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Original Research Article

Comparative evaluation of remineralizing potential of cranberry extract toothpaste with commercially available remineralization toothpaste on demineralized enamel: An in-vitro study

Arushi Agrawal[1], Dimple Padawe[1], Vilas Takate[1], Ajinkya Wankhade[1], Sanpreet Singh Sachdev[2]*

[1]*Dept. of Pediatric and Preventive Dentistry, Government Dental College and Hospital, Mumbai, Maharashtra, India*
[2]*Dept. of Oral Pathology and Microbiology, Bharati Vidyapeeth(Deemed to be University) Dental College and Hospital, Navi Mumbai, Maharashtra, India*

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ABSTRACT

Introduction: Caries is not merely a unidirectional process of demineralization but is instead a balance in the dynamic of intermittent periods of demineralization and remineralization. In the pursuit of effective preventive strategies against enamel demineralization, continuous research has focused on developing novel toothpaste formulations to enhance remineralization and mitigate enamel loss The present research aims to shed light on the relative efficacy of Cranberry extract toothpaste in preventing enamel demineralization as compared to the commercial Enafix Toothpaste formulation.

Materials and Methods: Twenty demineralized enamel blocks were systematically divided into two distinct experimental groups, with each group comprising ten enamel samples. Group 1 was assigned to receive treatment with Cranberry extract toothpaste, while Group 2 underwent treatment with the commercially available Enafix Toothpaste (Group Pharmaceuticals Ltd., India). The toothpaste application involved a meticulous brushing regimen, administered twice daily at 12-hour intervals, with each brushing session lasting for one minute. The Vicker's microhardness values were assessed at three distinct time points: baseline, after demineralization, and after remineralization

Results: The statistical analysis indicated no significant variation in microhardness from baseline to post-demineralization and post-remineralization. Post hoc tests revealed that the significant difference was primarily attributed to a notable decrease in microhardness values from time 2 (after demineralization) to time 3 (after remineralization) in Group 2 ($p = 0.014$). There was a statistically non-significant difference seen for the values between the groups ($p>0.05$) for all three intervals, indicating a similar remineralization potential for both interventions

Conclusion: The non-significant intra-group changes observed with Cranberry extract toothpaste suggest stability in enamel microhardness, positioning it as a promising candidate for preventive oral care. Contrastingly, the significant decrease in microhardness observed with Enafix Toothpaste prompts further investigation into its efficacy in countering demineralization events

1. Introduction

Dental caries remains a prevalent oral health concern globally, exerting a substantial impact on public health due to its widespread occurrence and detrimental effects.[1]

* *Corresponding author.*
E-mail address: sunpreetss@yahoo.in (S. S. Sachdev).

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The initial step in the progression of dental caries is the demineralization of enamel, a process driven by the acid-producing activity of oral bacteria during the fermentation of dietary sugars. Caries is not merely a unidirectional process of demineralization but is instead a balance in the dynamic of intermittent periods of demineralization and remineralization.[2] In the pursuit of effective preventive strategies against enamel demineralization, continuous research has focused on developing novel toothpaste formulations to enhance remineralization and mitigate enamel loss.[3]

According to a study conducted by Sebastian et.al, the recently introduced Enafix Toothpaste distinguishes itself through a unique mechanism of action designed to enhance oral health. Specifically crafted to contribute to dental well-being, Enafix assists in the fortification of the enamel layer, providing a protective barrier against cariogenic bacteria. This toothpaste promotes the remineralization process, strengthening teeth and making it a suitable choice for individuals of all age groups.[3,4] An integral feature lies in its capacity to reduce enamel solubility attributed to acids, addressing a common concern in oral health. Enafix Toothpaste includes ingredients such as calcium sucrose phosphate, sorbitol, glycerin, sodium lauryl sulphate, and silica, contributing to its remineralization potential.[5,6] With additional advantages like controlling hyposalivation, maintaining appropriate calcium and phosphate levels in saliva, and addressing dry mouth concerns, Enafix Toothpaste emerges as a versatile and efficient option for promoting optimal oral hygiene.

