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RESEARCH ARTICLE

Evaluation of Remineralizing Effects of CPP-ACP and Nanohydroxyapatite on Erosive Lesions of Enamel in Deciduous Teeth After Exposure to Acetaminophen Syrup: An *in vitro* Study

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Abstract:

Background:

Oral pediatric liquid medications are commonly used in children who suffer from chronic diseases. Due to acid components in their formulations, these medications may possess a high erosive potential to dental tissues. Erosion is one of the major causes of degradation and demineralization of the enamel. If demineralization causes are not inhibited, it progresses and completely degrades the enamel. So, the dentin is exposed, and, finally, the pulp will be engaged in the process.

Aim:

This study aimed to evaluate the remineralizing properties of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and nanohydroxyapatite on erosive lesions caused by acetaminophen syrup intake *in vitro*.

Methods:

The microhardness of 34 healthy human primary molars was determined prior to and following a seven-day, three-times-a-day, one-minute exposure to acetaminophen syrup. The teeth were then randomly divided into four groups and were immersed in artificial saliva, fluoride gel, nano-hydroxyapatite, and CPP-ACP for 10 minutes. Then, their final microhardness was measured, and repeated-measures ANOVA analyzed the data.

Results:

Enamel microhardness in two groups decreased after exposure to acetaminophen syrup and increased after applying remineralization agents, CPP-ACP, and nano-hydroxyapatite. The two groups did not show significant differences in terms of microhardness increase (p -value=0.141).

Conclusions:

According to the findings of this study, CPP-ACP and nano-hydroxyapatite can improve the microhardness of enamel, which has been reduced as a result of exposure to medicinal syrups.

Keywords: Deciduous teeth, Demineralization, Nanohydroxyapatite, CPP-ACP, Acetaminophen syrup, Microhardness, Dental enamel, Tooth remineralization.

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1. INTRODUCTION

1.1. Background

Dental erosion is the progressive, irreversible loss of dental hard tissues by a chemical process without bacterial involvement [1]. Erosion is a multi-factorial process that internal and external factors may cause. Internal factors are

associated with the contact of tissues of the tooth with the gastric acid, *e.g.*, in vomiting and reflux disorders. Acidic foods and drinks, as well as acidic medications, are known external agents [2]. Erosion in children may be associated with many clinical problems such as dental hypersensitivity, altered occlusion, eating difficulties, poor aesthetics, pulp exposure, and abscesses [3, 4].

Liquid pediatric medicines are common for children who suffer from chronic diseases [5]. All classes of pediatric drugs show acidic pH and high total sugar content [6]. Frequent

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intake of these drugs that have erosive potential can induce dental erosion [7 - 9].

Liquid oral medications are usually prescribed to ensure children's drug intake compliance. Acidic preparations are necessary for drug distribution, chemical stability, physiological adaptation, and flavor enhancement. These syrups include acidic components and can increase the risk of dental erosion due to prolonged and repeated consumption, high viscosity, and side effects of reduced salivation. Some in-vitro studies have reported that medical syrups can affect the hardness of the enamel and alter its morphology [8, 10, 11].

Hydroxyapatite is a highly histocompatible natural substance with beneficial qualities such as resemblance to the mineral phase of human hard tissues, biocompatibility, and poor solubility in humid settings, and it has numerous medical and dental applications [12]. Hydroxyapatite nanoparticles have superior bioactivity to larger crystals [13]. Hydroxyapatite nanoparticles are highly similar to the structure of enamel crystals. It shows a strong affinity to the tooth and strongly adsorbs on enamel surfaces [14]. In recent years, much research has shown that nano-hydroxyapatite can remineralize primary erosive lesions [15 - 17].

Casein phosphopeptide-amorphous calcium phosphate has been designed based on the stabilizing properties of milk and salivary proteins. High quantities of calcium, phosphate, and fluoride ions in casein phosphopeptides must be stabilized in solution in a bioavailable state to promote remineralization. [18].

Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) provides the teeth with a more highly amorphous and accessible form of phosphorus and calcium compared to the calcium and phosphate ions available in the saliva. In addition, saturation with phosphate and calcium ions causes the alkaline state in this compound and in the dental plaque if applied to the teeth [19]. Additionally, it inhibits demineralization and enhances remineralization of subsurface enamel lesions in human and animal trials. The results of studies have shown that CPP-ACP is effective in dentin remodeling [20 - 22]. CPP-ACP protects the enamel and prevents demineralization by increasing the degree of calcium and phosphate saturation when the pH decreases due to the erosive challenge [23].

