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ORIGINAL ARTICLE

Erosive Effects of Different Kinds of Beverages on the Enamel of the Extracted Teeth, Treated and Untreated by Fluoride Varnish

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ABSTRACT

Background: Fluoride varnish is often used to stop the irreparable damage caused by enamel erosion from acidic drinks, which is becoming a greater oral health problem.

Objective: To evaluate the erosive effects of various beverages on enamel microhardness of extracted teeth, treated and untreated with fluoride varnish.

Methodology: In this randomized experimental investigation, conducted over a period of six months from July 2021 to December 2021, 50 healthy permanent teeth that had been removed were split evenly between two groups: those that had fluoride varnish treatment and those that did not. Five subgroups (n=5) were created from each group and subjected to Coca-Cola, coffee, tea, milk, and sports drinks. Four times a day for seven days, teeth were submerged in 10 milliliters of the designated beverage for five minutes each time. They were then washed with deionized water and kept in fake saliva in between exposures. A Vickers microhardness tester was used to assess the surface microhardness before and after exposure. Scanning electron microscopy (SEM) was used to examine the surface morphology. Paired and independent t-tests with a 95% confidence interval were used for statistical analysis.

Results: Coca-Cola showed the most enamel degradation, with both the treated and untreated groups showing substantial decreases in microhardness ($p < 0.05$). Significant erosion was also produced by Sports Drink. Milk had no discernible erosive impact, whereas tea and coffee caused a mild but statistically significant weakening of the enamel. Although fluoride varnish decreased the loss of enamel hardness, it was unable to totally stop erosion brought on by acidic drinks.

Conclusion: Fluoride varnish offers some but not enough protection, and acidic drinks drastically weaken enamel. Milk is still non-erosive and may provide protection.

Keywords: Enamel erosion, fluoride varnish, acidic beverages, microhardness, Coca-Cola, sports drink, scanning electron microscopy.

INTRODUCTION

The hardest material in the human body is enamel, which is made up of around 90–95% hydroxyapatite, 3–4% water, and 1-3 percent organic content[1]. Even though enamel is strong, acidic environments, especially those with a pH lower than 5.5, cause it to demineralize[2]. Since enamel erosion cannot be stopped once it starts, prevention is an essential part of dental care[3]. Increasing salivary flow, eating meals high in calcium, and using fluoride to fortify the enamel surface are some preventative measures. Of them, fluoride varnish is generally seen as a very successful prophylactic measure[4].

The loss of tooth structure brought on by exposure to non-bacterial acids is known as dental erosion[5]. This may be brought on by internal issues like gastroesophageal reflux or external ones like drinking acidic drinks. The risk of erosion is greatly influenced by the frequency and mode of intake (e.g., gulping vs. sipping)[6]. Acidic drinks, including as fruit juices, sodas, and energy drinks, are often linked to enamel erosion, particularly when they are ingested without meals[7].

Regular exposure to acids softens and weakens enamel, which reduces the amount of time fluoride has to remineralize the surface. Thus, protecting the enamel before damage occurs should be the main goal of preventative measures[8]. In order to improve resistance to acid attack, fluoride varnish partly transforms hydroxyapatite into fluorohydroxyapatite by producing a covering that resembles calcium fluoride. Nevertheless, fluoride coverage is only around 40% of the enamel surface, even with ideal application[9].

Dental erosion has increased recently worldwide, particularly in kids and teenagers[10]. This increase is mostly caused by the rising intake of acidic beverages, especially sports drinks[11]. Even though tooth erosion is becoming more well recognized, not much study has been done in Pakistan on the subject. Although coffee, tea, milk, and sodas are often consumed beverages, little is known about their precise erosive effects in local settings, particularly when combined with or without fluoride varnish.

This research is to determine the protective function of fluoride varnish in these circumstances as well as the erosive effects of various drinks on the enamel of removed teeth.

Objective: To evaluate the erosive effects of various beverages on the enamel microhardness of extracted teeth, both treated and untreated with fluoride varnish.