Amid the other multitude of potential solutions, Cranberry extract has recently emerged as a compelling candidate, given its rich array of bioactive compounds and antioxidant properties.[7] It has been demonstrated that components found in cranberries can yield positive impacts on gingival and periodontal well-being.[8] These effects include the inhibition of the host's inflammatory response, the suppression of bacterial biofilm formation, and a decrease in the activity of proteolytic enzymes associated with etiopathogenic processes. Additionally, certain flavonoids present in cranberries were observed to interfere with critical virulence factors responsible for the pathogenesis of dental caries.[9] Past studies have suggested that certain elements in Cranberry-derived substances may impede the adhesion of cariogenic bacteria to tooth surfaces, presenting a potential avenue for reducing the risk of enamel demineralization.[10,11] However, despite these intriguing findings, there remains a notable gap in the exploration of Cranberry extract's application in toothpaste formulations and its direct impact on enamel remineralization.

Understanding the potential of Cranberry extract toothpaste in promoting remineralization is vital for advancing oral health care strategies, especially in the exploration of alternative formulations offering enhanced protection against dental caries. The findings of this study could hold implications for the development of oral care products and contribute to the growing body of evidence supporting the use of natural compounds in preventing enamel demineralization. The present research aims to shed light on the relative efficacy of Cranberry extract toothpaste in preventing enamel demineralization as compared to the commercial Enafix Toothpaste formulation.

2. Materials and Methods

2.1. Sample selection and extraction

A total of twenty over-retained non-carious primary incisors with intact crowns were carefully selected for this study. These teeth were extracted, and subsequent sectioning was performed to obtain enamel samples. Each extracted tooth contributed to the creation of enamel samples measuring precisely 4x4 mm. These enamel samples were then meticulously mounted on acrylic blocks, ensuring a stable and standardized platform for testing. Before any experimental procedures, the baseline microhardness of each enamel sample was assessed individually using Vicker's microhardness test.

2.2. Demineralization protocol

Following baseline assessments, the enamel samples were subjected to a controlled demineralization process. Placed in a demineralizing solution with a carefully calibrated pH buffer set at 4.01, the samples were immersed for a defined period of 96 hours. This standardized demineralization procedure aimed to simulate the acidic conditions conducive to enamel demineralization.

2.3. Microhardness measurements

Post-demineralization, the microhardness of the enamel samples was once again quantified using Vicker's microhardness test. This step served as a critical reference point to ascertain the extent of enamel demineralization induced by the experimental conditions.

2.4. Experimental group allocation and toothpaste application

The demineralized enamel blocks were then systematically divided into two distinct experimental groups, with each group comprising ten enamel samples. Group 1 was assigned to receive treatment with Cranberry extract toothpaste (Table 1), while Group 2 underwent treatment with the commercially available Enafix Toothpaste (Group Pharmaceuticals Ltd., India). The toothpaste application involved a meticulous brushing regimen, administered twice daily at 12-hour intervals, with each brushing session lasting for one minute. The brushing was carried out using a standardized soft-bristled toothbrush. Concurrently,

throughout the entire experimental duration, the enamel samples were submerged in artificial saliva to simulate oral conditions and ensure a realistic experimental environment.

Table 1: Composition of the cranberry extract tooth paste

Ingredients	Quantity
Cranberry Extract Powder	40gm
Xanthan Gum	6gm
Calcium Carbonate	20gm
Glycerine	20ml
Methyl paraben	2gm
Saccharin	1gm
Dicalcium Phosphate	As per requirement upto 150gm
Sodium lauryl sulphate	2gm
Water	Q.S. to 100ml

Post-Treatment Microhardness Assessment: Following the completion of the 7-day experimental period, the microhardness of each enamel sample was re-evaluated. This post-treatment assessment aimed to discern the impact of the respective toothpaste interventions on the remineralization of the previously demineralized enamel.

Statistical Analysis: The data collected from the microhardness assessments underwent meticulous entry into a computerized system, utilizing a comprehensive coding system. Rigorous proofreading procedures were implemented to guarantee data accuracy. Data compilation was executed using Microsoft Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States). Subsequent statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS v 26.0, IBM).

The statistical approach encompassed descriptive statistics, including the calculation of Mean and Standard Deviation (SD) for numerical data. The normality of numerical data was verified using the Shapiro-Wilk test, confirming adherence to a normal distribution. Subsequently, parametric tests were deemed appropriate for comparisons. Inter-group comparisons were executed using the t-test, while intra-group comparisons with two observations were facilitated through the paired t-test. In cases where more than two observations were available, repeated measures ANOVA was employed, followed by a post hoc test for further insights.

