of erosive foods and drinks.

Orange juice is among the most frequently consumed fruit juices worldwide. One orange averagely produces 90 g of juice, which has a pleasant fruity and sour taste and many nutritional benefits.[9,10] However, its acidic pH may adversely affect the tooth enamel and cause dental erosion.

How to cite this article: Mehrjoo M, Haghgoo R, Ahmadvand M. Effect of a nano-hydroxyapatite toothpaste on enamel erosive lesions of third molars induced by exposure to orange juice. Contemp Clin Dent 2024;15:17-21.

Submitted : 05-Mar-2023
Revised : 04-Aug-2023
Accepted : 13-Nov-2023
Published : 23-Mar-2024

Address for correspondence:
*Dr. Motahare Ahmadvand, No. 39, Italy Street, Enghelab Square, Tehran, Iran.
E-mail: motahare.ahmadvand@gmail.com*

Access this article online
Website: https://journals.lww.com/cocd
DOI: 10.4103/ccd.ccd_104_23
Quick Response Code:

Hydroxyapatite (HA) (Ca10[PO4]6[OH] 2) is an important biological substance and a main constituent of the mineral part of bone and tooth.[2,4] It is a suitable bioceramic for medical and dental applications as in dental implant coatings, orthopedic devices, alveolar ridge reconstruction, and drug delivery systems due to its optimal biocompatibility and possession of chemical and biological properties comparable to those of bone.[11] With the advances in nano-technology, nano-HA was also produced, which has higher solubility, higher surface energy, and more favorable biocompatibility and bioactivity than HA.[3,4] Nano-HA is the most similar compound to tooth structure. Nano-HA is increasingly used in dental materials due to its optimal biocompatibility, remineralization potential, and antimicrobial activity. Furthermore, nano-HA is more active than HA due to having a larger surface-to-volume ratio.[12] HA has several medical applications; however, information regarding its remineralizing effect on dental erosions is limited.[9]

Considering the adverse consequences of erosion and the need for treatment of erosive lesions, attempts are ongoing to find suitable remineralizing agents for erosive lesions. Accordingly, this study aimed to assess the effect of a nano-HA toothpaste on third molar enamel erosive lesions induced by exposure to orange juice.

Materials and Methods

This *in vitro* experimental study was conducted on extracted third molars with no caries, hypoplasia, enamel cracks, or erosions. The teeth had been extracted for purposes not related to this study.

The sample size was calculated to be 24, according to previous studies.[13-15] The collected teeth were inspected clinically and under a stereomicroscope at × 40 magnification to ensure the absence of caries (according to the World Health Organization criteria), wear, cracks, or hypocalcification. The teeth had been extracted within 1 month before the study onset, and stored in water in a glass container during this period. To prevent contamination, water was refreshed twice weekly. For the experiment, the teeth were mounted in autopolymerizing acrylic resin (Acropars, Iran) and the tooth surfaces were mechanically cleaned with a nonfluoridated prophy paste containing pumice (Associated Dental Product Ltd., Kemdent Works, UK) and low-speed hand-piece operating at 500–1500 rpm, to eliminate calculus and debris. For proper microhardness measurement, the surface of the samples was polished with grit silicon carbide abrasive papers of sizes 800, 1000, 2000, and 5000 (Matador Wasserfest, Germany) in the presence of water. It allowed the smoothed surface to be analyzed by a Vickers microhardness measuring device. After drying, the primary microhardness of the teeth was measured by a Vickers hardness tester (Shimadzu, Japan). For this purpose, the best point that was on a clean, flat surface free of any contamination and irregularity, for load application was identified under the stereomicroscope, and a 50 g load was applied within 10–15 s for assessment of microhardness.[16,17]

