



A Comparative Evaluation of Remineralization after an Erosive Challenge by Two Different Mouthwashes on Surface Roughness

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Abstract

Aims: The aims of this study were to evaluate the in vitro effect of a cocoa bean husk extract (CBHE) mouthwash in comparison with that of a fluoridated mouthwash on surface roughness of the enamel surface after subjecting the teeth to two types of energy drink challenge. **Materials and Methods:** Eighty (80) sound maxillary first premolar were collected and randomly divided into four groups, the teeth in the first and second groups were immersed in a tiger energy drink for 14 days and then one group in a CBHE and another in a fluoride mouth rinses for 7 days. While the remaining teeth in the third and fourth groups were immersed in a red bull energy drink for 14 days and then one group in a CBHE and another in a fluoride mouth rinses for 7 days. Enamel surface was assessed by a profilometer device at a baseline, after erosive challenge and after remineralization with the different treatment materials. **Results:** In all groups, there was a high statistically significant increase in surface roughness after erosive challenge. Meanwhile, there was a high statistically significant decrease in surface roughness in all groups after remineralization, with no significant differences between energy drinks or remineralization materials. **Conclusion:** Within the limits of the current study, cocoa bean husk extract and fluoride mouth rinses had an effective re-mineralizing ability after erosive energy drinks which have a destructive effect on tooth enamel. CBHE mouth wash showed a similar remineralization effect to fluoride groups.

الخلاصة

الأهداف: تهدف هذه الدراسة الى تقييم تأثير غسول الفم المستخلص من قشور الكاكاو ومن ثم مقارنته مع تأثير غسول الفم الفلورايد على خشونة سطح المينا بعد تعريض الأسنان لنوعين من مشروب الطاقة. **المواد وطرائق العمل:** تم جمع ثمانون (80) ضاحك أول سليم من الفك العلوي قلعت لغرض التقويم وقسمت عشوائياً إلى أربع مجاميع، غمرت الأسنان في المجموعتين الأولى والثانية في مشروب الطاقة (التايجر) لمدة 14 يوماً ثم غمرت إحدى المجاميع في مستخلص قشور الكاكاو والمجموعة الأخرى في غسول الفم الفلورايد لمدة 7 أيام. أما باقي الأسنان في المجموعتين الثالثة والرابعة تم غمرها في مشروب الطاقة (الريد بول) لمدة 14 يوماً ثم غمرت إحدى المجاميع في مستخلص قشور الكاكاو والمجموعة الأخرى في غسول الفم الفلورايد لمدة 7 أيام. ثم تم تقييم سطح المينا بواسطة جهاز اختبار الخشونة عند خط الأساس ، بعد التآكل وبعد إعادة التمدن بمواد المعالجة المختلفة. **النتائج:** في جميع المجموعات ، كانت هناك زيادة ذات دلالة إحصائية في خشونة السطح بعد اختبار التآكل. وايضا كان هناك انخفاض ذو دلالة إحصائية عالية في خشونة السطح في جميع المجموعات بعد إعادة التمدن ، مع عدم وجود فروق ذات دلالة إحصائية بين مشاريب الطاقة او مواد إعادة التمدن. **الاستنتاجات:** في حدود الدراسة الحالية ، كان لمستخلص قشور الكاكاو وغسول الفم الفلورايد قدرة فعالة على إعادة التمدن بعد مشروبات الطاقة التي كان لها تأثير مدمر على مينا الأسنان. كما وأظهر غسول الفم المستخلص من قشور الكاكاو تأثير إعادة تمدن مشابه لمجموعات غسول الفم الفلورايد.

