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Comparative study of the possible effect of bovine and some plant-based milk on cola-induced enamel erosion on extracted human mandibular first premolar (scanning electron microscope and X-ray microanalysis evaluation)

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ABSTRACT

Increased consumption of acidic soft drinks is becoming an important factor in the development of erosive wear. The potential of dairy drinks to protect enamel against dental erosion has been recorded. Recently, the demand for plant based milk beverages has been gaining popularity and used as an alternative to cow's milk.

Aim: reveal and compare the possible effects of bovine and three types of plant-based milk on enamel erosion caused by Coca-Cola®.

Material and method: 42 extracted premolars were distributed over three groups: Control negative group where teeth were not subjected to any treatment, Control positive group where teeth were subjected to Coca-Cola® and Experimental group where teeth were divided into four subgroups and subjected to Coca-Cola® then soaked in certain type of milk (bovine, soy, almond or oat milk). All groups were prepared for SEM analysis and EDAX.

Results: Coca-Cola® beverage significantly altered enamel superficial surface structure causing irregular surface, erosive lesions and cracks. Bovine and plant based milk has a reparative effect on eroded cervical buccal enamel.

Conclusion: Almond milk showed better results than other types of milk used concerning Ca and P levels as well as surface morphological alternations. Soy milk showed the least enamel remineralizing effect.

1. Introduction

Dental erosion is defined as a loss of dental hard tissue caused by acids, without bacterial involvement. Erosion may be caused by intrinsic or extrinsic factors. The extrinsic factors are related to acidic environment, diet, medication and life style [18,33]. Currently, the increased consumption of acidic foods and soft drinks is becoming an important factor in the development of erosive wear [16,17]. Coca-Cola®, as an example of soft drinks, was the world's most popular brand according to Interbrand's best global brand study of 2011, [11].

On the other hand, some studies showed the potential of dairy foods and drinks to protect enamel against dental erosion. [9] and [15] showed the rehardening effects of cow's milk and cheese in situ following the softening of human enamel with an acidic soft drink (cola drink).

Previous studies by Refs. [25] and [22] have shown that bovine milk possesses anticariogenic properties in animal caries models and can remineralize enamel subsurface lesions in vitro [19,21].

Recently soy, almond and Oat milk are used as an alternative to cow's milk especially for those have milk allergy or lactose intolerance and/or vegetarians. Plants based milk beverages can be made from cereals such as rice and oats, nuts such as almonds and legumes [2]. Soy and almond milk are popular examples of non-dairy milks in the USA while oat milk is common in the Europe.

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These beverages vary in taste and texture and are available in different formulations in the market [14].

The aim of the present study is to reveal and compare the possible effects of bovine milk and three types of plant-based milk on enamel erosion caused by coca-cola on human lower first premolars.

2. Materials and methods

Forty two sound (caries-free) human mandibular first premolars freshly extracted for orthodontic reasons were used in the present study. The extracted teeth were cleaned gently from residual debris and be washed thoroughly under running water and then they were examined under stereomicroscope to ensure the absence of caries, calculus, or surface defects. The apical foramen of each tooth was blocked with water proof acrylic varnish to prevent fluid entrance through it [6], then the teeth were divided into the following groups:

- **Control negative group (C-ve)**: seven teeth were placed in distilled water during the experimental period.
- **Control positive group(C+ve)**: seven teeth were placed in a beaker filled with 330 ml of Coca-cola® and immersed for 25 h and replaced every 5 h. This model allows for observation of demineralization within a reasonable time period as designed by Ref. [6].
- **Experimental group**: Twenty eight collected teeth were placed in a beaker filled with 330 ml of Coca-cola® and immersed for 25 h and replaced every five hours [6], then they were washed in water for seconds and dried by warm air [19], then they were randomly subdivided into 4 equal subgroups (7 premolars each) as follows:
- **Subgroup 1(SG1):** 7 teeth were immersed in a beaker filled with 125 ml of bovine milk (JUHAYNA® full cream); for 50 h and milk was replaced every 2 h as designed by Ref. [19].
- **Subgroup 2 (SG2):** 7 teeth were immersed in a beaker filled with 125 ml of Soy milk (Alpro® Soya Original); for 50 h and milk was replaced every 2 h.
- **Subgroup 3 (SG3):** 7 teeth were immersed in a beaker filled with 125 ml of almond milk (Alpro® Almond Original); for 50 h and milk was replaced every 2 h.
- **Subgroup 4 (SG4):** 7 teeth were immersed in a beaker filled with 125 ml of oat milk (Alpro® Oat Original); for 50 h and milk was replaced every 2 h.

