

ARE WE QUENCHING OUR THIRST AT THE EXPENSE OF OUR TEETH? AN IN VITRO STUDY TO EVALUATE CALCIUM RELEASE FROM THE ENAMEL SURFACE

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ABSTRACT:

Introduction:- Various beverages like tea, soft drinks and energy drinks contain high quantity of sugar and various additives and have a low pH, which may subject dental enamel to acid dissolution

Aims and Objectives:- To evaluate the effects of various commercial and domestic beverages on calcium release from enamel surface of teeth.

Materials and Method:- Enamel solubility was checked in 5 different beverages which were cola, mixed fruit juice, energy drink, tea and water. The calcium that was released into the beverages after 15min, 30 min and 60 min of exposure was determined using Calcium Reagent Set and measured using *uv-vis* Spectrophotometer.

Results:- Cola was found to be the most acidic with the lowest pH of 2.57. Mean calcium loss was found to be significant in soft drink. So, enamel solubility of teeth was the highest in cola drinks as compared to other beverages.

Keywords: Enamel solubility, Artificial beverages, Mean calcium loss.



INTRODUCTION:

In recent times when people are largely consuming fast food, “fast drinks” are also getting very popular, especially amongst young adults & teenagers. The companies manufacturing these drinks have very strong marketing strategies and use renowned faces to attract the audiences. These drinks are presumed to be innocent but contain high quantity of sugar and various additives and have a low pH, which may subject dental enamel to acid dissolution. Chronic consumption of such type of beverages affect the systemic health of individuals causing chronic diseases such as hypertension, diabetes and obesity. It also leads to increased prevalence of dental caries and dental erosion. Dental erosion is

progressive and irreversible loss of hard tissue due to chemical process, leading to increased enamel solubility.^[1] In addition, because of acidic content of these beverages, there is decrease in the pH of saliva below critical pH of 5.5, causing more rapid demineralization of enamel surface. The pathogenesis behind the erosion is high capacity of these drinks which dissolves more number of mineral ions from the tooth surface.

The calcium ions released from enamel surface are measured by various methods like mean weight loss, enamel microhardness, and using advanced techniques like spectrophotometry, semi and fully automatic analyzer. Soft drinks

and fruit juices have been extensively investigated over a long period of time for causing acid dissolution and erosion of dental enamel. Few studies have been conducted in the past to evaluate enamel solubility potential of these beverages by following the methodology of evaluating the weight loss as a proxy measure of enamel solubility.^[1,3,5,7] However, no study could be traced that evaluated the mineral loss in the concerned beverages.

So, with this background the present study was planned to evaluate the effects of various commercial and domestic beverages on calcium release from enamel surface of teeth.

Aim & Objectives: The aim of the present study was to evaluate the effect of commercial and domestic beverages on calcium release from the enamel surface.

MATERIALS AND METHODS:

This study was conducted after getting ethical clearance from Institutional Ethical Committee. This cross sectional study was carried out under following stages.

a) Selection and Cleaning of teeth:

Extracted, non-carious, permanent incisors and molars (25 each) were selected. These selected teeth were first cleaned properly prior to use with hydrogen peroxide for 10 seconds and then with water to remove stains (Figure 1). Five different sets of teeth each consisting of 1 incisor and 1 molar were

used to determine the rate of calcium released in each group.

b) Selection of beverages and pH measurement:

Current study tested the beverages which were divided into 5 major groups:

- Group 1 - Cola,
- Group 2- Mixed fruit juice,
- Group 3 - Energy drink,
- Group 4 -Tea
- Group 5 - Water.

Each beverage was evaluated by estimating its pH using digital pH meter.

c) Preparation of tooth- enamel surface:

Three coats of nail varnish was applied to the whole surface area of root (from cemento-enamel junction to apex), exposing only the surface of enamel which was done to prevent any release of calcium from the cementum. (Figure 2)

d) Determination of calcium:

The rate of calcium released into the beverages was analyzed and compared over a period of 1 hour. 10 ml of each beverage was taken into a separate clean beaker for each tooth individually. Tooth was then immersed into the sample and tested for calcium after 15min, 30 min and 60 min of exposure.

The calcium released into the beverages was determined by using Calcium Reagent Set (Accucare, Labcare Diagnostic Pvt. Ltd) and measured using uv-vis Spectrophotometer. (Figure

3) Determination at each intervals was carried out for all the five sets.

Data were entered into Excel sheet, and statistical analysis was performed using SPSS software.

RESULTS:

For Statistical analysis Kruskal-walis and Friedman test were performed. The pH of the selected beverages were measured (Table 1).

The pH of all test drinks ranged from 2.57 (cola) to 7.14(water). Even though all the beverages except water (control) were found to be acidic, cola was the most acidic with the lowest pH of 2.57. So, it is an important factor leading to dissolution of enamel.

When all these beverages were evaluated for determination of amount of calcium released at the time intervals of 15 min, 30 min and 60 min, for incisors it was found to be significant in group 1 (cola) (p value – 0.000080) (table 2) . This can be attributed to the extremely low pH of cola drinks as mentioned earlier. Mean calcium release was found to be negligible in water for both incisors and molars which confirmed that mineral water does not cause enamel dissolution (table 3). Whereas, the mean calcium released for both incisors and molars are found to be non significant in groups 2, 3, 4 and 5 at the time intervals of 15 min, 30 min and 60 min. (Graph 1)

DISCUSSION:

Fruit juices and other beverages are frequently consumed , especially by children and adolescents. So, the current study was intended to evaluate the enamel solubility potential of juices and soft drinks. Though occasional consumption of soft drinks will have negligible effect on the enamel, it is well recognized that long term consumption of soft drinks causes acid dissolution of enamel as most of these commercially available drinks have pH below the critical level.

