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Cabral Xavier, Alidianne Fábia; Nogueira Guimarães de Abreu, Mauro Henrique; Baptista da Costa Agra de Melo, João; Santos Anselmo, George Carlos; Leite Cavalcanti, Alessandro

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Original Article

Changes in Microhardness and Morphology of the Adamantine Structure as a Function of the Exposure Time to Different Drugs

Alidianne Fábia Cabral Xavier[1], Mauro Henrique Nogueira Guimarães de Abreu[2], João Baptista da Costa Agra de Melo[3], George Carlos Santos Anselmo[3], Alessandro Leite Cavalcanti[1]

[1]Department of Preventive Dentistry, School of Dentistry, State University of Paraiba, Campina Grande, PB, Brazil
[2]Department of Community and Preventive Dentistry, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
[3]Department of Mechanical Engineering, Federal University of Campina Grande, Campina Grande, PB, Brazil.

Author to whom correspondence should be addressed: Alidianne Fábia Cabral Xavier, Universidade Estadual da Paraíba, Centro de Ciências, Tecnologia e Saúde (CCTS), Curso de Odontologia, Av. Coronel Pedro Targino, Araruna, PB, Brasil. 58233-000. Phone: (+5583) 3373-1040. E-mail: alidianne.fabia@gmail.com.

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Abstract

Objective: To investigate the in vitro effect of four pediatric liquid medicines on the microhardness and morphology of the enamel of permanent teeth after different exposure times. **Material and Methods:** Claritin, Celestone, Amplictil and Vick syrup honey flavor were tested. Seventy tooth fragments were obtained, 50 embedded in acrylic resin and submitted to Knoop Hardness test (50 gf, 15 s, and 5 indentations) before and after immersion in drugs. The specimens were randomly divided into five groups (n = 10), four experimental and one control group (distilled water). The immersion cycle consisted of a single exposure in times of 5 and 15 minutes. Other 20 dental specimens were divided between groups and analyzed by Scanning Electron Microscopy. The level of statistical significance was set at 5% with a confidence interval of 95%. **Results:** The microhardness analysis showed no statistically significant difference between experimental groups ($p > 0.05$), although there was statistically significant difference within each group at different times ($p < 0.05$). The SEM micrographs showed distinct changes in the enamel morphology. **Conclusion:** The decreased in microhardness was dependent on the time of the immersion cycle and morphological changes were influenced by the type of drug and by the exposure time.

Keywords: Dental Enamel; Tooth; Hardness Tests; Pharmaceutical Preparations..

Introduction

Since the last decades, scientific evidence of epidemiologic character indicate a high prevalence of erosive lesions in children and adolescents and have greatly increased the interest in the pathophysiology of dental erosion [1-3].

The dynamics of the establishment of erosive wear is directly associated with histological changes in hard dental tissue [4]. In the early stages, the mechanical and physical properties of the tooth are modified due to the release of minerals to the erosive acid [4], whereas in the later stages, continuous exposure to acids causes loss of dental substrate, which in the enamel manifests through dissolution or abrasion of the previously softened region [5].

Acids may have intrinsic (when coming from gastric secretions) or extrinsic origin (from dietary, medical and occupational sources) [6]. The combined analysis of the individual chemical aspects of acid substances and the performance of erosion tests on the tooth structure has been widely used [7-9].

It has been suggested that, for enamel demineralization, the potential of hydrogen (pH) on the surface of this tissue should be less than 5.5 [4], and many liquid medications for pediatric use have pH lower than that considered critical for dissolution of the adamantine structure [10-16].

The in vitro assessment of the enamel structure exposed to the action of drug solutions revealed that they are able to reduce its hardness [8,9,12,16,17], increase its roughness [12], cause changes in its morphological structure [9,10,12,18,19] and induce calcium dissolution [20].

Based on these assumptions, this study aimed to comparatively assess the changes produced in the hardness and morphology of dental enamel in function of the exposure time to different pediatric drugs.

Material and Methods

Selection of Drugs

Four different drugs were selected (Claritin, Celestone, Amplictil and Vick syrup honey flavor) due to their previously analyzed physicochemical properties [13] (Table 1).

Table 1. Description of drugs tested according to the chemical characteristics presented and therapeutic class.

