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Susceptibility of Human Deciduous Enamel to Erosive Wear after Exposure to Commonly Prescribed Oral Pediatric Liquid Medicaments: An AFM Based *in vitro* Analysis

Sandleen Feroz[1,*], Sidra Aamir[1], Shazia Nawabi[2]

[1]Department of Dental Materials, Foundation University College of Dentistry, Islamabad, Pakistan
[2]Department of Prosthodontics, Qassim University, Kingdom of Saudia Arabia
*Corresponding author: sandaleenferoz@yahoo.com

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Abstract **Aim:** The aim of this *in vitro* analysis was to determine the effects of various commonly prescribed pediatric oral liquid medicaments on deciduous teeth. **Material and methods:** Thirty extracted human deciduous incisors were cleaned and then sectioned longitudinally resulting in 60 samples from a total of 30 tooth samples. After polishing all samples were randomly divided into three groups. Group 1: untreated group, Group 2: Immersion of samples in Paracetamol (sugar-free), Group 3: Immersion of samples in Chlorpheniramine. All the tooth samples in Group 2 and 3 were exposed to twenty cycles of 15 seconds immersion at 6 hours' interval. The root mean square roughness (Rrms) was calculated for surface roughness assessment from the AFM images. Anova and Tuckey's post hoc test were used to analyze the differences in mean roughness values between the three groups. **Results:** One way Anova showed a statistically significant differences (p-value of 0.00) between the mean roughness values of three groups. Furthermore, Tuckey post hoc test revealed a statistically significant difference between the surface roughness values of Group 2 and Group 3 (0.158±0.012) and between Group 1 and Group 2 (0.085±0.012), indicating that mean surface roughness values increased significantly after frequent exposures to pediatric oral liquids. **Conclusion:** The tested pediatric oral liquids could significantly increases the surface roughness values of all treated tooth surfaces giving encorughment to the erosive potential of these oral liquid medicaments towards more enamel loss.

Keywords: *Atomic Force Microscopy (AFM), remineralization, enamel demineralization, oral liquids*

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1. Introduction

Dental erosion also referred as acid erosion is a process of irreversible loss of tooth substance (chemical dissolution) due to the acids whose source of production could be extrinsic or intrinsic. Most commonly included intrinsic sources are recurrent vomiting, regurgitation or gastro esophageal reflux, whereas, extrinsic agents are acidic food and drinks (like carbonated drinks, sports drinks, wines etc) [1]. In oral cavity our teeth are continuously exposing to cycles of demineralization and remineralization, but this delicate cycle can easily be disturbed due to extensive consumption of acidic drinks which ultimately leads to dissolution of inorganic tooth substance [2]. This process of chemical dissolution, if not timely treated, may also cause tooth sensitivity, reduce tooth vertical dimension, pulpal exposure and inflammation (in severe cases) [3,4].

In developing countries like Pakistan, prevalence of dental erosion along with dental caries is very high not only in adults but in adolescent and children due to dietary habits and lack of standardized guidelines for diagnosis [5,6]. Studies showed that primary dentition is more prevalent to surface enamel loss mainly due to thin enamel layer and greater susceptibility to acidic dissolution as compare to permanent dentition [7,8,9]. Therefore, children on long term medications to treat various chronic diseases like asthma, epilepsy, allergies are more likely to show enamel surface loss due to dental erosion [10]. Mostly the drugs prescribed to children are in liquid form, colored, sweetened and flavored to mask their bitter tastes. Besides the main active ingredients various additives like flavoring agents, sweeteners, acids, preservatives and coloring agents are added in most pediatric liquid medications to minimize the unpleasant taste [11]. In order to further improve the taste of these pediatric liquid medications mostly acids are added [12,13]. This acidic contents also

serves as buffering agents and results in controlling the tonicity of the drug [12].

However, due to the presence of acidic content and fermentable carbohydrate, the frequent use of these flavored pediatric medications showed cariogenic potential [14-20]. A study conducted in 2007 concluded that about half of 97 most commonly prescribed liquid pediatric medications have an endogenous pH level below 5.5 and thus are capable of causing loss of enamel surface layer [12]. Additionally, these acidic formulations when consumed by children at night could further aggravate the process of erosion and dental caries [21].