1.2. Aim

The present study aimed to investigate the remineralization effects of nano-hydroxyapatite and CPP-ACP on the erosive lesions resulting from the consumption of acetaminophen syrup.

2. MATERIALS AND METHODS

This is an experimental laboratory investigation on 34 deciduous molar teeth that were previously diagnosed as caries-free (caries detection according to the criteria of WHO), wears, cracks, and hypo calcification in the clinical examination. This sample size was selected based on previous studies [17,20].

The study protocol was approved by the Ethics Committee of Shahed University (Ethics committee reference ID: IR.SHSHED.REC.1397.074). -The surface of the teeth was cleaned from debris by a mechanical method using fluoride-free prophylactic toothpaste with pumice, a low-speed (500-1500 rpm) handpiece, and a brassage-specific brush. [24] Then, a 55 mm square label was applied to the buccal surfaces of the teeth, and all other dental surfaces were covered with clear self-curing acrylic resin. The contact surface of all teeth, regardless of form, size, or group, is identical after hardening. During acrylation, the samples were placed in cold water to prevent changes due to the heat generated by the hardening of the acrylic resin. For proper microhardness measurement, the surface of the samples was polished (abrasive papers of sizes 800, 1000, and 2000; 3M Ind. & Com. Ltda; St Paul, MN, USA) in the presence of water. It allowed the smoothed surface to be analyzed by a Vickers microhardness measuring device. After polishing, the surface of the specimens was dried, and the initial microhardness was measured by the Vickers microhardness tester device (Type M, No g 5025, Shimadzu® Corporation, Japan). The best point for applying the pressure was determined. Because pressures of 20 and 50 g were used in earlier research to determine the microhardness of teeth, and the pressure of 50 g was shown to be the most appropriate [24], we likewise employed a pressure of 50 g and an 8-second time period. . This pressure was applied at three points, and the microhardness of each one was measured.

After measuring the initial microhardness, all teeth were placed in the Acetaminophen syrup (Corizan, Abidi® Co, Iran) by pH 5.33 for 1 min [25], three times/ day for seven days. Then, their microhardness was measured for the second time. Then, the teeth were randomly divided into the following groups [26]:

1- Group P.C. (positive control): 2 teeth were placed in fluoride gel (Pascadental, USA) for 10 min.

2- Group NC (negative control): 2 teeth were placed in artificial saliva (Biomaterials Analysis Laboratory of the Faculty of Dentistry, University of Tehran).

3- Group NHA (nanohydroxyapatite): 15 teeth were placed in the 10% nanohydroxyapatite solution (Yazd Pardis Research Company) for 10 min containing 4 g of nano-hydroxyapatite powder and 40 ml of distilled water [17].

4- Group CPP-ACP: 15 teeth were placed in the CPP-ACP (MI Paste, G.C. America) for 10 min [17].

Between phases and prior to microhardness determination, specimens were immersed in artificial saliva. [8]. Then, the microhardness of the teeth was measured for the third time. The microhardness changes within and among the groups were evaluated using the ANOVA test with SPSS software, version 23 (IBM Corp., Armonk, N.Y., USA), and adopting a significant level of 0.05. Multiple comparisons were also performed using the Bonferroni method. Normality of data was tested by Kolmogorov-Smirnova. The p-value was at least 0.2.

3. RESULTS

The mean first microhardness of 15 specimens of the NHA group was 28.303 kgf/mm², with a standard deviation of 51.52. After exposure to the acetaminophen syrup, the value was 252.3 kgf/mm² on average, with a standard deviation of 1.39. The third microhardness of these specimens was 291.24 kgf/mm², with a standard deviation of 82.52. The mean first microhardness of 15 specimens of the CPP-ACP group was 315.99 kgf/mm² with a standard deviation of 54.24, which reached 277.99 kgf/mm² with a standard deviation of 36.53 after exposure to acetaminophen syrup. The third mean microhardness of these specimens was 313.99 kgf/mm² with a standard deviation of 49.38. (Table 1, Fig. 1).