	Therapeutic Class			
Characteristics	Antihistaminic	Corticosteroid	Antipsychotic	Bronchodilator
Trade name	Claritin	Celestone	Amplictil	Vick syrup honey flavor
Active ingredient	Loratadine	Betamethasone	Chlorpromazine	Guaifenesin
Manufacturer	Mantecorp Ind. Quím.	Mantecorp Ind. Quím.	Sanofi-Aventis Farm.	Procter & Gamble Ind.
Pharmaceutical Form	Syrup	Elixir	Oral solution	Syrup
pH	2.43	2.87	2.58	4.86
Total Titratable Acidity	0.77%	0.34%	1.54%	0.45%
Total Soluble Solids	60.7%	48.6%	47.1%	53.2%
Total Sugars	51.7%	25.2%	23.0%	30.4%

Calculating the Number of Specimens

The sample size for comparison of means was calculated by considering the following parameters: 95 % confidence level = 1.96; 80 % test power = 1.29; standard deviation of 10.6 (SD1) and 11.5 (SD2) (final enamel microhardness after exposure to two different drugs) and considering an average difference of hardness to be identified equal to 16.

$$n=\frac{\{(z1-\propto/2+zb)(z1-\propto/2+zb)\ [(SD1)(SD1)]+[(SD2)(SD2)]\}}{(\text{difference between groups})^2}$$

$$n=\frac{(1.96+1.29)^2\ [(10.6)^2+(11.5)^2]}{(16)^2}$$

$$n=\frac{(10.5625)\ [(112.36)+(132.25)]}{256}$$

n= 10.09 = 10 independent samples

Preparation of Specimens

In the step of specimen preparation, 50 tooth fragments were obtained from 25 third molars. For this, a double-face diamond disk (XL 12205, Extec Corp., Enfield, CT, USA) "High concentration", 102 mm x 0.3 mm x 12.7 mm connected to a precision cutting apparatus (ISOMET Low Speed Saw, Buehler Ltd., Lake Bluff, IL, USA) was used. The first sectioning was made at the cementoenamel junction to separate the coronary and root portions, the latter being discarded. Then, the crown was sectioned parallel to the long axis in the mesiodistal direction to obtain two fragments (buccal and lingual / palatal). These were adapted to polyvinyl chloride (PVC) rings and embedded in acrylic resin (Vipi Ind. Com. Prod. Odontol. Ltda., Pirassununga, SP, Brazil).

After polymerization, the tooth surface was submitted to surface smoothing procedure by using abrasives sandpaper of different grain sizes (400 , 600, and 1200) (Extec Corp., Enfield, CT, USA) adapted to a rotating metallographic polishing machine (APL 4, Arotec Ind., Cotia, SP, Brazil). Then the samples were viewed in optical microscope model 444181 (Astro Optics. Division, Montpelier, USA) to verify that the surfaces were flat, polished and free of irregularities [12]. A cuboid area of 4x4 mm² in the center of the block was marked in order to delimit the region to be analyzed. The specimens were numbered and randomly divided into five groups of 10 elements each, which remained immersed in distilled water until the performance of the Knoop hardness test.

Initial Hardness Measures (Baseline)

The initial hardness was measured using Microhardness Tester FM - 700 (Future-Tech Corp., Fujisaki, Kawasaki-ku, Japan), fitted with a Knoop-type diamond indenter programmed to apply a static load of 50 gf in a time of 15 s. Five indentations were performed in the previously defined area, separated from each other by a distance of 100 µm, which average value was considered equivalent to the sample hardness value [12]. The specimens whose value ranged from 272 HK to 440 HK were selected [21]. Knoop hardness values were calculated by the software present in the equipment.

Immersion Cycle in Drugs

After initial microhardness measurements, the specimens were immersed in 100 mL of each of the solutions studied (G1: distilled water - pH 6.8 - Control; G2: Claritin; G3: Celestone; G4: Amplictil; and G5: Vick Syrup honey flavor). The erosive cycle consisted of a single exposure held under stirring (50 rpm) on a magnetic stirrer model 78HW - 1 (Coleman Equip. Laboratórios Com. e Imp. Ltda., Santo André, SP, Brazil) at different time intervals (T1 = 5 minutes and T2 = 15 minutes). At the end of the first five minutes, five specimens were removed from each group, keeping the five remaining until the period of 15 minutes was reached [19]. At the end of the erosive challenge, the specimens were individually washed with distilled water, dried with gauze and again submitted to microhardness analysis.