Recently many pharmaceutical companies have introduce sugar-free pediatric liquid medications but this reduction of sugary contents results in the addition of weak acids, to enhance their palatability concerns and other properties, which might cause tooth surface loss due to acid erosion [19]. However, there is still dearth of information regarding the erosive potential of most commonly prescribed sugar containing and sugar-free pediatric medications.

The aim of this *in vitro* analysis is to evaluate the effects of most commonly prescribed pediatric medications on enamel surface by the aid of Atomic Force Microscope Nano indentation.

2. Methodology

2.1. Sample Preparation

Thirty human deciduous incisors used in this *in – vitro* analysis were extracted from children 2-6 years old. The parents signed the written consent form that the extracted teeth could be used for the purpose of this experimental study. After removing the soft tissue debris by ultrasonic scalar further disinfection was done by immersion in sodium hypo chloride solution (5%) for one hour. The radicular portion of the tooth samples were separated from the coronal portion using slow speed cutting machine (Laizhou weiyi Co.Ltd Model DTQ-5) under running water. The labial portion of each incisor was further sliced longitudinally parallel to its long axis so that 60 tooth samples were obtained from 30 extracted primary incisors. The labial surfaces of enamel sections were polished using silica carbide paper (grades 600-1200) and then fixed in position within the preform Teflon molds (10mm x 8mm x 2mm) by flow able composite resin.

2.2. Oral Liquid Medicaments

Most commonly prescribed pediatric drugs to relieve some respiratory symptoms were chosen to serve as a demineralizing bath to induce dental erosion, i.e., Paracetamol and Chlorpheniramine. Paracetamol (Glaxo SmithKline Pakistan Limited) is classified as mild analgesic to treat fever, headache and is also available in "sugar-free" composition. To relieve the allergic symptoms and common cold an anti-histamine such as Chlorpheniramine (Ferozsons Laboratories LTD) is mostly prescribe. Pediatric oral liquids from two different brands were selected to make a comparison. The Chlorpheniramine group (**Bronochol syrup**) contains sugar while the Paracetamol **(Panadol Syrup**) belongs to sugar free type. All these drugs were purchased from local pharmacies of Islamabad, Pakistan.

Table 1. All the primary tooth specimens were assigned to following three groups

GROUPS	NO.OF SAMPLES	PEDIATRIC LIQUID MEDICAMENTS	APPLICATION FREQUENCY
1	20	No treatment	-
2	20	Paracetamol	20 cycles of 15 seconds immersion at 6 hours interval
3	20	Chlorpheniramine	20 cycles of 15 seconds immersion at 6 hours interval

2.3. Surface Roughness Assessment

For surface roughness evaluation images were taken with Atomic Force Microscopy Auto Probe CP 100 equipped with piezoelectric scanner of tapping mode. At least ten different film areas were used for root mean square (Rrms) calculation with a resolution of 256 x 256 pixels.

2.4. pH Measurement

The pH of the testing liquid medicaments was measured at room temperature (20 C) by using Digital pH meter. All the primary tooth specimens were then assigned to the following three groups (n=10) as shown in Table 1. The samples placed in Group 1 were not exposed to any treatment. However, to evaluate the erosive potential of medications enamel sections in Group 2 and 3 were subjected to twenty consecutive rounds of 15 seconds immersion in 10 ml of medicated liquids at 6 hour intervals at room temperature. Before and after exposure to different liquid medicaments all specimens were stored in a 100 ml deionizing water.

3. Statistical Analysis

Data was analyzed by using SPSS 11.O software. The mean roughness values of different groups were compared by using ANOVA. To determine the differences among three groups Tuckey's post hoc test were then performed.

Table 2. The pH of the tested pediatric liquid medicaments

GROUPS	Active Ingredient Concentration	Manufacturer of Drugs	pH (±SD) n= 5
Group 1	Control	Deionised water	7.09 ± 0.01
Group 2	Paracetamol	Glaxosmithkline Pakistan Limited	4.65±0.01
Group 3	Chlorpheniramine	Ferozsons Laboratories LTD	2.42±0.01

Table 3. Mean Surface Roughness Values of the experimental groups

Groups	Pediatric medicament Used	Rrms
Group 1	Control (No Treatment)	0.058±0.016
Group 2	Paracetamol	0.144±0.018
Group 3	Chlorpheniramine	0.301±0.054