The teeth were then immersed in artificial saliva (composed of 1.5 mmol/L calcium, 0.9 mmol/L phosphorous, 150 mmol/L KCl, and 0.05 mg/mL fluoride in 0.1 mol/L Tris buffer, and pH of 7)[18] for 7 days. During this period, the teeth were removed from artificial saliva on a daily basis at a specific time and immersed in 40 cc of orange juice (Sunich; Alifard, Iran) for 5 min to simulate intake of orange juice twice daily each time for 2.5 min. The pH of orange juice was measured to be 3.4. Orange juice was refrigerated before use and its temperature was 9°C at the time of immersion of specimens to simulate drinking cold orange juice in the clinical setting. Next, the teeth were removed from orange juice and placed back in artificial saliva. The teeth were randomly divided into three groups (n = 8) of (I) Pharmed toothpaste (Dr. Akhavi Lab Co., Iran) containing nano-HP, surfactants, silica, and extracts of chamomile, common sage, and myrtle, (II) a positive control group exposed to 1.23% sodium fluoride gel (Sina, Iran), and (III) a negative control group that remained in artificial saliva. The teeth were exposed to toothpaste and fluoride gel 10 min a day after exposure to orange juice for 7 days. After 7 days, the microhardness of the teeth was measured again, as explained earlier. No change or minimal reduction in the microhardness of specimens compared with their baseline microhardness would indicate optimal remineralizing efficacy of the performed intervention. Between phases and before microhardness determination, specimens were immersed in artificial saliva (pH of 7 and room temperature).

Statistical analysis

Data were analyzed using SPSS software, version 26 (IBM Corp., Armonk, N.Y., USA). Normal distribution of data was confirmed by the Shapiro–Wilk test ($P > 0.05$), and homogeneity of variances was approved by Levene's test ($P > 0.05$). Thus, comparisons were made by paired t-test, analysis of variance (ANOVA), and Bonferroni test at 0.05 level of significance.

Results

Table 1 presents the measures of central dispersion for the microhardness (Vickers hardness number) of specimens in the three groups before and after the intervention. The paired t-test showed a significant reduction in the microhardness of specimens after the intervention in the artificial saliva group ($P = 0.000$) and fluoride gel group ($P = 0.002$). However, no significant reduction was noted in the microhardness of the nano-HA group compared with the baseline ($P = 0.132$).

ANOVA was then applied to compare the microhardness of specimens in the three groups, which revealed no significant difference in baseline microhardness of the three groups (P = 0.299). However, the difference in microhardness was significant among the three groups after the intervention (P = 0.000). Pairwise comparisons by the Bonferroni test were then performed [Table 2], which showed that the nano-HA group had significantly higher secondary microhardness than the other two groups (P = 0.000 for both). However, the secondary microhardness of fluoride gel and artificial saliva groups was not significantly different (P = 1.000).

Discussion

Considering the adverse consequences of erosion and the need for treatment of erosive lesions, attempts are ongoing to find suitable remineralizing agents for erosive lesions. Accordingly, this study assessed the effect of a nano-HA toothpaste on third molar enamel erosive lesions induced by exposure to orange juice. Within-group comparisons showed a significant reduction in the microhardness of specimens after the intervention in artificial saliva and fluoride gel groups. However, no significant reduction was noted in the microhardness of the nano-HA group compared with the baseline. Between-group comparisons revealed no significant difference in the microhardness of the three groups at baseline. However, after the intervention, the microhardness of the nano-HA group was significantly higher than that of other groups. However, the difference in secondary microhardness between the fluoride gel and artificial saliva groups was not significant.