Scanning Electron Microscopy

For morphological analysis, 20 tooth fragments were divided into five groups and exposed to test solutions as described above. After the immersion cycle, the specimens were submitted to the drying procedure, fixed in stubs with double-sided adhesive carbon tape (Electron Microscopy Sciences Inc., Washington, PA, USA) and covered with gold layer on a vacuum metallizing device (Quick Coater SC -701, Sanyu Electron Co., Tokyo, Japan). The specimens were examined in a Scanning Electron Microscope (Model SSX-550/Superscan, Shimadzu Corp., Kyoto, Japan), operated at 15 kV, with surfaces examined along their entire length. Readings were made with magnification of 1000x and 2000x. The findings of micrographs were analyzed by visual and qualitative comparison.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 18. The significance used was 0.05 with 95% confidence level. Initially, the normality of data distribution has been investigated (Shapiro-Wilk test). For microhardness analysis at the initial time, ANOVA test for independent samples was used and for times T1 and T2, the Kruskal-Wallis test was used. Comparison of microhardness values between T1 and T2 within each group was performed by applying the Mann-Whitney test, while for T0 vs. T1 and T0 vs. T2 combinations, the t test for paired samples was applied, except for T0 vs. T2 in Group 3, in which the Wilcoxon test was used.

This study followed ethical guidelines recommended by the Brazilian legislation and was approved by the Human Research Ethics Committee of the State University of Paraiba (CAAE 0020.0.133.000-11).

Results

The normality analysis of samples and residues showed normal distribution ($p > 0.05$) of microhardness data at the initial time (T0). The overall average hardness of dental blocks was 374.83

HK and there was no statistically significant difference between the mean values of groups evaluated (p = 0.065) (Table 2).

Table 2. Distribution of mean microhardness values (T0) according to the group.

Group	Mean (SD)	p-value
G1	380.43 (23.31)	
G2	383.82 (12.88)	
G3	362.02 (18.84)	>0.05*
G4	363.87 (13.38)	
G5	381.91 (18.70)	

*ANOVA/TUKEY

Microhardness residues in time T1 showed non-normal distribution (p < 0.05), with no statistical significance (p = 0.793) between microhardness values of the different experimental groups at T1 (Table 3).

Table 3. Median and minimum and maximum microhardness values in time T1.

Group	Median	Minimum	Maximum	p-value
G2	325.74	293.50	339.82	
G3	315.62	310.74	336.84	>0.05**
G4	315.90	310.32	335.22	
G5	324.72	306.60	336.60	

**KRUSKAL-WALLIS

Non-normal distribution was observed for G2/T2 (p = 0.001) and for microhardness residues at time T2 (p < 0.05). After 15 minutes of acid challenge, no statistically significant difference between experimental groups (p = 0.179) (Table 4) was observed.

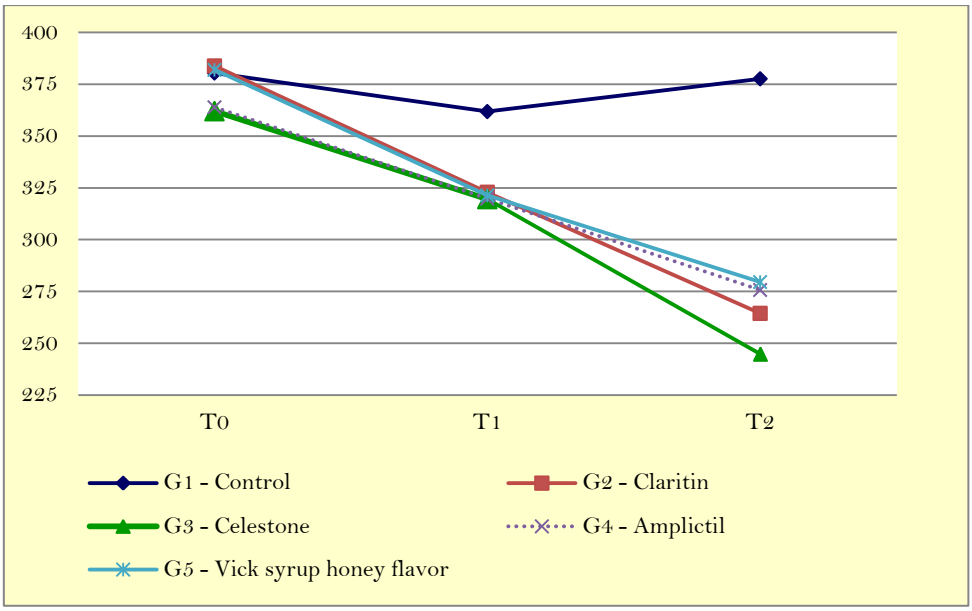
Table 4. Median and minimum and maximum microhardness values in time T2.

