In-Vitro Evaluation of the Effect of Addition of Xylitol to Carbonated Diet Soda on Enamel Microhardness of Permanent Teeth

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Abstract

Background and Aim: High consumption of carbonated diet soda is the most common etiologic factor for dental erosion. This study aimed to assess the efficacy of addition of Xylitol to carbonated diet soda to prevent enamel microhardness reduction in permanent teeth.

Materials and Methods: This in-vitro experimental study was conducted on 40 human impacted third molars that had been surgically extracted and were free from caries, erosion, cracks or hypocalcification. For correct measurement of microhardness, surface of samples was polished with 5000 grit abrasive paper and microhardness was measured using Vickers microhardness testing machine. The teeth were then divided into 4 groups and immersed in 40 ml of 4 different solutions for 5 minutes (regular coke as the first control, coke zero, coke zero plus 10 g Xylitol and tap water as the second control). Understudy surface was a 5x5 mm square on the distal surface of teeth. At the end of experiment, microhardness of teeth was measured again. Changes in microhardness were compared in each group with paired t-test and between groups with ANOVA.

Results: Based on the obtained results, microhardness decreased after immersion of specimens in regular coke, coke zero and coke zero plus 25% Xylitol by -38.66±24.87, -26.1±16.65 and -19.5±23.52, respectively and these reductions were statistically significant (P1=0.001)(P2=0.001)(P3=0.005). Change in microhardness was -7.4±9.17 in specimens immersed in tap water and was not statistically significant (P4=0.5). The reduction in microhardness of enamel exposed to Xylitol was significantly less than the other two test groups.

Conclusion: Addition of Xylitol to carbonated diet soda reduced the amount of tooth erosion but could not prevent it.

Key Words: Erosion, Enamel, Microhardness, Xylitol

Introduction

Dental erosion is defined as irreversible destruction of tooth structure mediated by a chemical reaction without the involvement of bacteria. Acid exposure is usually the main cause of erosion [1]. Acids may have intrinsic or extrinsic sources. An important cause of erosion is sudden increase in consumption of non-alcoholic soft drinks, diet soda and juices [2]. Studies have indicated that acidic carbonated beverages have high affinity to enamel and play a role in occurrence of erosion [3-5]. Consumption of non-alcoholic carbonated soft drinks is rapidly growing. Shenkin et al, in their study demonstrated that the affinity of carbonated soft drinks to enamel is more than that of saliva and other non-sugary drinks i.e. orange juice [6]. Thus, replacement of sodas with less acidic drinks can be effective for reducing enamel destruction [3]. In some diet car-
bonated soft drinks such as coke zero, sucrose has been eliminated and replaced with some other sweeteners to reduce the calorie content. Xylitol is a five-carbon sugar alcohol. It is not fermentable by S. mutans and consequently does not cause a pH drop in the oral cavity. Also, Xylitol interferes with the glycolytic pathway, prevents the consumption of Sucrose by S. mutans and inhibits their growth and proliferation [7]. The majority of recent studies have shown that continuous consumption of Xylitol decreases the cariogenicity of bacteria. It has also been demonstrated that Xylitol can react with metal ions such as calcium and oxyacids. Xylitol competes with water for primary hydration of calcium and has a stabilizing effect on salivary calcium and phosphate levels. It increases the pH of dental plaque and leads to remineralization [8, 9]. Investigations have confirmed that Xylitol increases enamel microhardness [10, 11]. Effects of Xylitol on tooth remineralization have been evaluated in products such as gums, syrups, mouth rinses, juices, and lollipops. However, the efficacy of Xylitol in carbonated soft drinks has not been evaluated so far. Considering all the above, the purpose of this study was to evaluate the effect of Xylitol in carbonated diet soda on enamel microhardness of permanent teeth.

Materials and Methods

This in-vitro experimental study was conducted on 40 impacted third molars that had been surgically extracted. On clinical examination, the teeth were free from caries, erosion, cracks or hypocalcification.

After collection, the teeth were stored in brand new glass containers filled with Tehran tap water at room temperature for 3 months. In order to prevent surface contamination or changes, the water in the containers was changed twice a week. Tooth surfaces were cleaned from calculus and debris by using fluoride-free prophylactic paste containing pumice, pumice wheel and low speed hand piece operating at 500-1500 rpm.