Each tooth was washed under running water for 3 min and blotted dry in open air then it was mounted on the SEM holder using removable adhesive. The middle of the cervical third of enamel on buccal surface was adjusted to be examined and to measure the surface (Ca) and (P) weight % using FEI/Inspect (S) scanning electron microscope attached with energy dispersive X-ray analyzer (SEM-EDXA Unit, Main Defense Chemical Laboratory). For the scanning electron microscopic examination, the collected teeth were examined at 30 kV using the secondary electron LFD detector under the magnification (X 1000) and (X4000) with a (spot size 4.7–5.5 nm) in each magnification. Surface (Ca) and (P) weight % were measured with energy dispersive X-ray analysis (EDXA) with S-UTW detector (EDXA Inc., Mahwah, NJ, USA). The count rate of the EDXA detector were between 1800 and 2000 counts per second. with a resolution of 132.14 eV [8]. Statistical analysis was used for data management and analysis so paired T-test was used to compare the values before and after immersion in different products, one way analysis of variance (ANOVA) followed by Tukey type non-parametric post hoc test are used for multiple comparisons, to determine differences between groups and Ca and P contents.

3. Results

3.1. Histological results

3.1.1. Control −ve group

Examination by SEM of the control −ve group, cervical third of the buccal surface, revealed a smooth regular enamel surface with no evidence of erosive areas, distinctive cracking or any surface defects. A plenty of well-defined perikymata grooves and ridges, few enamel rod ends and areas of rodless enamel are clearly observed (Figs. 1A & 2A).

3.1.2. Control positive group (cola group)

Examination by SEM of the control +ve group, cervical third of the buccal surface, revealed irregular enamel surface with some evidence of erosive lesions, darkened areas of irregular outline and areas of ill-defined enamel structure and apparently observed irregular grooves of variable length (Figs. 1B and 2B).

3.1.3. Subgroup 1 (milk group)

Examination by SEM of the cervical third of buccal surface in this group, revealed apparent small light sporadic areas with large erosive areas (Figs. 1C and 2C).

3.1.4. Subgroup 2(soy group)

Examination by SEM of cervical third of the buccal surface in this subgroup, revealed irregular enamel surface with porous defects seemed to be lined by white deposits (Fig. 1D) and minute cracks could be detected (Fig. 2D).

3.1.5. Subgroup 3 (almond group)

This subgroup revealed almost regular smooth enamel surface with diffused deposits no obvious cracks, small areas of defect still present (Figs. 1E and 2E).

3.1.6. Subgroup 4 (oat group)

This subgroup revealed sporadic globular pattern of deposits, recovered erosive enamel surface, deposits on the enamel surface fill the irregularities of the enamel however there are areas of erosive defects still exist (Figs. 1F and 2F).

3.2. Statistical analysis

The mean surface Ca & P wt% in C-ve was higher than that in C+ve group. However mean surface C wt% in C+ve group was higher than in C-ve group. These differences were statistically highly significant. There was also statistically significant difference between mean surface Ca/P ratio in C-ve and C+ve group as shown in Fig. 3A.

There were significance differences between the different beverages regarding the change in mean surface Ca, P and C wt%. Also, all subgroups showing increase in mean Ca and P contents and decrease in C content compared to C+ve. SG3 showing the highest mean Ca wt% followed by SG4 then SG1 and the lowest value was for SG2. SG3 showing the highest mean P wt% followed by SG1 then SG4 and the lowest value was for SG2. SG2 showing the highest mean C wt% followed by SG4, then SG1 and SG3 respectively. Mean Ca/P ratio was highest in SG2 compared to other groups as shown in Fig. 3B.

4. Discussion

Tooth structure undergoes continuous remineralization and

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