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## Comparison of erosive potential of butter milk, tender coconut and a commercially available energy drink on human enamel: An *in vitro* study

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### Abstract

**Background:** Increased consumption of energy drinks by youth population leading to increased prevalence of erosion calls for exploring safe and culturally acceptable alternatives. Hence a study was planned to test the erosive potential of butter milk, tender coconut and a commercially available energy drink on human enamel *in vitro*.

**Methods:** An *in vitro* study was conducted by selecting 30 extracted human central incisors based on selection criteria. Three enamel sections (specimens) were taken from each selected tooth and were randomly assigned to three interventional groups *viz.*, Group A-Energy drink, Group B-Butter milk, Group C -Tender coconut water) followed by immersion in test solution for 15 minutes and placement in artificial saliva for rest of the day. This was done for 15 days duration. Pre and post 15 days test evaluation of microhardness of enamel was done assessing Knoop's hardness value. Iodide permeability test was performed to measure the level of enamel demineralization. Student's paired t test and oneway Anova tests were employed for statistical analysis fixing significance level at  $p < 0.05$ .

**Results:** There was a significant decrease in enamel hardness in all the interventional groups ( $p < 0.05$ ) after intervention compared to baseline values. Reduction in enamel hardness was highest in energy drink group ( $8.12 \pm 1.42$ ) followed by Buttermilk group ( $2.53 \pm 0.23$ ) and Tender coconut water group ( $0.39 \pm 0.18$ ). However, the difference between groups was not statistically significant ( $p > 0.05$ ).

**Conclusion:** Tender coconut water may be considered as safe alternative energy drink for consumption.

**Keywords:** Erosion, dental enamel, energy drinks, coconut, buttermilk

### 1. Introduction

Tooth wear is a cumulative multifactorial lifetime process, which to a large extent is irreversible. Tooth enamel is the teeth's outer covering, which provides a protective barrier for the tooth. It will not recover its original properties once worn away. Dental erosion is a relatively wasting disorder of dental hard tissues introduced by adoption of modern lifestyle. It is defined as "loss of tooth substance by chemical processes not involving bacteria caused by a variety of extrinsic and intrinsic factors"<sup>[1]</sup>. The frequent exposure of tooth surface to acids present either in food substances or due to regurgitation of gastric contents into the oral cavity will lead to demineralization of tooth<sup>[2]</sup>. Intrinsic factors includes from involuntary gastrointestinal disturbances such as gastroesophageal reflux disease (GERD), bulimia during pregnancy<sup>[2]</sup>. Extrinsic factors such as environmental factors, medicaments, diet, lifestyle and its related behavior must also be considered important in the etiology of dental erosion<sup>[3]</sup>. The extrinsic factors such as acidic beverages which come in contact with the teeth are known to trigger dental erosion effects because of their pH below the critical pH of dental enamel<sup>[2, 3, 4]</sup>. Erosion is found initially in the enamel and, if unchecked, may proceed to the underlying tooth structures. Depending upon the severity and length of exposure it may even lead to the total destruction of the dentition.

Dental erosion appears to be a growing public health problem in several countries and the increasing levels are thought to be due to higher consumption of acidic beverages like sugary carbonated drinks and fruit juices<sup>[4]</sup>. Buffering capacity of the drink also plays a significant role in the cause of dental erosion.

However, it is more worrying when this condition is found in an alarming proportion among children <sup>[5]</sup>. If the prevalence of this condition is not controlled and stabilized, children may suffer from severe tooth surface loss, tooth sensitivity, over closure, poor aesthetics, or even dental caries in the affected teeth <sup>[5]</sup>. An increasing interest in erosion as a major cause of tooth surface loss is reflected by several more recent focused publications and seems to be a public health concern for the dental profession in this millennium. Thus, it is vital to understand the causes of tooth erosion as well as the preventive measures that can protect the teeth.

The consumption of energy drinks has become a lifestyle among India's youth. Red Bull energy drink is a functional beverage with a unique combination of ingredients. It has been specially developed for times of increased mental and physical exertion. The Red Bull brand held an 88% off-trade value share in energy drinks as a whole <sup>[6]</sup>. In India butter milk and tender coconut are commonly used beverages that are culturally acceptable and economically feasible. But it is not clear whether these beverages have any adverse effect on dental enamel. Hence an *in vitro* study was conducted to evaluate and compare the erosive potential of butter milk, tender coconut and an energy drink (red bull) on human enamel.