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INTRODUCTION

Dental erosion is a chronic pathological condition, which involves dental hard tissue loss as a result of chemical phenomena^(1,2). Acidic exposure in the tooth causes drawing the mineral ions from enamel and produces surface damage to the enamel^(3,4). Many experimental results displayed that erosive beverage could initiate the dissolution of phosphate and calcium substances of dental samples and made an alteration in the surface morphology when exposed to these beverages⁽⁵⁾. One way to upsurge the hardness and repair the damaged enamel demineralization is to reestablish the damaged structure with remineralization process. A supersaturated environment is being offered by the re-mineralizing materials, which supporting mineral gain, and are accepted as a possible non-invasive process for the effective controlling of early lesions⁽⁶⁾. Many remineralization agents are available, and the most popular and widely used is fluoride⁽⁷⁾. High consumption of fluoride leads to increasing the occurrence of dental fluorosis and poisoning, so it must be given with proper dosage and careful technique^(8,9).

At present time modern approaches in Medicine and Dentistry prefers utilizing ingredients derived from nature. Therefore, the development of extracts and powders from plants having therapeutic properties has become essential and needs

to be more developed because it is assumed that drugs gained from natural materials are comparatively safe and inexpensive^(8,10).

Cocoa or *Theobroma cacao* is a natural supply, which can be used in dentistry, the cocoa extract has theobromine (3,7-dimethylxanthine) which is a primary alkaloid present in chocolates together with tea and other foods, which is estimated to be an alternative to fluoride^(11,12). The surface roughness is an important property in aesthetics,⁽¹³⁾ it represents the finer irregularity of surface textures that are characteristic in the materials or production process⁽¹⁴⁾.

It forms the need for a suitable quantitative tool for accurate assessment of such roughness. A tool, mainly prepared for this kind of estimation is a profilometer, which is a commonly accepted method to assess surface textures. It is a widespread method to analyze surface configuration and comprises a non-invasive approach. Moreover, in this system the whole roughness is quantified by a metric average value, which allows a statistical evaluation⁽¹⁵⁾.

The aims of this *in-vitro* research are:

1. To investigate and assess the effect of an erosive challenge of two types of energy drinks in terms of surface roughness.
2. To investigate and assess the effect of a CBHE mouthwash then compare it with fluoride mouthwash on surface roughness

of human dental enamel.

MATERIALS AND METHODS

Teeth Sample Collection:

Approval of study was from the Scientific Research Committee / Department of Pedo. Ortho. Preventive Dentistry / College of Dentistry / University of Mosul. Eighty (80) sound maxillary first premolars were assembled from Al-Noor dental center and some private clinics in Mosul city from patients aged between (12-15) years old extracted for orthodontic purposes, then the teeth were examined under 10X magnifying lens to ensure that the teeth are free from cracks, caries or any other defects ⁽¹⁶⁾.

Teeth Sample Preparation

The teeth were stored in a plastic container with 0.1% thymol solution at 4°C to preclude bacterial growth until their use ⁽¹⁷⁻¹⁹⁾. Before using the teeth, they were prepared by cleaning them with non-fluoridated pumice and white rubber prophylactic cup using a low speed hand piece and they were rinsed in tap water. Then the crowns were disconnected from the roots by employing a diamond disc bur in the high speed hand piece and cooled down with water. The crowns were then framed in cylindrical plastic tubes (16mm diameter×14mm depth) with cold cure acrylic resin by exposing the outer buccal enamel surface, and then the teeth samples were polished by using the universal polishing machine.

Materials: Tiger energy drink (Free lines For General Trading Co. LLC/Jordan) and Red bull energy drink (Red bull GmbH/Austria) which were used for erosive attack in addition to Cocoa bean husk (from which the CBHE was prepared) and Fluoride mouth wash which were used in remineralization after the erosive attack of the groups of the sample.

Cacao Bean Husk Extracts Preparation (CBHE):

The proportions of the materials used in this study were made according to the method followed by Matsumoto *etal* (2004), Srikanth *etal* (2008), RohiniDua *etal* (2017) ^(20- 22) in the following steps:

The ground husks of the cacao beans (1.0 kg), were firstly mixed with 5 g of cellulose then in 4.75 L of distilled water at 50°C for 4 hours. After that ethanol was added up to 50% (v/v final concentration) and the output mixture was refluxed for 1 hour then was filtrated with Whatman 42 filter paper. The ethanol was removed subsequently by evaporation and the aqueous solution lyophilized to get a powder. This process produced 120 gm of powdered extract. The powder was liquefied in distilled water to get a solution with a final concentration of 1 mg/ml ^(20- 22). The cocoa bean husk bag (Micacao/USA) that was used in this study contained 113.36 gm and the materials that treated with it was made as proportion and it yielded 13 gm.