MATERIAL AND METHODS

Study Design and Setting: This randomized experimental trial was conducted at Isra Dental College in collaboration with Mehran University of Engineering and Technology. The study was designed to evaluate the erosive effects of different beverages on the enamel of extracted teeth, both treated and untreated with fluoride varnish.

Study Period: The study was conducted over a period of six months, July 2021 to December 2021, starting immediately after the approval of the research summary. The extended duration was necessary to accommodate the time required for sample preparation, collection, experimental procedures, and analysis.

Study Population: Fifty removed human permanent teeth, comprising canines, lateral incisors, first premolars, and maxillary and mandibular central incisors, made up the research population. To guarantee enamel homogeneity, teeth were chosen at random and checked for inclusion and exclusion criteria.

Inclusion and Exclusion Criteria: Extracted complete permanent central incisors, lateral incisors, canines, and first premolars from male and female patients of any age that were non-carious met the inclusion criteria. Teeth that were damaged or fragmented, exhibited attrition, abrasion, or erosion, or were carious were not included.

Sample Size and Sampling Technique

Sample Size Calculation:

$$\text{Sample size} = \frac{\text{Population size}}{1 + \text{Population size x e}^2}$$

Final sample size: 50 teeth

Sampling Technique: The 50 removed teeth were split evenly into two groups (n = 25 each) using a randomized sample procedure. Group I was treated with sodium fluoride (NaF) varnish, whereas Group II was left untreated.

Data Collection Procedure: After receiving ethical permission, 50 healthy removed teeth were acquired from Isra Dental College's Oral Surgery Department. Following ISO/TS 11405:2003 requirements, teeth were disinfected for one week in a 0.5% chloramine T trihydrate solution. Teeth were cleaned, scaled, and then kept at 4°C in distilled water.

Treatment Protocol: NaF varnish was applied to Group I using a brush on all surfaces and left to dry for 4 hours. Group II remained untreated.

Erosive Challenge: Five teeth were assigned to each of the five liquids (Coca-Cola, Coffee, Tea, Milk, and Red Bull) in each set of 25 teeth. Four times a day, for five minutes each time, the teeth were submerged in ten milliliters of the designated beverage. The specimens were kept in artificial saliva (pH 7.0) between cycles and overnight after being properly washed with deionized water after each exposure. For seven days in a row, this procedure was carried out every day, with drinks and fake saliva being replaced at the end of each cycle. During demineralization cycles, samples were stored in hermetically sealed containers to preserve acidity and stop gas loss in carbonated beverages.

Assessment of Erosion Potential: Surface Microhardness Test (SMH): Surface microhardness (SMH) was measured both before and after the erosive cycles using a Vickers microhardness tester (JT Toshi, Tokyo, Japan). The hardness value was calculated using the formula: $HV = 1.854 \times L / d^2$, where HV represents the Vickers hardness number, L is the applied load in kilograms, and d is the diagonal length of the square indentation in millimeters.

Scanning Electron Microscope (SEM) Analysis: The enamel's surface morphological alterations were investigated using a JEOL JSM-820 Scanning Electron Microscope (SEM). To improve conductivity and allow for high-resolution surface analysis, the tooth specimens were gold-coated using a vacuum evaporator (JEE-420, JEOL Ltd., Japan) before imaging.

Statistical Analysis: The independent sample t-test was used in the statistical analysis, which was conducted using SPSS version 2001, to compare the enamel microhardness values of the fluoride-treated and untreated groups. Enamel microhardness was the dependent variable, whereas the beverage type and fluoride therapy were the independent factors. The findings' significance was assessed using a two-tailed test and a 95% confidence interval.

Ethical Approval: The study protocol was reviewed and approved by the Research Ethics Committee of Isra Dental College prior to initiation. All procedures adhered to ethical standards and institutional guidelines for the use of extracted human teeth in research.