In the present study it was observed that enamel solubility is more in cola drinks as compared to energy drinks and other beverages which is not in accordance with the study done by Von Fraunhofer and Rogers (2006) who found that , enamel solubility is greater in non- cola drinks. Whereas, Owens BM and Kitchens M (2007) and Kuldeep Dhanker et al (2013), Tadakmadla J et al(2014), Zimmer S et al(2015), Panda A et al(2017) found that enamel solubility is significant in cola drinks in comparison to other fruit juices, energy drinks, tea and other non cola beverages which is similar to the results obtained in our study. Tea is the most popular beverage in India consumed with maximum frequency. So we have chosen tea as one of our study groups for testing enamel solubility. Though the p value was found to be significant, however, for tea, it can be inferred that it is a safer drink as compared to other beverages.

In the present study, the minimum time interval for evaluation of calcium solubility was 15 minutes since it is the

minimum time required for the release of measurable calcium level to be sensed by the spectrophotometer. Even though, the beverages are not retained for such long periods in the oral cavity, the acidic environment is maintained for almost **25 mins** which further contributes to the enamel dissolution. Hence, the teeth were immersed in test drinks for 30 minutes and 60 minutes to simulate the effect of these beverages in chronic consumers of these drinks.

There are certain limitations of the study such as the in vitro design limiting it to be implied in clinical conditions, lack of sophisticated instruments which can be used to measure released calcium ions in the beverages. In the oral cavity, saliva plays an important role in counteracting the decreased pH by the action of buffering agents. As it is an in-vitro study with smaller sample size we cannot correlate these results for direct implementation in clinical scenario. So,

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further studies need to be conducted with bigger sample size and saliva simulation for more accurate results.

CONCLUSION:

Though these beverages are very appealing and readily available, they pose an increased risk of enamel solubility of teeth and dental erosion. It is proven that they are hazardous for the general health as well. Enamel solubility of teeth is the highest in cola drinks as compared to other beverages, which is more in chronic consumers of these drinks. Hence, the health professionals play a major role in educating the population about its deleterious effects. It can be prevented by reducing time of exposure of beverages in the oral cavity. People must rinse the mouth after consumption of these drinks so that the oral flora maintains at neutral pH and erosive potential of these drinks is reduced.

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TABLES:

Sr. No.	Beverages	pH
I	Cola	2.57
II	Mixed fruit juice	3.98
III	Energy drink	3.27
IV	Tea	5.00
V	Water	7.14

Table 1: pH of beverages

Group		I0	I15	I30	I60	P value
1	Mean	0.1800	0.2060	0.2440	0.3100	0.00008
	SD	0.000	0.0114	0.02302	0.06245	
2	Mean	0.0600	0.0800	0.100	0.1260	<0.0001
	SD	0.000	0.00707	0.0100	0.01817	
3	Mean	0.0400	0.0720	0.1040	0.1360	<0.0001
	SD	0.000	0.01643	0.01342	0.01517	
4	Mean	0.0300	0.0620	0.1000	0.1200	<0.0001
	SD	0.000	0.01924	0.01414	0.01817	
5	Mean	0.2400	0.2400	0.2400	0.2400	
	SD	0.000	0.000	0.000	0.000	

Table 2: Amount of calcium release from incisors using different beverages at different time interval

Group		M0	M15	M30	M60	P value
1	Mean	0.1800	0.2380	0.3100	0.3720	0.00008
	SD	0.000	0.02168	0.05099	0.05404	
2	Mean	0.0600	0.1060	0.14800	0.1740	<0.0001
	SD	0.000	0.01673	0.027748	0.02510	
3	Mean	0.0400	0.0900	0.1300	0.1760	<0.0001
	SD	0.000	0.01225	0.018708	0.04219	
4	Mean	0.0300	0.0740	0.10400	0.1320	<0.0001
	SD	0.000	0.02510	0.020736	0.02168	
5	Mean	0.2400	0.2400	0.2400	0.2400	
	SD	0.000	0.000	0.000	0.000	

Table 3: Amount of calcium release from molars using different beverages at different time interval

FIGURES:



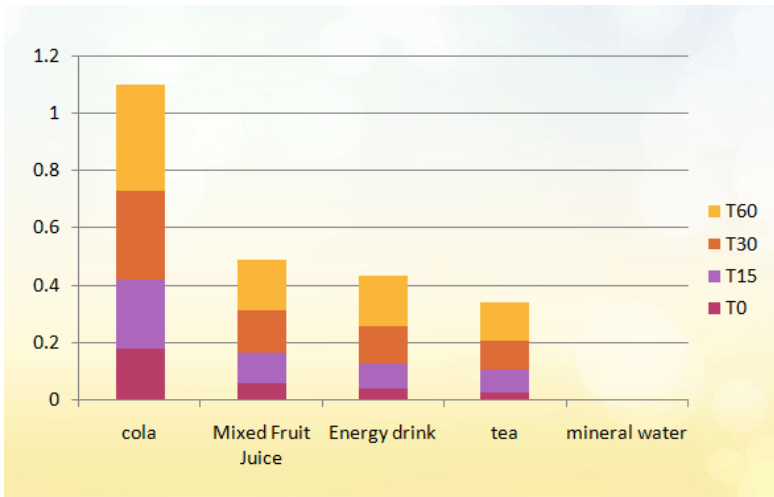
Figure 1: Selection and cleaning of extracted non carious teeth



Figure 2: Preparation of tooth surface



Figure 3: Calcium release is evaluated by Calcium Reagent Kit and measured by uv-vis Spectrophotometer.



Graph 1: The Rate of Calcium Released from Enamel Surface of Teeth Immersed in Beverages