When applied on the tooth surface, HA creates a thin crystalline self-organized, strong coating on the enamel surface, which bonds to the enamel.[4] The manufacturer of Pharmed toothpaste claims that it has cariostatic effects, resolves tooth hypersensitivity by gradual obstruction of denuded dentinal tubules, gradually repairs the eroded enamel, whitens the teeth, has antimicrobial activity, and prevents gingival inflammation.[19] The present results confirmed the optimal remineralizing efficacy of this toothpaste for erosions caused by orange juice. The present results were in agreement with the findings of Haghgoo *et al.*,[13] and Yaberi and Haghgoo,[15] who showed that nano-HA had the potential to remineralize erosive lesions despite some methodological differences. They used a nano-HA solution instead of toothpaste and the specimens were exposed to the materials for a longer period, compared with the present study. Furthermore, artificial saliva was used in the present study while they placed the specimens in the oral cavities of candidates. The present results were also consistent with the findings of another study by Haghgoo *et al.*,[20] who demonstrated that all toothpastes containing nano-HA, irrespective of their concentration, increased the microhardness of demineralized teeth. Consistent with the current findings, Haghgoo *et al.*,[14] in another study, indicated a significant increase in microhardness of all specimens following exposure to nano-HA solutions. Moreover, Juntavee *et al.*[21] reported that nano-HA in both forms of toothpaste and gel had greater efficacy than fluoride varnishes for remineralization of incipient caries. Their results were similar to the present findings. Similarly, Saudi and Ibrahim[22] showed that nano-HA toothpaste successfully remineralized incipient erosive lesions. They also used fluoride as the control group and showed that nano-HA and fluoride had comparable potential in remineralizing incipient erosive lesions. However, fluoride gel in the present study did not show optimal remineralizing efficacy.

Table 1: Measures of central dispersion for the microhardness (Vickers hardness number) of specimens in the three groups before and after the intervention (*n*=8)

Group	Minimum	Maximum	Mean	SE	SD	Variance
Artificial saliva						
Baseline	256.66	453.33	370.37	22.18	62.74	3937.28
After intervention	174.33	258	234.85	10.39	29.41	865.09
Fluoride gel						
Baseline	286.33	402.33	346.99	14.16	40.05	1604.14
After intervention	159.33	291.66	234.70	15.61	44.17	1951.22
Nano-HA toothpaste						
Baseline	289	458	391.08	21.14	59.8	3576.65
After intervention	289	442.33	337.03	18	50.92	2593.74

SE: Standard error; SD: Standard deviation; Nano-HA: Nano-hydroxyapatite

Table 2: Pairwise comparisons of the secondary microhardness of the three groups (after the intervention) by the Bonferroni test

Group (I)	Group (J)	Mean difference	SE	P	95% CI	
					Lower bound	Upper bound
Artificial saliva	Fluoride gel	0.147	21.233	1.00	−55.087	55.382
Nano-HA	Artificial saliva	102.185	21.233	0.000	46.951	157.419
Nano-HA	Fluoride gel	102.332	21.233	0.000	47.098	157.567

SE: Standard error; CI: Confidence interval; Nano-HA: Nano-hydroxyapatite

Abdou[23] confirmed that nano-HA solution remineralized demineralized enamel, and had a significantly higher efficacy than fluoride for this purpose. Nakamura *et al*.[16] demonstrated that nano-HA toothpaste with and without fluoride both enhanced remineralization; however, this enhancement was significantly greater in the nano-HA toothpaste + fluoride group. Mielczarek and Michalik[24] revealed the optimal efficacy of nano-HA in stopping the progression of erosive lesions. Furthermore, Min *et al*.[25] confirmed optimal efficacy of nano-HA in the prevention of tooth wear.

In the present study, the selection of fluoride gel for the positive control group was because of the fact that some previous studies showed optimal efficacy of fluoride for remineralization of erosive lesions.[16,22] Furthermore, the selection of artificial saliva for the negative control was because artificial saliva has no positive effect on erosive lesions and does not change the microhardness of teeth either.[26-29] In the present study, the microhardness of specimens after exposure to orange juice and before exposure to remineralizing agents was not assessed since a reduction of microhardness following exposure to orange juice has been previously confirmed.[3,5,9]

Previous studies conducted on remineralizing agents applied materials for long periods on the surface of specimens, which is not clinically feasible.[30,31] Thus, in the present study, the materials were applied for only 10 min on the surface of teeth once daily to increase the clinical generalizability of the results, which was a strength.