## 2. Methodology

An *in-vitro* Experimental Study was planned to test the erosive potential of Butter milk, Tender coconut and Red bull on extracted human central incisors teeth samples using Knoop hardness test and Iodide permeability test. Ethical clearance was obtained from Institutional Ethical Review Board, where the study was conducted.

Materials used in the study were freshly extracted central incisors, Butter milk (Nandini Dairy products, Karnataka), fresh tender coconut water available in the local market, Red bull (Austrian Red Bull GmbH), pH meter (pHep, Hanna Instruments, VIC), Artificial saliva (Saleva), Knoop hardness indenter (MHT-10 Microhardness Tester) and Potassium iodide.

### 2.1. Assessment of pH of beverages

An array of butter milk packets, fresh tender coconut and Red bull cans were purchased from commercial outlets. The baseline pH of each beverage was determined using a pH meter immediately after opening the can/polythene bag. All measurements were taken at room temperature. The pH of all the beverages before they were poured into respective beakers was recorded on five different time intervals (5, 10,15,20,25 minutes) and results were averaged to obtain a mean pH.

### 2.2. Preparation of enamel specimens

Thirty human central incisors which were extracted due to periodontal problems were used to examine the erosive potential of selected beverages on dental enamel. The teeth were disinfected in formalin solution (10%), immediately after extraction. They were rinsed with tap water, cleaned with pumice slurry and air-dried. Teeth having restorations, dental caries, fluorosis, abrasion, erosion, cracks, hypoplastic areas were excluded. Teeth were stored in artificial saliva till the commencement of the experiment. This was done to prevent dehydration. Crowns were separated from roots using hard tissue microtome. Immediately after the separation five indentations are created in each tooth on sound enamel surface at a distance of 100  $\mu$ m to assess the micro hardness of enamel using Knoop diamond indenter. These indentations

acted as reference points for assessing the post surface hardness of enamel. Each crown was cut longitudinally into three equal parts using carborundum discs. Each half of tooth was mounted on acrylic resin for further experimentation. So each tooth resulted in a total of three equal halves for immersion in the three different beverages used in the present study. First half of the tooth mounted on acrylic resin was immersed in Butter milk, second half was immersed in Tender coconut and the last half of same tooth was immersed in Red Bull energy drink.

### 2.3. Intervention

About 50ml of each beverage was taken in sterilized borosilicate beakers. Enamel specimens were soaked in beakers containing any one of the respective beverages for about fifteen minutes every day for 15 days. Since beverages like butter milk and tender coconut cannot be preserved for a long duration of time, fresh beverages were taken every day for the experimentation. Fresh Red bull tins were also purchased every day in order to maintain the pH. Once the enamel specimens were taken out from their respective beverages they were immersed immediately in artificial saliva (*Saleva*) throughout the study period to simulate oral environment and to prevent dehydration. The same procedure was carried out for 15 days after which the specimens are cleaned using distilled water and subjected to enamel hardness tests. Knoop hardness and Iodide permeability tests were performed to evaluate the enamel surface loss.

### 2.4. Assessment of surface hardness of enamel

The embedded enamel specimens were fixed on a glass slide with double-sided adhesive tape. Hardness measurements were performed with a Knoop diamond under a load of 50 g. Indentations were made with the long axis of tooth parallel to the vertical border of the window at intervals of 50 mm. The length of the indentations was measured with an optical analysis system. As the length of the indentations in enamel was time-dependent, it was measured immediately after the experimental procedure. Iodide permeability test was performed to measure the level of enamel demineralization. Enamel samples were soaked in potassium iodide for thirty minutes and the amount of iodide recovered provides information on the pore volume of the test sample.