Group	Median	Minimum	Maximum	p-value
G2	266.78	251.18	275.54	
G3	270.54	138.38	275.92	>0.05**
G4	285.12	231.56	293.88	
G5	297.74	257.96	297.74	

**KRUSKAL-WALLIS

Graph 1 shows the microhardness values in T0 and after the performance of acid challenges (T1 and T2), and it was observed that there was a constancy in the average values of the control group and a similar decrease in the microhardness values of the experimental groups.

Statistically significant difference (p < 0.05) was observed for all experimental groups when comparing means at time T0 versus T1 and T0 versus T2. Similarly, statistically significant difference (p < 0.05) was observed in the analysis between microhardness values at times T1 and T2 (Table 5).



Graph 1. Variation in the average values of the enamel microhardness according to the group and time interval.

Table 5. Comparison between average microhardness values between times T1 and T2 for each experimental group.

Comparison T1 *versus* T2	p-value
G2	0.002***
G3	0.009***
G4	0.008***
G5	0.002***

***WILCOXON/PAIRED T TEST

The findings regarding micrographs revealed that the tooth surface exposed to the drugs showed different morphological standards. For group exposed to Claritin, it was found widespread demineralization in the centers of prisms in time T1 and formation of craters in time T2 (Figure 1). Dental specimens submitted to the action of Celestone showed intense destruction of prisms and formation of craters in T1 and T2 (Figure 2). The images relating to the Amplictil group demonstrated demineralization with formation of depressions in T1 and craters in T2 (Figure 3). For group Vick Syrup Honey flavor, irregular dental surface was observed (Figure 4).

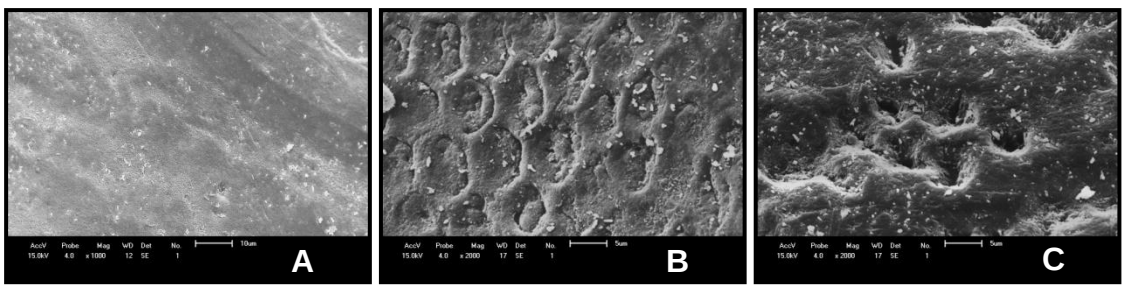


Figure 1. Micrographs of permanent enamel at baseline (A - 1000x) and after immersion in Claritin in times of 5 (B - 2000x) and 15 minutes (C - 2000x).

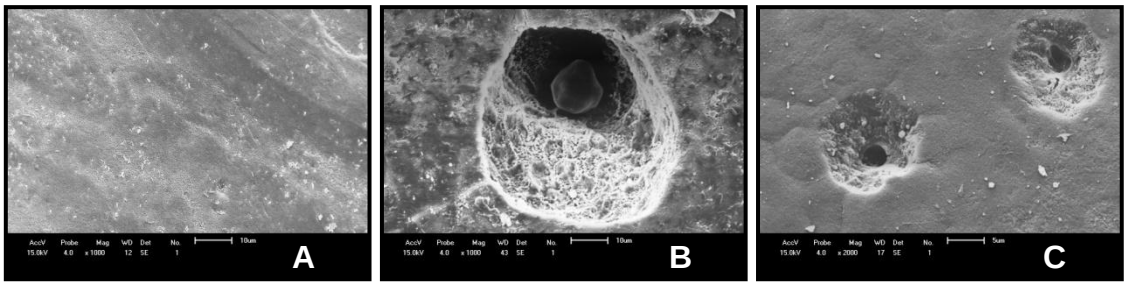


Figure 2. Micrographs of permanent enamel at baseline (A - 1000x) and after immersion in Celestone in times of 5 (B - 2000x) and 15 minutes (C - 2000x).