In the current study, electron microscopic assessment of the enamel surface before and after the intervention was not performed, which was a limitation of this study. Electron microscopic assessment of enamel surface after erosion and after remineralization with nano-HA toothpaste can provide valuable information, and should be performed in future studies. Furthermore, the efficacy of fluoride in combination with nano-HA toothpaste for remineralization of enamel lesions was not evaluated in this study. Future studies are required to assess the efficacy of nano-HA toothpaste in addition to fluoride. Moreover, the remineralizing efficacy of Pharmed toothpaste can be compared with other remineralizing agents, such as casein phosphopeptide amorphous calcium phosphate.

Conclusion

The present results showed that Pharmed toothpaste containing nano-HA has optimal efficacy for remineralization of enamel erosive lesions induced by exposure to orange juice.

Recommendation

It is recommended that the effects of nano-HA toothpaste with and without fluoride on the microhardness of deciduous and permanent teeth are also investigated.

Ethical statement

The study protocol was approved by the Ethics Committee of Shahed University, Tehran, Iran (Ethics committee reference ID: IR.SHSHED.REC. 1400.025)

Limitation

It was difficult to collect 24 permanent third molar teeth with no caries, hypoplasia, enamel cracks, or erosions.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ramos-Gomez F, Crystal YO, Ng MW, Tinanoff N, Featherstone JD. Caries risk assessment, prevention, and management in pediatric dental care. Gen Dent 2010;58:505-17.
2. Fallahinejad Ghajari M, Nabavi Razavi S. Comparing the erosive effect of Iranian soft drinks with standard samples; a calcium ion analysis. Arch Oral Biol 2012;57:525-30.
3. Grando LJ, Tames DR, Cardoso AC, Gabilan NH. *In vitro* study of enamel erosion caused by soft drinks and lemon juice in deciduous teeth analysed by stereomicroscopy and scanning electron microscopy. Caries Res 1996;30:373-8.
4. Heshmat H, Banava S, Mohammadi E, Kharazifard MJ, Mojtahedzadeh F. The effect of recommending a CPP-ACPF product on salivary and plaque pH levels in orthodontic patients: A randomized cross-over clinical trial. Acta Odontol Scand 2014;72:903-7.
5. Odebunmi EO, Dosumu OO. Fermentation studies and nutritional analysis of drinks made from water extract Hiscus sabdariffa Galyx (SOBO), juices of citrus science (Orange), *Ananas comosus* (pineapple). J Food Technol 2007;5:198-204.
6. Kitasako Y, Cochrane NJ, Khairul M, Shida K, Adams GG, Burrow MF, *et al.* The clinical application of surface pH measurements to longitudinally assess white spot enamel lesions. J Dent 2010;38:584-90.
7. Faria AI, Gallas-Torreira M, López-Ratón M. Mandibular second molar periodontal healing after impacted third molar extraction in young adults. J Oral Maxillofac Surg 2012;70:2732-41.
8. West NX, Hughes JA, Addy M. The effect of pH on the erosion of dentine and enamel by dietary acids *in vitro*. J Oral Rehabil 2001;28:860-4.
9. Smith AJ, Shaw L. Baby fruit juices and tooth erosion. Br Dent J 1987;162:65-7.
10. Johansson AK, Lingström P, Imfeld T, Birkhed D. Influence of drinking method on tooth-surface pH in relation to dental erosion. Eur J Oral Sci 2004;112:484-9.
11. Ajami B, Ebrahimi M, Karbasi S. Investigation of the erosive effect of carbonated drinks on deciduous enamel micro hardness. J Islamic Dent Assoc 2016;18:51-7.
12. Rostam Miri L, Saeedi Asl M, Safari R. Evaluation of physicochemical, sensory and shelf life of beverages prepared from soy protein hydrolyzed by alkalase enzyme. J Dent Technol 2018;10:403-10.
13. Haghgoo R, Haghgou HR, Abbasi F, Tavakkoli M. The effect of nano-hydroxyappatite solution on the permanent tooth remineralization following exposure to soft beer (*in situ*). J Dent Med 2015;27:233-40.