Group Design and Methods

The specimens were divided randomly into four groups 20 specimens/group with the treatment materials as following:

First group (T-CBHE) : n=20 immersed in tiger energy drink for 14 days and then in cocoa bean husk extract solution for 7 days.

Second group (T-F): n=20 immersed in tiger energy drink for 14 days and then in fluoride mouth rinse for 7 days.

Third group (R-CBHE) : n=20 immersed in red bull energy drink for 14 days and then in cocoa bean husk extract solution for 7 days.

Fourth group (R-F): n=20 immersed in red bull energy drink for 14 days and then in fluoride mouth rinse for 7 days.

All samples were demineralized daily - for 2 minutes each time – 4 times, along one single hour, by 2 beverages for athletes:

Tiger or Red bull energy drinks figure (1). For the immersion of each sample, 250 ml of each drink was employed. Between the action intervals of beverages, the samples were saved in artificial saliva. The samples were introduced in energy drinks under analysis for 14 days ⁽²³⁾. Following the last immersion in acid drinks, the samples were rinsed with distilled water and dried, then re-measured. Remineralization was conducted according to a modification of a method described by Puig-Silla *etal* (2009) ⁽²⁴⁾, each group was dipped twice a day for 30 seconds in 200 mL of the respective mouth wash as seen in figure (2) for 7 days, then cleaned with distilled water perfectly and replaced in the artificial saliva bath. The mouth rinses employed were changed daily. After the last immersion the samples were washed, dried then introduced to the measuring.



Figure (1): Erosive Challenge with Tiger and Red bull Energy Beverage.

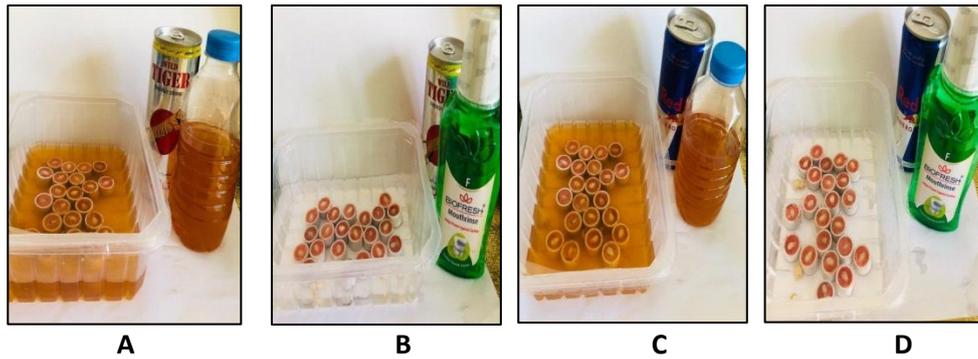


Figure (2): Remineralization Step with Fluoride and Cocoa Bean Husk Extract Mouth Rinses: **A-** CBHE Mouth Rinse after Tiger Energy Beverage. **B-** Fluoride Mouth Rinse after Tiger Energy Beverage. **C-** CBHE Mouth Rinse after Red bull Energy Beverage. **D-** Fluoride Mouth Rinse after Red bull Energy Beverage.

Surface Roughness Test:

A profile meter (Mitutoyo /Tokyo,Gapan) was used to measure the surface roughness (SR) of the enamel surface samples with magnification of 50X. The investigation was conducted at a Technical Institute / Mosul University. Surface roughness was measured by the arithmetical average of surface which showed the maximum and minimum lines drawn at the highest peak and lowest valley found within a central line along the area ⁽²⁵⁾. A section of typical length is sampled from the mean line on the roughness chart. In the Y direction, the distance between the Maximum peak (Rp) and valley (Rv) of the sampled line is measured. The value is calculated in micrometer (μm) ⁽²⁶⁻²⁸⁾. The cutoff value or reference length was modified to act at 0.8 mm. Three measurements of surface roughness were executed for each sample, and the mean of these readings was used for the statistical analysis ^(29, 30).