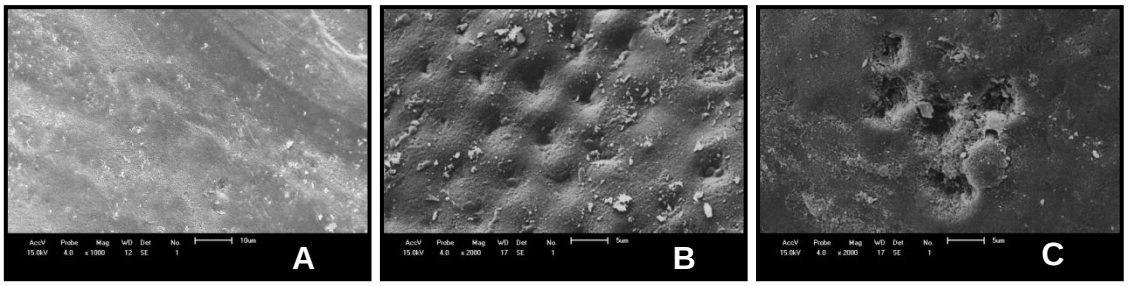


Figure 3. Micrographs of permanent enamel at baseline (A - 1000x) and after immersion in Amplictil in times of 5 (B - 2000x) and 15 minutes (C - 2000x).

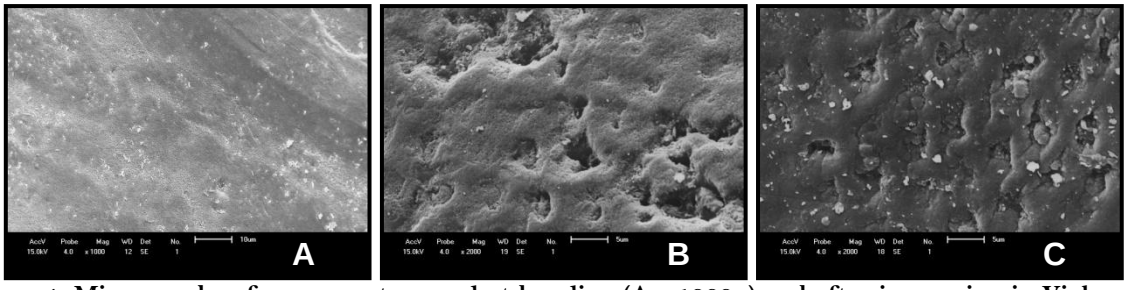


Figure 4. Micrographs of permanent enamel at baseline (A - 1000x) and after immersion in Vick syrup honey flavor in times of 5 (B - 2000x) and 15 minutes (C - 2000x).

Discussion

The assessment of the mechanical and morphological characteristics of dental enamel submitted to the action of pediatric liquid medicines belonging to the therapeutic class of corticosteroids, antipsychotics, bronchodilators and antihistamines made dental erosion a clearly observable event in this study, which confirms the finding of different in vitro studies [8-10,12,16-20], that although have made use of different experimental protocols, also highlighted the demineralizing potential of pediatric drugs.

Thus, it is assumed that, despite the different dosing regimens adopted, pediatric patients who need the administration of these substances will present an increased risk of erosive lesions. Many medications are administered in times distant from meals as well as at night or even during sleep, during which salivary flow is reduced and may thus increase the intensity of erosive challenge [12]. It should also be pointed out that these children may very likely be exposed to other erosive sources, such as the intake of acidic foods and drinks and / or the occurrence of gastric disorders, intensifying both quantitative and qualitatively acid challenges imposed to the tooth structure.

The methodological definition of this study, with regard to the dental substrate used, was based on the report that human tissues are the choice for conducting laboratory research, despite the recognized and increasing difficulty in acquiring adequate supply [22]. Only samples of erupted and semi-erupted third molars newly extracted and obtained from young patients were included in this research in order to minimize variations between specimens. To optimize the use of these substrates, two dental blocks of each tooth, one from the buccal surface and the other from the lingual / palatal surface were obtained. In contrast, in a recent study [19] bovine samples were used to analyze the erosive effect of an antihistamine formulation, which might have occurred because they are more easily acquired [22] and especially because they have the advantage that from a single bovine incisor, four or five specimens can be obtained [23].