RESULTS

After seven days, the average initial Vickers hardness number (VHN) for teeth without fluoride varnish exposed to sports drinks dropped to 345, suggesting a moderate erosive potential—higher than tea, coffee, and milk, but lower than Coca-Cola. Following the trial, the initial VHN for teeth coated with fluoride varnish decreased from 388 to 352. Figures 1 and 2 demonstrate that there was no discernible difference between teeth treated with fluoride and teeth not treated with sports drinks ($P > 0.05$). Among all the drinks examined, Coca-Cola caused the most erosion on teeth without fluoride varnish, with an initial VHN of 400 that decreased to 340 after seven days.

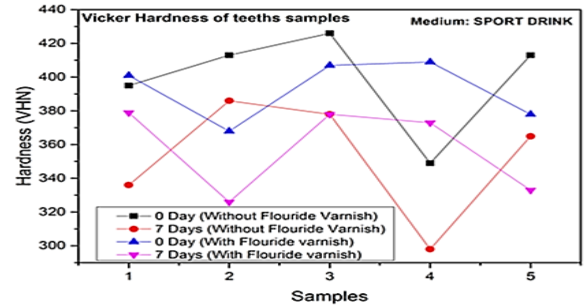

Figure 1: Line graph comparing initial and final enamel hardness in fluoride-treated and untreated teeth after exposure to sports drink.

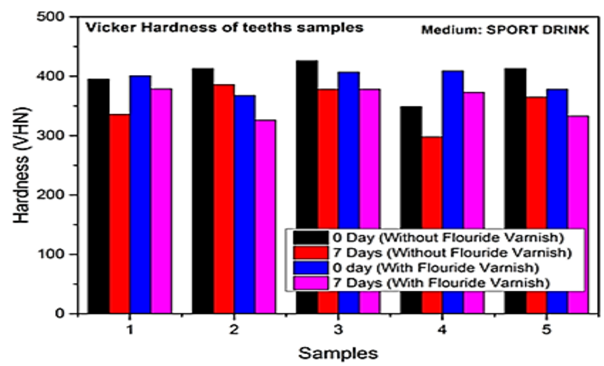

Figure 2: Bar graph showing enamel hardness reduction in fluoride-treated vs. untreated teeth following sports drink immersion.

After the trial, the average Vickers hardness number for teeth treated with fluoride varnish and exposed to Coca-Cola dropped from 370 VHN to 333 VHN. Figures 3 and 4 demonstrate that, despite a small difference between the treated and untreated groups, the outcome was not statistically significant ($P > 0.05$).

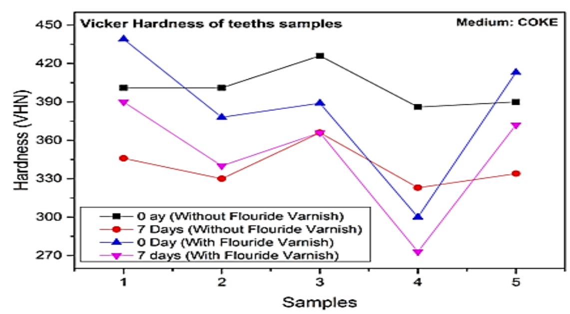

Figure 3: Line graph comparing pre- and post-exposure enamel hardness in teeth treated and untreated with fluoride varnish after coke immersion.

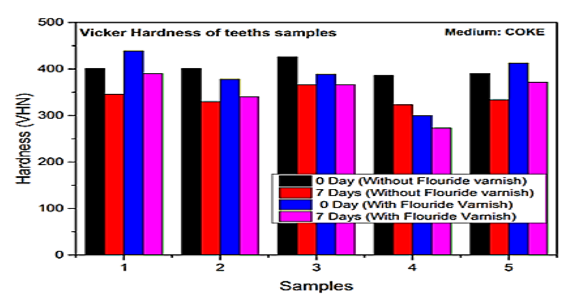

Figure 4: Bar graph illustrating enamel hardness changes in fluoride-treated and untreated teeth after exposure to coke.