14. Haghgoo R, Mehran M, Ahmadvand M, Ahmadvand MJ. Remineralization effect of eggshell versus nano-hydroxyapatite on caries-like lesions in permanent teeth (*in vitro*). J Int Oral Health 2016;8:435-9.
15. Yaberi M, Haghgoo R. A comparative study of the effect of nanohydroxyapatite and eggshell on erosive lesions of the enamel of permanent teeth following soft drink exposure: A randomized clinical Trial. J Int Oral Health 2018;10:176-9.
16. Nakamura M, Khairul M, Shida K, Adams GG, Burrow MF, Reynolds EC, *et al*. The effect of toothpaste containing nano-hydroxyapatite based on nanotechnology on remineralization of human enamel. J Dent 2016;38:584-90.
17. Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent 2008;36:74-9.
18. Alexandria AK, Vieira TI, Pithon MM, da Silva Fidalgo TK, Fonseca-Gonçalves A, Valença AM, *et al. In vitro* enamel erosion and abrasion-inhibiting effect of different fluoride varnishes. Arch Oral Biol 2017;77:39-43.
19. Manchery N, John J, Nagappan N, Subbiah GK, Premnath P. Remineralization potential of dentifrice containing nanohydroxyapatite on artificial carious lesions of enamel: A comparative in vitro study. Dent Res J (Isfahan) 2019;16:310-7.
20. Haghgoo R, Rezvani MB, Haghgoo HR, Ameli N, Zeinabadi MS. Evaluation of Iranian toothpaste containing different concentrations of nano-hydroxyapatite on the remineralization of incipient carious lesions: *In vitro*. J Dent Med 2015;27:254-8.
21. Juntavee A, Juntavee N, Sinagpulo AN. Nano-hydroxyapatite gel and its effects on remineralization of artificial carious lesions. Int J Dent 2021;2021:7256056.
22. Saudi R, Ibrahim MA. Comparison between nano-hydroxyapatite and CPP-ACPF in remineralizing early carious lesions (*in vitro* study). BAU J Creat Sustain Dev 2020;2:7.
23. Abdou A. Surface morphology of erosive enamel lesions remineralized using casein-Phosphopeptide amorphous calcium phosphate versus nano-hydroxyapatite in comparison to sodium fluoride by using the Atomic force microscope: An *in-vitro*. J Dent Res 2018;4:103-10.
24. Mielczarek A, Michalik J. The effect of nano-hydroxyapatite toothpaste on enamel surface remineralization. An *in vitro* study. Am J Dent 2014;27:287-90.
25. Min A, Manton DJ, Shen P. The effect of toothpaste containing nano-hydroxyapatite based on nanotechnology on remineralization of human enamel Caries Res 2011;(41):377-86.
26. Rirattanapong P, Vongsavan K, Tepvichaisillapakul M. Effect of five different dental products on surface hardness of enamel exposed to chlorinated water *in vitro*. Southeast Asian J Trop Med Public Health 2011;42:1293-8.
27. Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization – An *in vitro* study using scanning electron microscope and DIAGNOdent. Indian J Dent Res 2011;22:77-82.
28. Devlin H, Bassiouny MA, Boston D. Hardness of enamel exposed to Coca-Cola and artificial saliva. J Oral Rehabil 2006;33:26-30.
29. Lussi A, Megert B, Eggenberger D, Jaeggi T. Impact of different toothpastes on the prevention of erosion. Caries Res 2008;42:62-7.
30. Huang SB, Gao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion *in vitro*. Biomed Mater 2009;4:034104.
31. Kim MY, Kwon HK, Choi CH, Kim BI. Combined effects of nano-hydroxyapatite and NaF on remineralization of early caries lesion. Key Eng Mater 2007;330:1347-50.