The exposure time of dental fragments to acid medicines was 5 and 15 minutes; therefore, it was found that the time interval is critically important for the occurrence of changes in the adamantine structure for both response variables. The four drugs tested reduced the surface microhardness similarly, since there was no significant difference between groups at T1 and T2 ($p > 0.05$). However, the exposure time of five minutes was sufficient to cause significant decrease in microhardness, and it was found that the longer the exposure time of enamel blocks to the drug, the lower the hardness values. Unlike these findings, a previous study [19] found no significant difference in roughness values after time of 5, 15 and 30 minutes of exposure to antihistamine medication. This divergence may be explained not only by the type of substrate used, as in this work human teeth were used; in another study bovine teeth were used, but also by the method used [19].

The literature showed no consensus on the duration of acid challenges, nor on the exposure time to the drug solution [8-10,12,16-19]. However, it is believed that if the erosive challenge is longer, the outer layer of the softened surface will possibly be completely dissolved, resulting in permanent loss of the mineralized enamel structure [24], often making the analysis of post-immersion findings difficult [10].

To simulate the clinical situation as much as possible, extended erosion periods should be avoided [25,26]. Moreover, it is necessary that the exposure time produces a measurable change by the chosen method. Therefore, parameters are often intentionally chosen to exaggerate the actual clinical conditions [23].

Another issue to be considered is that in most tests, a single exposure to the product is recommended, followed by immediate erosion assessment, while in some studies, multiple exposures were performed [7-9]. The advantage of using a series of repeated challenges is that it increases the chances of obtaining reliable results, as changes can be detected after multiple exposures, even if not identified after a single exposure [7]. In this study, single exposure was performed, unlike other experiments, which performed several exposures over 12 days [12], 14 days [8,17] and 28 days [9].

The erosive effect evaluated by Scanning Electron Microscopy (SEM) revealed the occurrence of different erosion patterns between the different experimental groups, and this variation depends on the type of drug tested and the exposure time. Changes in the morphology of post-

immersion dental enamel to different drug solutions were also observed by other authors [10,12,18,19].

Hypothetically, it is assumed that the erosive potential of drugs is related to low pH values. Thus, it was observed that for Group 2 (Claritin) in the time of five minutes, widespread demineralization was observed, especially in the center of prisms, giving the aspect of honeycomb compatible with type-I standard described previously [27]. On the other, when using time of 15 minutes, it was observed the formation of an irregular pattern in which flat areas, depressions and craters appear together. For Group 3 (Celestone), the formation of craters in both time periods studied was observed. The images obtained allowed supposing that the dissolution of the tooth structure did not occur only in a superficial way as for the other groups, showing that this drug showed a more aggressive action.

SEM micrographs of samples exposed to Amplictil (Group 4) showed distinct structural features, and for the exposure time of five minutes, there was formation of shallow depressions and for the time of 15 minutes, there was formation of craters distinct from those observed for Celestone (Group 3).

Vick syrup honey flavor (Group 5) resulted in fainter changes in the enamel structure, which is possibly due to the fact that this drug had the highest pH value. This group showed an irregular dissolution pattern, in which for the time to five minutes, there are areas of supposedly aprismatic nature and early removal of the central portion of prisms, while for the time to 15 minutes, a generalized roughness was observed on the enamel surface, showing no similarities with the prismatic morphology, which is consistent with type-III pattern of Silverstone [27].

The formation of each pattern depends on how the acid acts on the axes of prisms and on the orientation of crystals; furthermore, all patterns may occur on enamel surface treated with different acids applied in identical conditions [27].

Even considering the possibility of overestimating the demineralization occurrence, according to the above, it appears that in vitro models are extremely useful, as they can be run in a short period of time, providing reliable information on the erosion mechanism, although not replicating the oral environment with all the biological changes known to influence tooth erosion [28].

Conclusion

The drugs tested reduced the microhardness of the enamel of permanent teeth, which is dependent on the exposure time and the morphological changes were influenced by the different drug studied, as well as by the exposure time.

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