After seven days, the average Vickers hardness number (VHN) for teeth without fluoride varnish exposed to tea dropped from 380 to 350. This decrease was not enough to imply that tea is a major contributor to tooth decay in our society. The initial VHN for teeth coated with fluoride varnish was 365; after the trial, it dropped to 342. Figures 5 and 6 demonstrate that there was no significant difference between the treated and untreated groups (P > 0.05), suggesting that fluoride varnish is not very successful in stopping tea-induced erosion.

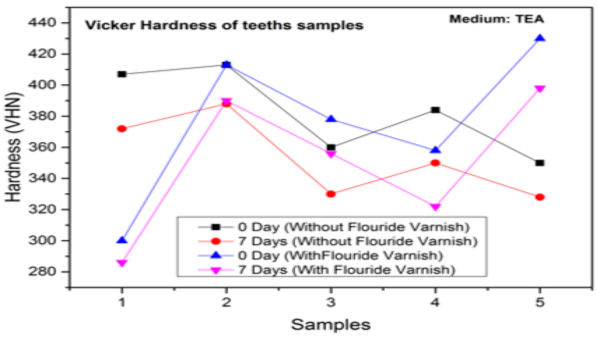

Figure 5: Line graph depicting changes in enamel hardness in treated and untreated teeth following tea exposure.

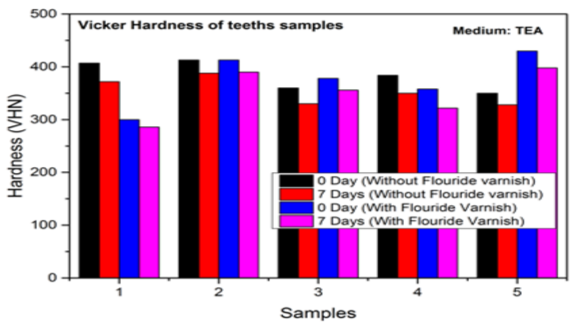

Figure 6: Bar graph comparing enamel hardness reduction in fluoride-treated and untreated groups after tea immersion.

Teeth without fluoride varnish exposed to coffee had an initial average Vickers hardness number (VHN) of 398, which dropped to 359 after seven days. This decrease was insufficient to draw the conclusion that coffee seriously erodes teeth. The initial VHN for teeth coated with fluoride varnish was 397; after the trial, it decreased to 376. Figures 7 and 8 demonstrate that, despite a little difference between the treated and untreated groups, the outcome was statistically inconsequential (P > 0.05). This implies that fluoride varnish provides only a limited level of defense against erosion brought on by coffee.

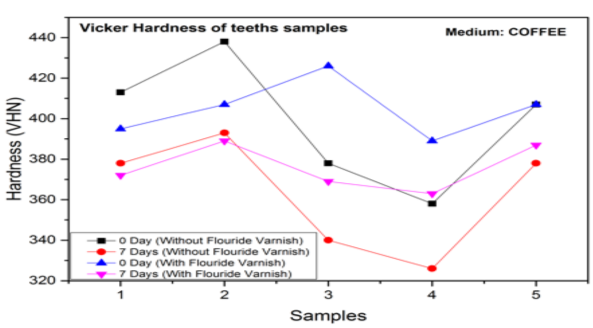

Figure 7: Line graph showing enamel hardness before and after coffee exposure in teeth with and without fluoride varnish

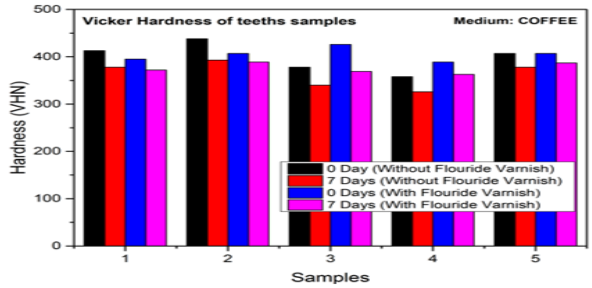

Figure 8: Bar graph demonstrating the effect of fluoride varnish on enamel hardness loss after coffee exposure.