Absence of enamel defects, microscopic caries or cracks was ensured by evaluation of samples under stereomicroscope with 40x magnification.

For mounting the teeth, a square-shaped label measuring 5x5 mm was placed on the distal surface of teeth. In order to avoid the confounding effect of monomer and its polymerization heat, cold mounting polyester resin was used. The teeth were placed in the container and catalyst was added to the polyester liquid resin in the container. After curing that took 24 hours, specimens were polished with 5000 grit abrasive paper to create a smooth surface for evaluation with microhardness testing machine (G-M 5037, Shimadzu, Japan). For microhardness measurement, 50 g force was applied to three points located at the corners of a triangle and the microhardness values were recorded. The teeth were then divided into 4 groups of 10. The first group specimens were immersed in 40 ml of regular coke as the first control, second group specimens were soaked in 40 ml of coke zero, third group in 40 ml of coke zero plus 10 g of Xylitol (25% Xylitol) and the fourth group in 40 ml of tap water (district 6, Tehran) as the second control.

The pH of solutions was measured by a pH-meter (CH-1901 Herisau, Metrohm Ltd., Switzerland). The teeth were immersed in 40 ml of the respective solutions for 5 minutes that had been poured into the containers after opening the bottle. In group 3, immediately after the pouring of coke zero, 10 g of Xylitol (Merck, Germany) was added. The teeth were immersed in solutions simultaneously. In order to simulate clinical setting, the temperature of solutions was adjusted at 9°C. The solutions were gently stirred during this time period.

After completion of immersion time, the teeth were rinsed with water and a technician blinded to the group allocation of teeth measured their microhardness.

Change in microhardness was compared in each group by paired t-test and between groups by ANOVA. Microhardness value was variable at different points and the probe of the machine caused porosities at the test site making it impossible to carry out a second measurement at the same point. In order to resolve this problem, microhardness was measured in three points on the surface of each sample. These three points were selected with the closest proximity of one another;which was 20 micron. The mean microhardness in these 3 points was measured before and after the intervention (immersion in solutions). Wilcoxon signed rank test was applied for baseline microhardness analysis of 30 points which revealed no significant difference. The same statistical test was used for mi-
crohardness analysis of 30 points after the immersion of samples in solutions which showed no significant difference either. Therefore, although the re-evaluation of microhardness of primary points was not feasible, lack of significant difference between the microhardness of tested points indicated that the microhardness of each point was indicative of the surface microhardness.

Results
This study evaluated the effect of regular coke, coke zero, coke zero plus 25% Xylitol and tap water as the control on enamel microhardness of teeth. The mean microhardness change in the understudy points was statistically analyzed using paired t-test and p<0.05 was considered statistically significant.

The mean primary (baseline) microhardness of 10 samples in group one was 338±22.1 that reached 299.3 ± 16.3 after immersion in regular coke equal to 88.4% of the primary microhardness value. Paired t-test indicated that this reduction was statistically significant.

The mean baseline microhardness of 10 specimens in group two was 336.1 ± 35.1 that reached 310 ± 16.3 after immersion in coke zero equal to 92.1% of the primary microhardness value. Paired t-test indicated that this reduction was statistically significant.

The mean primary (baseline) microhardness of 10 samples in group 3 was 335.7±24.8 that reached 335.7±24.8 after immersion in coke zero plus 25% Xylitol equal to 94.1% of the primary microhardness value. Paired t-test indicated that this reduction was statistically significant (p=0.005).

The mean baseline microhardness of 10 samples in group 4 was 329.1±36.2 that reached 326.7±35.1 after immersion in tap water equal to 97.7% of the primary microhardness value. This reduction was not statistically significant. (p=0.5)

ANOVA demonstrated significant differences in microhardness of teeth after immersion in regular coke, coke zero, and coke zero plus 25% Xylitol. The greatest reduction in microhardness observed after immersion in regular coke followed by coke zero and coke zero plus 25% Xylitol (Table 1).