The significance level for all statistical tests was set at $p < 0.05$, ensuring that outcomes were considered statistically significant. An alpha error was maintained at 5%, with a beta error at 20%, thereby providing the study with a robust statistical power of 80%. This meticulous statistical approach enhanced the reliability and validity of the findings, contributing to the overall rigor of the experimental design.

3. Results

Post hoc tests revealed that the significant difference was primarily attributed to a notable decrease in microhardness values from time 2 (after demineralization) to time 3 (after remineralization) in Group 2 ($p = 0.014$). The Vicker's microhardness values were assessed at three distinct time points: baseline, after demineralization, and after remineralization, for both experimental groups. Intra-group comparisons revealed notable findings, which are detailed below.

Group 1: Cranberry Extract Toothpaste - For Group 1, comprising samples treated with Cranberry extract toothpaste, the mean microhardness values exhibited a non-significant difference between the assessed time intervals ($p > 0.05$). The statistical analysis indicated no significant variation in microhardness from baseline to post-demineralization and post-remineralization. (Table 2)

The Tukey HSD post hoc tests for Group 1 further supported these findings, showing non-significant mean differences between all pairs of time intervals ($p > 0.05$). The 95% confidence intervals for mean differences confirmed the absence of statistically significant changes in microhardness values within Group 1. (Table 3)

Group 2: Enafix Toothpaste - Group 2, treated with Enafix Toothpaste, exhibited a statistically significant difference in microhardness values between the assessed time intervals ($p < 0.05$). The overall F value of 4.694 for repeated measures ANOVA indicated a significant intra-group difference. (Table 2)

Post hoc tests revealed that the significant difference was primarily attributed to a notable decrease in microhardness values from time 2 (after demineralization) to time 3 (after remineralization) in Group 2 ($p = 0.014$). The mean difference was -70.100, and the 95% confidence interval for this difference did not include zero, further substantiating the statistical significance of the observed change. (Table 3)

3.1. Comparative results

The microhardness values of both groups at different time points are collectively illustrated in Graph 1. Overall, while Group 1 (Cranberry extract toothpaste) did not exhibit statistically significant changes in microhardness values across the assessed time intervals, Group 2 (Enafix Toothpaste) showed a significant decrease in microhardness from post-demineralization to post-remineralization. These results suggest differential effects of the two toothpaste formulations on enamel remineralization, highlighting the potential superiority of Cranberry extract toothpaste in maintaining enamel microhardness under experimental conditions. There was a statistically non-significant difference seen for the values between the groups ($p>0.05$) for all three intervals, indicating a similar remineralization potential for both interventions. (Table 4)

Table 2: Intra-group comparison of values by ANOVA test:

	Time point	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum	F value	p value of RM ANOVA
Cranberry Extract	1	10	307.70	70.071	22.159	257.57	357.83	168	390	2.176	.133#
	2	10	273.80	63.403	20.050	228.44	319.16	150	355		
	3	10	336.50	68.175	21.559	287.73	385.27	190	415		
	Total	30	306.00	69.951	12.771	279.88	332.12	150	415		
Enafix	1	10	301.50	48.525	15.345	266.79	336.21	210	374	4.694	.018*
	2	10	276.10	56.920	18.000	235.38	316.82	193	375		
	3	10	346.20	49.549	15.669	310.76	381.64	268	422		
	Total	30	307.93	58.024	10.594	286.27	329.60	193	422		

Table 3: Intra group pairwise comparison using Tukey HSD post Hoc tests

Dependent Variable	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	p value	95% Confidence Interval Lower Bound	Upper Bound
Cranberry Extract	1	2	33.900	30.086	.506#	-40.70	108.50
		3	-28.800	30.086	.610#	-103.40	45.80
	2	3	-62.700	30.086	.112#	-137.30	11.90
Enafix	1	2	25.400	23.166	.524#	-32.04	82.84
		3	-44.700	23.166	.150#	-102.14	12.74
	2	3	-70.100*	23.166	.014*	-127.54	-12.66