There was no discernible erosive impact, since the initial average Vickers hardness number (VHN) for teeth without fluoride varnish exposed to milk was 400. After seven days, this value slightly dropped to 398. The initial VHN for teeth coated with fluoride varnish was 388, then it decreased slightly to 386. Figures 9 and 10 demonstrate that there was no discernible difference between the treated and untreated groups (P > 0.05), indicating that fluoride varnish had no effect on the hardness of enamel when exposed to milk.

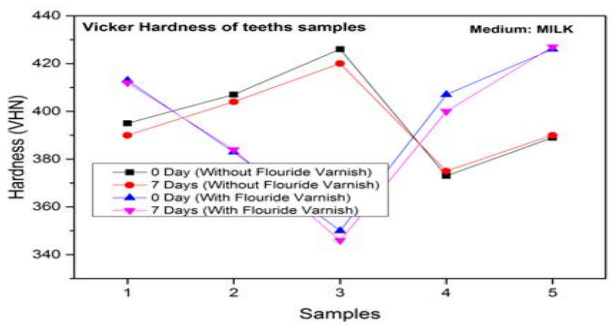

Figure 9: Line graph comparing enamel hardness in fluoride-treated and untreated teeth before and after milk exposure

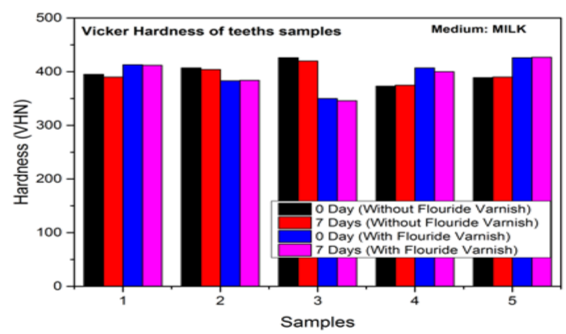

Figure 10: Bar graph showing enamel hardness changes in teeth treated and untreated with fluoride varnish after immersion in milk.

The enamel hardness (Vickers Hardness Number, or VHN) for five drinks is shown in Table 1 before and after exposure, along with averages, standard deviations, and significant values. With a mean hardness loss from 400.80 to 339.80 (mean difference = 61.0, p = 0.000), Coca-Cola showed the most enamel degradation. Significant erosion was also brought on by sports drinks (mean decrease = 46.6, p = 0.001). While milk had a minor impact (mean drop = 2.2, p = 0.240), suggesting no major erosive potential, tea and coffee induced considerable enamel weakening (mean drops = 29.2 and 35.8 respectively, both p = 0.000). These findings demonstrate that although milk is safe for enamel, fizzy and acidic drinks considerably weaken enamel.

Table 1: Pre- and Post-Treatment Enamel Microhardness (VHN) for Teeth Exposed to Different Beverages (Without Fluoride Varnish)

Beverage	Pre-Hardness (Mean ± SD)	Post-Hardness (Mean ± SD)	Mean Difference	p-value	Interpretation
Coca-Cola	400.80 ± 15.58	339.80 ± 16.86	61.00	0.000	Highly significant enamel erosion
Tea	382.80 ± 27.82	353.60 ± 26.21	29.20	0.000	Moderate erosion, statistically significant
Coffee	398.80 ± 31.24	363.00 ± 28.50	35.80	0.000	Moderate erosion, statistically significant
Milk	398.00 ± 19.88	395.80 ± 16.98	2.20	0.240	No significant erosion
Sports Drink	399.20 ± 30.15	352.60 ± 35.96	46.60	0.001	Significant enamel erosion

Table 2: Paired Samples t-Test for Pre- and Post-Treatment Enamel Microhardness Following Exposure to Different Beverages (Without Fluoride Varnish)

Pair	95% Confidence Interval of the Difference	t	df	Sig. (2-tailed)
	Upper Mean Difference			
Coke	68.999	21.173	4	.000
Tea	36.191	11.597	4	.000
Coffee	43.424	13.038	4	.000
Milk	6.625	1.380	4	.240
Sports Drink (SD)	61.307	8.797	4	.001