### 2.5. Measurement of enamel microhardness

Measurement of enamel microhardness was done by single operator blinded to the treatment of the enamel specimens. The length of the indentations made on each tooth was measured using a micrometer mounted on a microscope attached to the hardness tester. Each specimen was measured two times. Pre-test reading was taken for all the enamel specimens mounted on an acrylic resin which constitutes pre-test reading. Post-test reading was obtained using the same enamel specimens which were daily immersed for 15 days in three different beverages (butter milk, tender coconut and Red bull drink)

### 2.6. Statistical Analysis

Data was entered systematically in Microsoft Excel spreadsheet and master table was prepared. Statistical analysis was done using SPSS 19.0 software. Statistical significance was set at the 5%. Student's paired t test and one-way ANOVA test was applied for intra and inter group comparisons respectively.

### 3. Results

There was a significant decrease in enamel hardness in all the interventional groups ( $p < 0.05$ ) after intervention compared to baseline values. Reduction in enamel hardness was highest in

energy drink group ( $8.12 \pm 1.42$ ) followed by Buttermilk group ( $2.53 \pm 0.23$ ) and Tender coconut water group ( $0.39 \pm 0.18$ ) however the difference between groups was not statistically significant ( $p > 0.05$ ). (Table 1)

**Table 1:** Result of Inter and Intra-group comparison of Enamel Hardness

Groups	Pre-test enamel hardness (Mean Knoop hardness number)	Post-test enamel hardness (Mean Knoop Hardness number)	95% Confidence Interval of the Difference	Student's t value (p-value)
Group A (Energy Drink)	322.49±9.49	314±10.82	9.69 – 6.53	11.03* (0.00)
Group B (Buttermilk)	322.49±9.49	319.96±2.67	4.27 – 0.78	3.11* (0.008)
Group C (Tender coconut)	322.49±9.49	322.1±9.31	0.65 – 0.06	2.65* (0.019)
One-way Anova value (P value)	F=2.31 (p=0.11), degrees of freedom = 2,			

\*Statistically significant

### 4. Discussion

Present *in vitro* study compared the erosive potential of Red bull, Butter milk and Tender coconut on tooth enamel. The baseline hardness of untreated enamel specimens in our experiment was similar to result of a previous study [7]. There was significant reduction in enamel hardness in all groups post intervention. Highest decrease in hardness of enamel was observed in specimens that were immersed in Red bull energy drink, followed by Butter milk and Tender coconut. Since the present study is the first of its kind which compared the erosive potential of energy drink with butter milk and tender coconut on tooth enamel, valid comparisons could not be done. The reason for high erosive potential of Red bull energy drink could be that the pH of red bull is less when compared to the other two beverages used in the study. Demineralization is a process in which acids reach a susceptible site on hydroxyapatite crystal surface of enamel thereby leading to calcium and phosphate dissolution into the surrounding aqueous phase between the crystals [8].

The selection of the beverages in the present study was based on availability of products in the market. Consumption of sports drinks has been on the rise in groups of people other than athletes. There is a rise in consumption of soft drinks, sport drinks and energy drinks among children and adolescents too [6]. So it important for the dentists to understand the erosive potential of these beverages in order to create awareness about it among the susceptible population. The study focused on indigenously available products that might be suggested as alternatives to acidic beverages. Butter milk and tender coconut water were selected because they are most commonly used beverages in India [9, 10]. Fresh tender coconut contains glucose, fructose and minerals like iron, potassium, magnesium and calcium [9]. It has many medicinal properties [11]. In dentistry tender coconut is most commonly used as a transporting media for an avulsed tooth [12]. Buttermilk is a fermented dairy ingredient widely used across various sectors of population because of its emulsifying capacity and its positive impact on flavor. The functional property of Butter milk is that it helps in digestion of food because of its probiotic nature. The pH of buttermilk ranges from 4.41-4.83 [10]. Hence, it was chosen for the study to check its erosive potential on human enamel.

Demineralization of the enamel was measured as change in surface hardness with a Knoop hardness diamond under a load of 50 g. It has been shown that this method is suitable to determine small changes in surface microhardness and of the erosive attack [13]. Indentations were made at intervals of 50 mm on enamel specimens in such a manner that each baseline indentation was followed by the corresponding post-treatment indentation. This indentation pattern was chosen in our

experiment in order to maintain the homogeneity between baseline and post-test measurements.