Despite changes in microhardness over the course of the trial, these changes were similar in both groups (NHA and CPP-ACP), and no significant difference in microhardness was

seen between the two groups (p-value=0.141). Since changes were significant over time, the value of microhardness was compared in pairs three times in Bonferroni's Paired test (Table 2). The value of microhardness decreased significantly at the second time compared to the first time (p-value=0.001) and it increased (p-value=0.001) after intervention (p-value=0.002). However, after treatment, the microhardness did not show significant changes compared to the initial microhardness (p-value>0.999).

Table 1. Changes in the rate of microhardness in experimental groups.

Experimental Groups	Number	Primary Evaluation		After Demineralization		After Intervention	
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
NHA	15	303.3	52.2	252.3	39	291.2	52.8
CPP-ACP	15	316	54.2	278	53.3	314	38.5

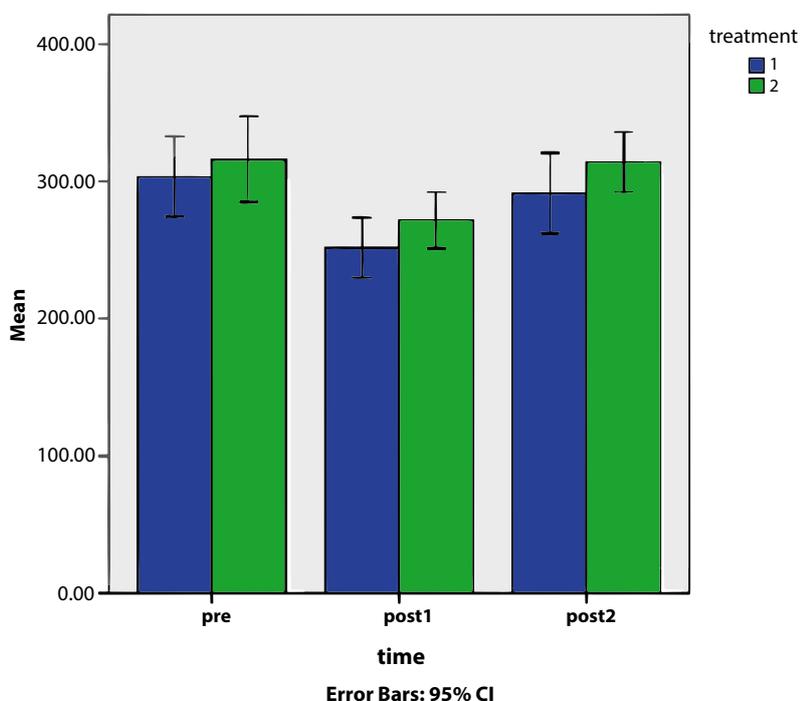


Fig. (1). Histogram of the primary, secondary and third microhardness in experimental groups. (Blue: NHA group, green: CPP-ACP group).

Table 2. Comparison of primary, secondary and third microhardnesses.

Microhardness		Mean Difference	Std. Error	Sig.	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
Primary	Secondary	44.488	10.642	.001	17.326	71.651
	Third	7.020	11.322	1.000	-21.878	35.919
Secondary	Primary	-44.488	10.642	.001	-71.651	-17.326
	Third	-37.468	9.670	.002	-62.150	-12.786
Third	Primary	-7.020	11.322	1.000	-35.919	21.878
	Secondary	37.468	9.670	.002	12.786	62.150

4. DISCUSSION

The effect of nano-hydroxyapatite and CPP-ACP on the microhardness of enamel in deciduous molar teeth following acetaminophen syrup exposure was investigated in this study due to the associated complications of the medication's effects on the enamel and the search for a substance capable of remineralizing the medication.

The study's findings indicate that acetaminophen syrup considerably reduces the enamel's microhardness.

Given that the medical syrups lower the pH, it may explain the reduction of the microhardness after exposure to acetaminophen [27].

Mahmoud and Omar, in their study, showed a positive association between Oral pediatric liquid medications and erosion. These results are in agreement with our study [11]. The results of the study by Babu also showed that in the exposure of enamel in deciduous molar teeth to the four pediatric syrups (amoxicillin, metronidazole, acetaminophen, ibuprofen + acetaminophen), the highest reduction in the microhardness of enamel was observed for the amoxicillin syrup and the lowest for the ibuprofen +acetaminophen [8].

The findings of the study by Lakshay Dhawan are consistent with our study. Despite that, in the study by Lakshay Dhawan, the time of placement of the specimens in the three types of syrup was 1 min, three times/ day for 28 days [25].