Table 3: Paired Samples Analysis of Enamel Microhardness Pre- and Post-Beverage Exposure with Fluoride Varnish Application

Beverage	Pre-Hardness Mean ± SD	Post-Hardness Mean ± SD	Mean Difference	Std. Deviation	Std. Error Mean	95% CI (Lower)
Coke	383.80 ± 52.40	348.20 ± 45.70	35.600	10.574	4.729	22.471
Tea	375.80 ± 50.96	350.40 ± 46.96	25.400	8.706	3.894	14.590
Coffee	404.80 ± 14.18	376.00 ± 11.45	28.800	16.053	7.179	8.868
Milk	395.80 ± 29.98	393.80 ± 31.04	2.000	3.464	1.549	-2.301
Sports Drink	392.60 ± 18.47	357.80 ± 26.05	34.800	9.418	4.212	23.106

Table 4: Paired Samples t-Test for Pre- and Post-Hardness of Enamel After Beverage Exposure with Fluoride Varnish

Pair	Paired Differences	T	df	Sig. (2-tailed)
	95% Confidence Interval of the Difference			
	Upper Mean Difference			
Pre-Hardness Coke Results – Post Hardness Coke Results	48.729	7.529	4	.002
Pre-Hardness Tea Results – Post Hardness Tea Results	36.210	6.524	4	.003
Pre-Hardness Coffee Results- Post Hardness Coffee Results	48.732	4.012	4	.016
Pre-Hardness Milk Results – Post Hardness Milk Results	6.301	1.291	4	.266
Pre-Hardness SD Results – Post Hardness SD Results	46.494	8.262	4	.001

The findings of the paired samples t-test evaluating the variations in enamel microhardness before and after exposure to different drinks throughout a seven-day erosive cycle are shown in Table 2. Strong erosive potential was indicated by statistically significant decreases in enamel hardness for Coca-Cola (Mean = 68.999, p =.000), Tea (Mean = 36.191, p =.000), Coffee (Mean = 43.424, p =.000), and Sports Drink (Mean = 61.307, p =.001). Milk, on the other hand, shown a non-significant decrease in enamel hardness (Mean = 6.625, p =.240). This is probably because of its protective makeup, which includes proteins, calcium, and phosphate that work with the hydroxyapatite surface to prevent enamel demineralization. These results demonstrate that although milk may provide a preventive advantage, acidic liquids without fluoride's protective action dramatically lower enamel hardness.

A thorough comparison of enamel microhardness before and after exposure to various drinks over a seven-day period, with fluoride varnish applied, is shown in Table 3. Twenty-five observations in all were examined. The mean and standard deviation (SD) for the pre- and post-hardness measurements, together with the computed mean difference and confidence intervals, are included in the paired samples statistics. Despite the presence of fluoride, Coke had the largest mean decrease in hardness (35.600), followed closely by Sports Drink (34.800), Coffee (28.800), and Tea (25.400). The calcium, phosphate, and casein content of milk, on the other hand, reinforced its protective qualities with a negative lower limit in the confidence interval and a small, non-significant mean difference (2.000). These findings suggest that while fluoride varnish reduces enamel degradation, acidic drinks such as Coke and Sports Drinks continue to have a significant impact.

Even with the application of fluoride varnish, the results of the paired samples t-test show statistically significant decreases in enamel hardness following 7 days of exposure to Coke (t = 48.729, p =.002), Tea (t = 36.210, p =.003), Coffee (t = 48.732, p =.016), and Sports Drink (t = 46.494, p =.001) (table 4). A significant difference between pre- and post-exposure readings is confirmed by the fact that all of these p-values are less than 0.05. Milk, on the other hand, did not exhibit any discernible change in hardness (t = 6.301, p =.266), indicating that its protective effect is probably caused by the calcium and phosphate it contains. Five samples (df = 4) served as the basis for each test.

