Dental Erosive Potential of Non-nutritive Sweeteners (Aspartame, Acesulfame-k, Sucralose), and Steviol Glycosides in Carbonated Drinks

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Background: The link of acidic beverage consumption and dental erosion is a well-known fact. However, the substitution of sucrose and high-fructose corn syrup (HFCS) by either non-nutritive sweeteners (NNS) or Steviol Glycosides (SG) in carbonated soft drinks remains unclear. **Objective**: To assesses the dental erosion potential related to NNS (Aspartame, Acesulfame-K, Sucralose), and SG in carbonated soft drinks on 60 extracted healthy premolar teeth. **Methods**: Samples were randomly distributed into five groups: Group I: Classic Coke™, Group II: Light Coke™, Group III: Stevia Coke™, Group IV: Mineral Water, Group V: Distilled Water. Initially, pH, titratable acidity and calcium content of each tested drinks were determined. Standardize enamel surface area of premolar teeth were exposed in selected five beverages in 5-minute duration, three times in 4 hours interval daily for four consecutive days. The erosive potential was evaluated by measuring calcium released and teeth weight loss. **Results:** All tested carbonated soft drinks showed significance differences $p<0.05$. Light Coke™ showed the highest dental erosion followed by Classic Coke™ and Stevia Coke™. **Conclusion:** Substitution of sweeteners types in acidic beverages do give a different influence on teeth erosion. SG can be proposed as future sweetener substitute in acidic beverages to reduce dental erosion.

1. Introduction

Dental erosion is a chronic, localized and painless pathological loss of dental hard tissues due to a chemical process that is not involve of microorganisms (1, 2). The well-known main external factor of tooth erosion is due to frequent consumption of low pH beverages that below the critical pH value (3). In the last few decades, a huge increase in the turnover of the acidic beverages has been witnessed (4) such as 'sugar free', 'low-sugar' and 'healthy' acidic beverages products to meet customers demand (5, 6). These sugar free beverages contain artificial sweetener either non-nutritive sweeteners (NNS) or steviol glycoside (SG) as a substitutional of sucrose and high fructose corn syrup (HFMC) (6, 7).

In 2001, a research showed that sugar-free soft drinks producing much more erosion than sugar soft drinks (9). The proposed factors of dental erosion brought by sugar-free soft drinks is the chemical inherent or multiple content of acids contain in the sugar-free soft drinks (5, 9). The controversial issue of dental erosion brought by artificial sweeteners beverage is still debatable till the year 2011 (10). Therefore, the aim of this in vitro study was to assess the dental erosion potential related to NNS (Aspartame, Acesulfame-K, Sucralose) and SG in carbonated soft drinks on 60 extracted healthy premolar teeth. Selection of the premolar teeth in this in-vitro study is mainly following the cohort properties of inclusion which is non-carious extracted teeth that will be used instead of the carious teeth (11).

2. Materials and Method

2.1 Preparation of healthy extracted premolar teeth

60 non-carious extracted premolar teeth were cleaned initially and covered with three layers of nail varnish leaving 30 mm^2 enamel surface area (Figure 1). Tooth specimens will be divided into 3 sets of 5 groups (n=4 per set group) labelled as Group I (Classic Coke™), Group II (Light Coke™), Group III (Stevia Coke™), Group IV (Mineral Water) and Group V (Distilled Water, DH_2O).

Fig. 1: A tooth specimen that was covered by varnished leaving an exposed standardize enamel window (30 mm^2)

2.2 Characterisation of soft drinks

2.2.1 Measurement of pH

50ml of freshly opened tested drinks were placed into clean separated beakers. The pH of the beverages was measured three times using a calibrated pH electrode.

2.2.2 Determination of titratable acidity (TA) and calcium content

50ml of freshly opened tested drinks were placed in a clean separated beaker and in small increments, 0.5 M of sodium hydroxide (NaOH) were added to each drink. Changes in the pH of the tested drinks were monitored and the volume of NaOH required to increase the pH of the soft drink to the pH 5.0, 7.0 and 10.0 were recorded. Similar steps were repeated three times.