This study showed that the microhardness of teeth demineralized with acetaminophen syrup was increased after exposure to nano-hydroxyapatite and CPP-ACP, and nano-hydroxyapatite and CPP-ACP were able to remineralize the demineralized teeth.

Hydroxyapatite is known as a remineralizing agent when applied to the surface of the enamel [28]. Nanohydroxyapatite is a material that is most similar to the tooth structure. It decreases microleakage in dental cavities, is biocompatible, and has antimicrobial properties with remineralization potential [15]. Considering the hydrophilic property of the nano-hydroxyapatite crystal, it is a moisturizing agent on the dental surface, which creates a strong layer on the enamel those bonds to the tooth crown [29].

The availability of calcium and phosphorus ions is necessary for the progress of remineralization. The fluorapatite and fluorohydroxyapatite crystals are created in the presence of calcium, phosphorus, and fluoride ions [30]. The microhardness increase in the CPP-ACP group may be due to the amorphous and sedimentable form of calcium and phosphorus ions. On the other hand, saturation with calcium and phosphorus ions converts acidic to alkaline [31]. Also, the results of studies show that ACP-CCP can serve as a calcium source with remineralization capability. The material is designed to stabilize calcium and phosphate ions on the teeth' surface [32 - 35].

Studies by Rezvani [20] in 2015 and Haghgoo [21] in 2017 showed that the mean microhardness of the enamel of the teeth increases when exposed to the CPP-ACP, which is consistent with our study, but in the study of Rezvani, the permanent teeth were exposed to the coke and whey was compared with CPP-

ACP, and in the study of Haghgoo, erosive lesions were created on the permanent molar teeth and TCP was compared with CPP-ACP.

In 2018, Haghgoo showed that nanohydroxyapatite increased the microhardness of the enamel of healthy third molar teeth after exposure to the beer in an in-situ manner, which is consistent with our study, but in this study, the eggshell was compared with nano-hydroxyapatite in an in-situ manner, which is inconsistent with our study [24].

De Carvalho's studies showed that the microhardness of the demineralized enamel increases after the application of nano-hydroxyapatite, in which permanent molars were examined [36]. Additionally, recent research showed hydroxyapatite deposition on polymeric composite resin that could prevent secondary caries on the margins of restorations. [37] Another in vitro and in vivo study demonstrated the high potential of remineralization of the enamel of deciduous teeth when nanostructured microparticles were used in Biomimetic Hydroxyapatite toothpaste [38]. The time of the exposure of specimens to the nano-hydroxyapatite in Mielczarek's study [39] was a 3-week period, in the study of Haghgoo [24], it was 10min, two times/day for ten days, and in another study by the same author, it was 10min [36].

In all studies on this material so far, relatively long exposure times have been applied; however, from the clinical viewpoint, this long period is prohibiting and impossible under clinical conditions. Therefore, in the present study, 10 min was considered for the exposure to remineralizing agents because longer periods would be impossible for patients to use and are not consistent with the clinical criteria [30, 31].

Previous studies [8, 32] with different concentrations of nanohydroxyapatite on the remineralization of initial enamel lesions showed that a concentration of 10% nanohydroxyapatite has a higher remineralization capacity than lower concentrations, but there is no significant difference with higher concentrations.

Some unexplored variables can have a significant influence on the oral environment. The use of probiotics [40] And natural compounds. [41] Can modify Clinical and Microbiological Parameters. These compounds could have an effect also in combination with hydroxyapatite. All these variables should be considered in future clinical trials.

No research has been done on the effect of remineralizing agents on erosive lesions of deciduous teeth caused by medicinal syrups. This study investigated the effects of nano-hydroxyapatite and CPP-ACP on erosive lesions of the enamel of deciduous teeth resulting from the medical syrups. Our study had some limitations, such as finding 34 healthy primary molars.

CONCLUSION

According to the findings of this in vitro study, CPP-ACP and nano-hydroxyapatite can improve enamel's microhardness, which has been reduced as a result of exposure to medicinal syrups.

RECOMMENDATION

It is recommended that the effects of other medical syrups on the microhardness of deciduous and permanent teeth are also investigated. It is also recommended that the effect of different exposure times for the remineralizing agents be investigated.

ETHICAL STATEMENT

The study protocol was approved by the Ethics Committee of Shahed University, Tehran, Iran (Ethics committee reference ID: IR.SHSHED.REC.1397.074)

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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None.

CONFLICT OF INTEREST

The authors state that they have no financial or other potential conflicts of interest.

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