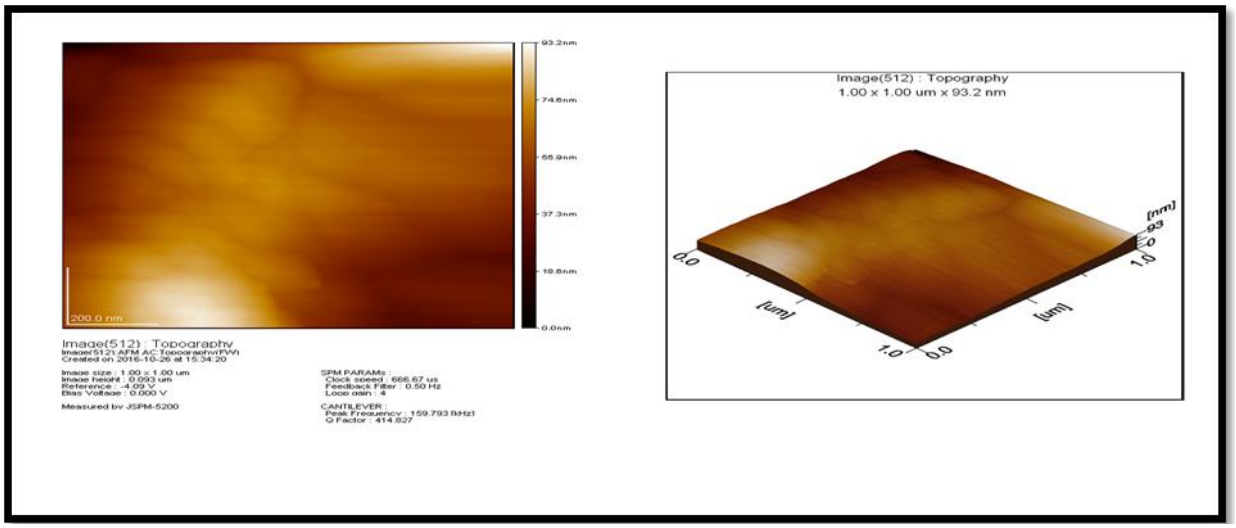

Figure 1. GROUP 1 (Control Group)

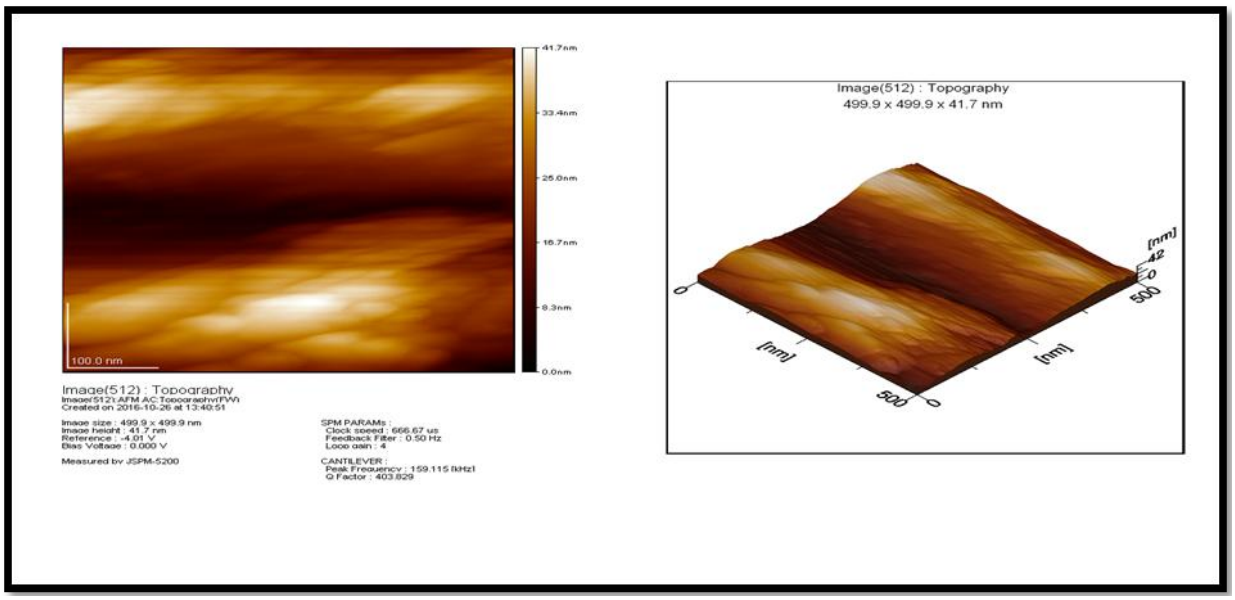

Figure 2. GROUP 2 (Paracetamol)