<table>
<thead>
<tr>
<th>Group number</th>
<th>Study group</th>
<th>Mean and SD</th>
<th>ANOVA value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regular coke</td>
<td>-38/66±24/87</td>
<td>0/014=2,1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Coke zero</td>
<td>-26/1±16/65</td>
<td>0/001=3,1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Coke zero plus Xylitol</td>
<td>-19/5±23/52</td>
<td>0/001=4,1</td>
<td>0/018=4,3</td>
</tr>
<tr>
<td>4</td>
<td>Tap water</td>
<td>-7/4±9/17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion
Erosion is defined as the irreversible destruction of enamel by chemical agents without the involvement of microorganisms [1]. In most cases, the main cause of erosion is exposure to acids especially in nonalcoholic beverages [1, 2]. Prevalence of erosion has greatly increased in the recent years due to the growing consumption of acidic carbonated soft drinks [3]. Replacement of these drinks with less erosive beverages seems logical. This study evaluated the effect of regular coke, coke zero and coke zero plus 25% Xylitol on enamel microhardness of permanent teeth. The obtained results revealed that the enamel microhardness of teeth decreased after immersion in regular coke, coke zero and coke zero plus 25% Xylitol and this reduction was higher in regular coke than in the latter two groups.

Enamel microhardness reduction after exposure to coke zero plus 25% Xylitol was less than that in regular coke and coke zero groups. This finding may be attributed to the hydration of calcium ions...
and their super-saturation preventing further release of calcium ions [8, 9]. However, various studies have used different methods for quantitative assessment of demineralization making comparisons in this respect difficult. Lippert compared enamel microhardness reduction after exposure to coke and lemonade and showed that the effect of coke in this respect was greater than that of lemonade which is in accord with the present study results [12].

Haghgoo et al. demonstrated that enamel microhardness of permanent teeth decreased to a greater extent after immersion in Zamzam Cola compared to Delester (a type of nonalcoholic beer) [4]. In the present study, enamel microhardness reduction was greater in regular coke compared to coke zero groups.

Coke zero plus Xylitol reduced the enamel microhardness less than coke zero which is probably due the fact that Xylitol interferes with enamel decalcification. However, in the study by Haghgoo, acrylic resin was used for mounting of teeth; whereas, in the present study cold mounting system with no monomer was applied. Acrylic resin monomer can act as a confounding factor.

According to the results of Chunmuang et al, enamel microhardness reduction due to immersion in orange juice was greater than that in orange juice plus Xylitol [10] which is in agreement with the present study findings. Scheinin et al, in their study revealed that premineralized bovine enamel microhardness increased as the result of exposure to Xylitol in candy [11]. In the current study, coke zero plus Xylitol reduced enamel microhardness but this reduction was significantly less than that due to regular coke. In this study, Xylitol was added to an acidic solution and evaluation was carried out under in-vitro conditions; whereas, Scheinin’s study was performed in-vivo and the buffering capacity of saliva and the differences between bovine and human enamel can be responsible for the observed differences between the two studies. According to the results of an insitu study by Creanor, bovine enamel microhardness increased after exposure to Xylitol in chewing gum [13]. The obtained different results may be explained by the buffering capacity of saliva and the differences between bovine and human enamel.

In a study by Devlin et al, reduction in microhardness after immersion in coke was greater than that in the present study [14] which is probably due to the longer exposure time to coke in their study (1, 2, 3 and 5 hours) in comparison to 5 minutes exposure time in the present study.

In the current investigation, we tried our best to eliminate all the confounding factors. In Brown et al, study, non-carious sound areas of the carious teeth were used as the study specimens [15]; whereas, we excluded carious teeth with highly susceptible enamel surfaces from our study. We used impacted teeth in this study because we wanted to eliminate the confounding effects of oral and nutritional habits that may impact on the developed teeth.

In this study, 5000 grit abrasive paper was used which is the softest abrasive paper available in the market and by doing so the unwanted removal of the hypermineralized surface enamel was minimized [16]. Collection of sound teeth with no enamel defects was among the limitations of this study.

Furthermore, this study evaluated the enamel microhardness under in-vitro conditions. Similar studies are recommended to be performed insitu in the oral environment.

**Conclusion**

Based on the present study results, addition of Xylitol to carbonated soft drinks reduces dental erosion but cannot prevent it.

**References**