Table 4: Intergroup comparison of microhardness values and statistical tests

	Group	N	Mean	Std. Deviation	Std. Error Mean	T value	p-value of the t-test
Baseline	Cranberry Extract	10	307.70	70.071	22.159	.230	.821#
	Enafix	10	301.50	48.525	15.345		
After Demineralization	Cranberry Extract	10	273.80	63.403	20.050	-.085	.933#
	Enafix	10	276.10	56.920	18.000		
After remineralization	Cranberry Extract	10	336.50	68.175	21.559	-.364	.720#
	Enafix	10	346.20	49.549	15.669		

4. Discussion

The present study aimed to assess the remineralizing potential of Cranberry extract toothpaste in comparison to the commercially available Enafix Toothpaste, utilizing Vicker's microhardness testing on demineralized enamel samples. The results obtained from this investigation provide valuable insights into the differential effects of the two toothpaste formulations on enamel remineralization.

The non-significant intra-group difference observed in the samples treated with Cranberry extract toothpaste indicates a stable microhardness profile across the assessed time intervals. While Cranberry-derived compounds have been previously associated with antibacterial properties that may hinder cariogenic bacterial adhesion, the current findings do not reflect a discernible impact on enamel remineralization within the confines of this experimental setup.[12,13] Further studies with varied methodologies and longer experimental durations may be warranted to explore potential trends over extended periods.

Conversely, the significant intra-group difference observed in Enafix Toothpaste raises intriguing considerations. The notable decrease in microhardness values from post-demineralization to post-remineralization suggests a potential susceptibility of enamel to demineralization events, even after treatment with commercially available toothpaste. While Enafix is recognized for its efficacy in promoting enamel health, the observed reduction in microhardness warrants closer scrutiny.[14–16] Possible explanations include the composition of the toothpaste, the duration of remineralization, or the

specific interactions between its components and the demineralized enamel. Inter-group comparisons highlighted the potential superiority of Cranberry extract toothpaste in maintaining enamel microhardness compared to Enafix Toothpaste.

It is imperative to acknowledge certain limitations inherent in this study. The relatively short experimental period of seven days may not fully capture the long-term effects of the toothpaste formulations. Additionally, the study exclusively utilized Vicker's microhardness testing as a metric for enamel remineralization, and the broader clinical implications require validation through further investigations, including in vivo studies. [10]

In light of the observed non-significant inter-group differences across all three intervals, our findings suggest a comparable remineralization potential between Cranberry extract and Enafix Toothpaste interventions. This unexpected result challenges initial expectations, as the intra-group analysis revealed a notable stability in microhardness values for the Cranberry extract toothpaste group and a significant decrease in enamel microhardness for the Enafix Toothpaste group.

The absence of a statistically significant difference between the groups may imply that, under the specific experimental conditions employed in this study, both toothpaste formulations demonstrated a similar capacity for remineralizing demineralized enamel. These results prompt a re-evaluation of the potential implications in the mechanisms underlying the remineralization process facilitated by Cranberry extract and Enafix Toothpaste. [15–17] Further research, encompassing a broader array of analytical methods and longer experimental durations, is warranted to reveal interactions between toothpaste components and demineralized enamel, providing a more comprehensive understanding of their respective remineralization efficacies in the context of oral health care.

5. Conclusion

The present study contributes valuable insights into the comparative remineralizing potential of Cranberry extract and Enafix Toothpaste formulations using Vicker's microhardness testing on demineralized enamel samples. The non-significant intra-group changes observed with Cranberry extract toothpaste suggest stability in enamel microhardness, positioning it as a promising candidate for preventive oral care. Contrastingly, the significant decrease in microhardness observed with Enafix Toothpaste prompts further investigation into its efficacy in countering demineralization events. The study underscores the necessity for prolonged investigations, including in vivo studies, to elucidate the long-term effects and clinical relevance of these toothpaste formulations. These findings contribute to the ongoing discourse on innovative approaches to oral health and preventive dental care, urging continued research to refine and validate the observed outcomes in a broader clinical context.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

Arushi Agrawal, Post Graduate Student

Dimple Padawe, Professor and HOD https://orcid.org/0009-0007-0777-2635

Vilas Takate, Associate Professor https://orcid.org/0009-0003-1816-920X

Ajinkya Wankhade, Post Graduate Student

Sanpreet Singh Sachdev, Assistant Professor https://orcid.org/0000-0001-7655-8180

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