Considering the findings of erosive potential of energy drink in the present study, indigenous beverages may well represent an alternative to energy drinks for the prevention and control of dental erosion. Further research and controlled clinical trials are necessary to evaluate the erosive potential of various energy drinks in the market.

### 5. Conclusion

There was significant reduction in enamel hardness post exposure to energy drink, buttermilk and tender coconut water. However, the reduction was highest in energy drink group indicating its high erosive potential. Hence, tender coconut water may serve as an effective alternative to energy drinks in prevention of dental erosion among population.

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### 7. References

- Saads Carvalho T, Lussi A. Chapter 9: Acidic Beverages and Foods Associated with Dental Erosion and Erosive Tooth Wear. *Monogr Oral Sci.* 2020;28:91-98. doi: 10.1159/000455376, 2019, PMID: 31940633.
- Lussi A, Carvalho TS. Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. *Monogr Oral Sci.* 2014;25:1-15. doi: 10.1159/000360380. Epub 2014 Jun 26. PMID: 24993253.
- Li H, Zou Y, Ding G. Dietary factors associated with dental erosion: a meta-analysis. *PLoS One.* 2012;7(8):e42626. doi: 10.1371/journal.pone.0042626. Epub 2012 Aug 31. Erratum in: *PLoS One.* 2016;11(8):e0161518. PMID: 22952601; PMCID: PMC3432030.
- Lussi A, Carvalho TS. Analyses of the Erosive Effect of Dietary Substances and Medications on Deciduous Teeth. *PLoS One.* 2015;10(12):e0143957. doi: 10.1371/journal.pone.0143957. PMID: 26700481; PMCID: PMC4689448.
- Salas MM, Nascimento GG, Huysmans MC, Demarco FF. Estimated prevalence of erosive tooth wear in permanent teeth of children and adolescents: an epidemiological systematic review and meta-regression analysis. *J Dent.* 2015;43(1):42-50. doi: 10.1016/j.jdent.2014.10.012. Epub 2014 Nov 8. PMID: 25446243.

6. Alsunni AA. Energy Drink Consumption: Beneficial and Adverse Health Effects. *Int J Health Sci (Qassim)*. 2015;9(4):468-74. PMID: 26715927; PMCID: PMC4682602.
7. Lussi A, Kohler N, Zero D, Schaffner M, Megert B. A comparison of the erosive potential of different beverages in primary and permanent teeth using an *in vitro* model. *Eur J Oral Sci*. 2000;108(2):110-4. doi: 10.1034/j.1600-0722.2000.90741.x. PMID: 10768723.
8. Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental erosion--an overview with emphasis on chemical and histopathological aspects. *Caries Res*. 2011;45(Suppl 1):2-12. doi: 10.1159/000325915. Epub 2011 May 31. PMID: 21625128.
9. Bhagya D, Prema L, Rajamohan T. Therapeutic effects of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats. *Asian Pac J Trop Med*. 2012;5(4):270-6. doi: 10.1016/S1995-7645(12)60038-8. PMID: 22449517.
10. Sodini I, Morin P, Olabi A, Jiménez-Flores R. Compositional and functional properties of buttermilk: a comparison between sweet, sour, and whey buttermilk. *J Dairy Sci*. 2006;89(2):525-36. doi: 10.3168/jds.S0022-0302(06)72115-4. PMID: 16428621.
11. Syafriani R, Sukandar EY, Apriantono T, Sigit JI. The effect of coconut water (*Cocos nucifera* L.) and an isotonic drink on the change of heart rate frequency in the rats induced hypertension. *Procedia Chemistry*. 2014;13:177-80.
12. Sanghavi T, Shah N, Parekh V, Singbal K. Evaluation and comparison of efficacy of three different storage media, coconut water, propolis, and oral rehydration solution, in maintaining the viability of periodontal ligament cells. *J Conserv Dent*. 2013;16(1):71-4. doi: 10.4103/0972-0707.105303. PMID: 23349581; PMCID: PMC3548351.
13. Schlueter N, Hara A, Shellis RP, Ganss C. Methods for the measurement and characterization of erosion in enamel and dentine. *Caries Res*. 2011;45(Suppl 1):13-23. doi: 10.1159/000326819. Epub 2011 May 31. PMID: 21625129.