DISCUSSION

This research assessed the erosive potential of five popular drinks—milk, tea, coffee, sports drinks, and Coca-Cola—on removed human teeth with and without the use of fluoride varnish. According to the results, Coca-Cola significantly decreased the microhardness of teeth without treatment, from 400.80 ± 15.58 VHN to 339.80 ± 16.86 VHN (mean loss = 61.00; p = 0.000). Even teeth treated with fluoride exhibited erosion, dropping from 370 to 333 VHN, albeit the varnish somewhat mitigated the loss of hardness. These results are in line with earlier research that found colas with high titratable acidity and low pH to be among the most erosive drinks[12]. In a similar vein, another research discovered that cola-based beverages softened the surface of enamel more than milk or fruit juices[13].

Both the treated and untreated groups saw considerable enamel erosion as a result of sports beverages. VHN dropped from 399.20 ± 30.15 to 352.60 ± 35.96 in untreated teeth (mean drop = 46.60; p = 0.001). Although treated teeth survived somewhat better, there was still significant erosion shown in the decline from

388 to 352 VHN. These results are consistent with other studies that showed that the acidic components of sports and energy beverages, as well as their regular usage in sporting environments, led to enamel breakdown[14]. Similar to Coca-Cola, fluoride varnish highlighted the limits of varnish application in high-acid situations by offering some protection but failing to totally stop the erosive process.

With statistically significant decreases in VHN in untreated teeth (tea: from 382.80 to 353.60, $p = 0.000$; coffee: from 398.80 to 363.00, $p = 0.000$), tea and coffee demonstrated mild enamel degradation. Although there was a modest decrease in enamel loss in the treated groups, the changes were not statistically significant ($p > 0.05$). Despite being acidic, tea and coffee have a relatively low erosive potential, according to earlier studies, particularly when eaten without sugar or additions[11,15]. Our study's modest fluoride varnish protection against tea and coffee erosion is consistent with other research that found fluoride varnishes work better against strong acids than slightly erosive drinks[16].

On the other hand, milk had very little influence on the integrity of enamel. Fluoride-treated teeth revealed equally negligible changes, with the VHN of untreated teeth dropping from 398.00 ± 19.88 to 395.80 ± 16.98 (mean loss = 2.20; $p = 0.240$). These findings corroborate earlier research that found milk's neutral pH and calcium and phosphate ion concentration, which may promote remineralization, have a protective effect[17,18]. In this instance, fluoride varnish did not provide a discernible extra benefit, underscoring the fact that milk is naturally non-erosive.

Overall, our results show that fluoride varnish provides some protection against enamel degradation, especially for very acidic liquids. It does not, however, totally stop enamel from softening. This restriction is crucial, particularly in light of how often and for how long people are exposed to acidic beverages in daily life. In order to maintain the health of enamel, public awareness should also stress reducing consumption of acidic drinks, even if fluoride treatment is still a helpful preventative measure.

Study Strengths and Limitations: The controlled experimental design with consistent exposure intervals and repeated cycles that mimic actual beverage intake, as well as the use of SEM analysis and microhardness testing to thoroughly evaluate enamel degradation, are two of this study's strong points. A comparative viewpoint on the erosive potential of many popular drinks is provided by their inclusion. Nevertheless, the study's in vitro design limits its ability to accurately simulate the intricate oral environment, including salivary flow, pellicle production, and individual differences in oral hygiene. The sample size for each subgroup was small, which could have limited statistical power, and the very brief exposure length might not accurately represent long-term impacts.

CONCLUSION

The results show that acidic drinks, especially sports drinks and Coca-Cola, dramatically lower enamel microhardness and alter the surface morphology, which is a symptom of erosion. Although applying fluoride varnish reduces enamel damage, it does not provide total defense against erosion brought on by acid. Conversely, milk has no erosive properties and could even be protective. These findings highlight how crucial it is to limit intake of acidic beverages and use fluoride treatments in addition to preventative measures to maintain the integrity of enamel.

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