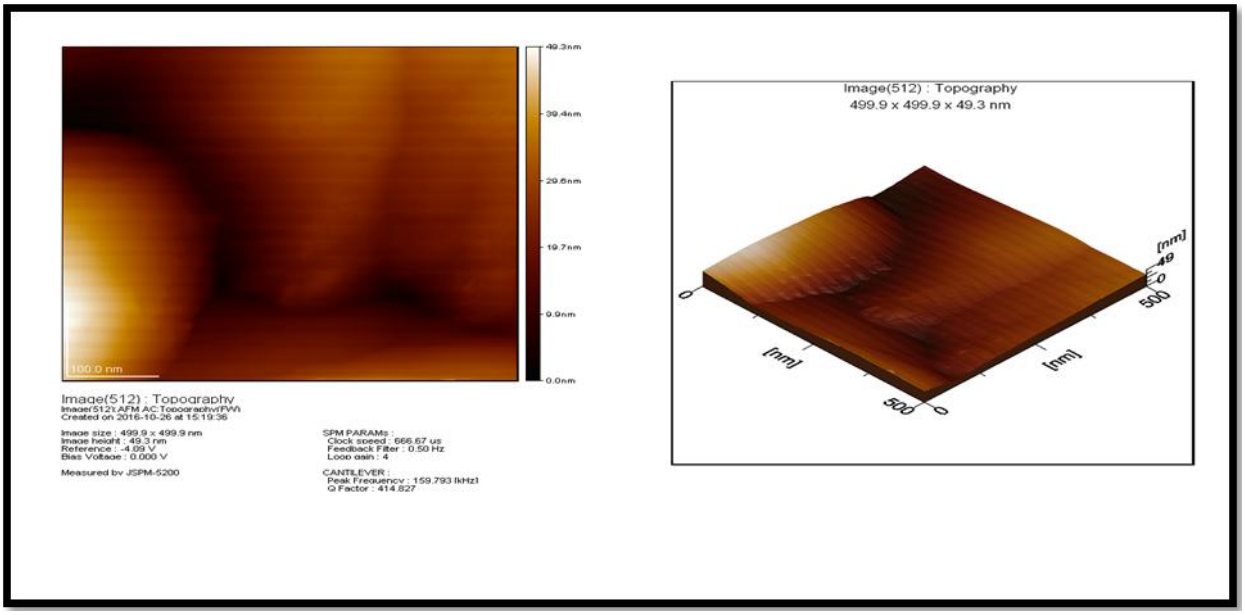

Figure 3. GROUP 3 (Chlorpheramine)