2.2.3 Determination of calcium content

200µl of the tested drink was added to a clean beaker containing 1 ml of potassium hydroxide (1.25 N KOH) and 100µl of calcon. 1% Ethylene diamine tetra acetic acid (EDTA) was added in a small increment volume until the solution appeared as blue color. The volume of EDTA required to produce the end point was recorded. This step was repeated on a standard control which contains a known amount of calcium chloride ($CaCl_2$) (0.1 mg/ml). Based on the volume of EDTA used to complex with a known amount of calcium (standard control), the concentration of calcium present in the drinks were calculated.

2.3 Assessment of tooth weight loss and rate of calcium released

The initial weight (Wo) of the tooth specimens were taken followed by immersion in a separated beaker containing 50ml of the respective beverages at 70rpm (constant stirring) for 5 minutes. 200µl of the drinks were pipetted out and the concentration of the calcium in the drinks were determined. Specimens were washed and left to dry in the oven (30ºC for 2 hours) and were re-weighted (W_1). The weight loss due exposure to the beverages was calculated. These processes were repeated three times in 4 hours interval daily for four consecutive days.

2.4 Statistical Analysis

Statistical analyses were determined by repeated measure ANOVA with Pillai's Trace in multivariate tests and Bonferroni adjustments to estimate marginal means for all drinks.

3. Results

All tested carbonated soft drinks showed significance differences $p<0.05$. Classic Coke^{TM} was the most acidic amongst the soft drinks (pH 3.25). Light Coke^{TM} and Stevia Coke^{TM} have pH values of 3.53 and 3.52 respectively. While, mineral water and DH_2O were recorded at pH 7.24. As for TA, all respective drinks showed almost equivalent TA to reach pH 5.0. Light Coke^{TM} and Stevia Coke^{TM} showed the same values of NaOH to increase the pH to 7.0 (0.34 ml) while Classic Coke^{TM} showed less TA (0.33 ml). Calcium content for Light Coke^{TM} and Classic Coke^{TM} were 26.67 µg/ml. As for Stevia Coke^{TM}, mineral water and DH_2O, the calcium content was 13.33 µg/ml. The assessment of weight loss and rate of calcium released from the enamel surface displayed the specimens immersed in Light Coke^{TM} have the highest enamel weight loss and calcium released followed by Classic Coke^{TM} and Stevia Coke^{TM} in descending order (Table 1).

Tested drinks	Tooth weight loss (mg)	Calcium Released (µg/min)
Light Coke	55.93 ± 0.76	44.46 ± 8.30
Classic Coke	35.88 ± 0.80	16.67 ± 9.23
Stevia Coke	35.49 ± 1.01	11.94 ± 8.76
Mineral Water	32.94 ± 1.14	5.56 ± 6.50
DH_2O	30.52 ± 0.57	5.56 ± 10.16

Table 1: The weight loss (mg) and rate of calcium released (µg/min) from the enamel surface upon exposure to the selected drinks. Results represent as mean ± standard deviation of teeth specimen

4. Discussion

All the carbonated soft drinks were below the critical pH (pH 5.5). Classic Coke^{TM} demonstrated the lowest pH than other drinks. The lower the pH, the higher the number of hydrogen ions. However, pH is not a reliable indicator since it does not measure amount of undissociated acid (1, 12). Characterizing the erosive potential of drinks is best done by TA (1, 13). Light Coke^{TM} has the higher TA than Stevia Coke^{TM} because it contains phosphoric acid and citric acid (14). Stevia Coke^{TM} having the lowest TA thus it neutralised fastest.

As dental erosion is a demineralization process, calcium will release from the enamel surface following exposure to the carbonated soft drinks which also leads to tooth weight loss. Teeth immersed in Light Coke^{TM} showed the highest amount of calcium released and weight loss. This can be correlate with the high TA of Light Coke^{TM}. Previous study by Low et al in 2008 proposed that there is direct relationship between the weight loss of the tooth and loss of calcium ions. The complexity of the erosive potential and the fact that in vitro studies cannot totally reproduced in the clinical condition since there are many individual factors affecting the environment of oral cavity such as the salivary conditions and real buffering capacity (15). In conclusion, substitution of sweeteners types in acidic beverages do give a different influence on teeth erosion. SG can be proposed as future sweetener substitute in acidic beverages to reduce dental erosion.

5. References

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