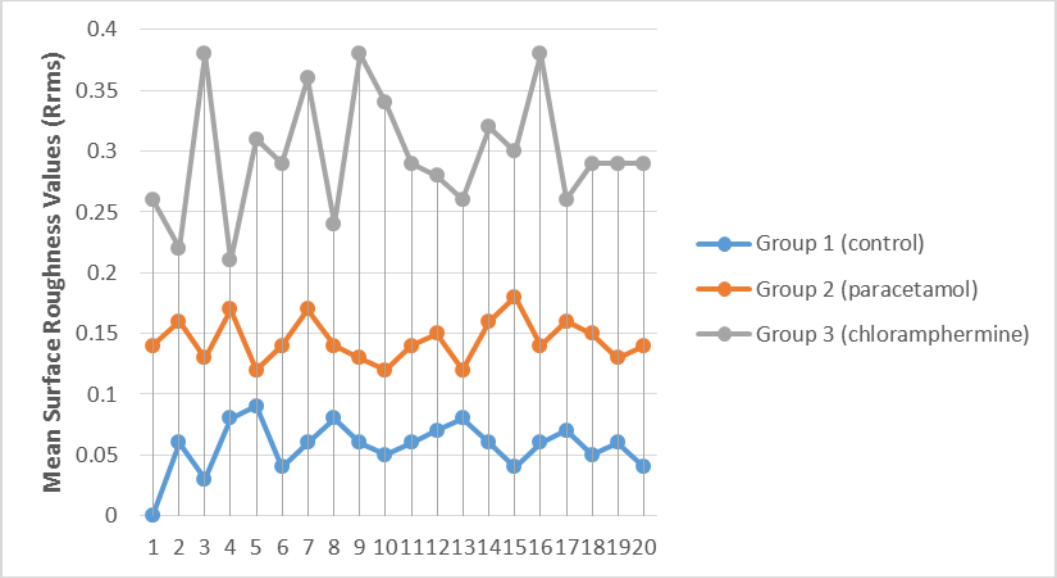

Figure 4. The mean surface roughness values of Group 1, Group 2 and Group 3

4. Results

4.1. pH Values

The pH value of all three groups have been shown in the Table 2. The values indicate that all the pediatric oral medicaments exhibited an acidic pH.

4.2. Mean Surface Roughness Values

The mean surface roughness values have been shown in Table 3. One way Anova showed a statistically significant differences (p-value of 0.00) between the mean roughness values of three groups. Furthermore, Tuckey post hoc test revealed a statistically significant difference between the surface roughness values of Group 2 and Group 3 (0.158±0.012) and between Group 1 and Group 2 (0.085±0.012), indicating that mean surface roughness values increased significantly after frequent exposures to pediatric oral liquids.

Figure 1 shows untreated tooth samples (Group 1). Figure 2 and Figure 3 reveals enamel surfaces after exposure to two different oral medicaments (Group 2 & 3)

5. Discussion

The erosive effects of medicated paediatric oral liquids using AFM has not been addressed in literature till now. The bleaching effects of various agents were first studied with the aid of AFM by Hegedus et al who compared the results obtained from SEM with those of AFM [22]. AFM is an important modern tool to study the effects of various paediatric liquid medicaments on enamel surfaces as it provides high resolution images. The topographic features of polished tooth surfaces were observed by using tapping mode AFM (TM AFM) and net differences in the mean surface roughness values between exposed and un exposed enamel surfaces were calculated. Thus AFM based nano indentation helps to accurately identify the remineralized and demineralized tooth surfaces [1].

The erosive effects of two different, most commonly prescribed, paediatric liquid medicaments were studied and then compared with untreated tooth surfaces. The results suggested a statistically significant difference with a p – value of 0.00 indicating the demineralizing effect of these drugs on tooth surface. Enamel subsurface, more frequently seen on the deciduous teeth surfaces, is less mineralized than enamel surface. In order to minimize the natural enamel surface variations between different teeth, which respond in a different manner to acidic dissolution, polished tooth specimens were used [23]. However, these polished enamel surfaces exhibit more dissolution of surface layer as compared to natural tooth surfaces in the oral cavity. In this *in vitro* analysis, two most commonly used liquid formulations were used. These pediatric medicaments contained many other constituents besides the active ingredient such as flavouring agent, sweeteners, bulk materials, preservatives and coloring agents etc. Acidic content of these liquid medicaments not only improved palatability but also helped to maintain chemical stability, tonicity and physiological compatibility. Most commonly added acid in these formulations was citric acid. According to studies frequent exposure to citric acid has been linked directly to tooth erosion due to its ability cause acidic dissolution of hydroxyapatite crystals. Similarly, lactic acid at low pH conditions proven to be more erosive than citric and maleic acid.

The deciduous tooth surfaces of Group B & C were exposed to 20 cycles of 15 seconds immersion in their respective drugs. The enamel sections in group B were exposed to sugar free composition of Paracetamol (pH 4.6) exhibit lower values of mean surface roughness values (0.144±0.018) as compared to mean surface roughness (Rrms) values of tooth samples in Group C (0.301±0.054).

Mean surface roughness assessment of enamel surfaces revealed that both sugar containing and sugar-free liquid medications have erosive potential. However, more extensive clinical studies are required in this regard to minimize the risk of erosion during frequent consumption of these liquid formulations by children.

6. Conclusion

Within the limitations of this in vitro analysis, it can be concluded that all the samples showed increase in the values of surface roughness after exposure to all the tested medicated pediatric oral liquids. Thus there is a possible association between the formulation of theses liquid medicaments and tooth surface erosion.

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