RESULTS

Table (1) clarifies the comparison of SR mean values at baseline, erosive attack and remineralization stages in each group by one way analysis of variance (ANOVA) test, and the results displayed that there was highly statistically significant differences $p \leq 0.01$ in all groups between and within groups for all types of treatment. Table (2) shows the mean values of SR in each group, there was a statistically significant increase in mean SR after an erosive attack in both type of energy drinks in comparison with the baseline values. Then there was a statistically significant decline in mean values of SR after being exposed to the two types of remineralization mouthwashes. Table (3) displays the mean value and Duncan multiple range test of SR in each stage, different small letters of the Duncan multiple range test indicate there are a significant difference among groups in remineralization stage, with no significant differences among groups

within baseline or erosive challenge stages. Table (4) shows the comparison between groups within each stage by one way analysis of variance (ANOVA) test

and the results displayed that there was no statistically significant differences between and within groups in the same stage.

Table (1): ANOVA Test of SR Mean Values Between the Variables in Each Group.

Roughness		Sum of Squares	Df	Mean Square	F	Sig.
T-CBHE	Between Groups	16.414	2	8.207	248.218	.000**
	Within Groups	1.885	57	.033		
T-F	Between Groups	16.661	2	8.331	277.613	.000**
	Within Groups	1.710	57	.030		
R-CBHE	Between Groups	16.383	2	8.192	275.580	.000**
	Within Groups	1.694	57	.030		
R-F	Between Groups	15.056	2	7.528	259.954	.000**
	Within Groups	1.651	57	.029		

Df: degree of freedom **Highly Statistically Significant Difference $p \leq 0.01$.

Table (2): Mean Values and Duncan's Multiple Range Test of SR in Each Group According to stage.

Variables	Groups				
		T-CBHE	T-F	R-CBHE	R-F
Baseline	Mean	.660c	.670c	.655c	.688c
	N	20	20	20	20
	Std. Deviation	.175	.175	.160	.160
Erosive Attack	Mean	1.950a	1.951a	1.934a	1.915a
	N	20	20	20	20
	Std. Deviation	.227	.225	.222	.225
Remineralization	Mean	1.329b	1.329b	1.252b	1.295b
	N	20	20	20	20
	Std. Deviation	.084	.132	.117	.100

N: Number of the specimens, Std. Deviation: Standard Deviation. Duncan's Multiple Range Test, Different small letters Indicate Statistically Significant Difference Within the Same Column (Vertically).

Table (3): Duncan's Multiple Range Test According to Groups of Each Stage

Groups	variables	Baseline	Erosive	Remineralization
T-CBHE	Mean	.660 a	1.950 a	1.329 a
	N	20	20	20
	Std. Deviation	.175	.227	.132
T-F	Mean	.670 a	1.951 a	1.329 a
	N	20	20	20
	Std. Deviation	.175	.225	.084
R-CBHE	Mean	.655 a	1.934 a	1.252 b
	N	20	20	20
	Std. Deviation	.160	.222	.117
R-F	Mean	.688 a	1.915 a	1.295 ab
	N	20	20	20
	Std. Deviation	.160	.225	.100

Means with Different Small Letters are Statistically Significant (vertically).

Table (4): ANOVA Test of SR Mean Values Between Groups within Each Stage.

Groups within each stage		Sum of Squares	df	Mean Square	F	Sig.
Baseline	Between Groups	.013	3	.004	.152	.928
	Within Groups	2.149	76	.028		
Erosive	Between Groups	.017	3	.006	.111	.954
	Within Groups	3.867	76	.051		
Remineralization	Between Groups	.081	3	.027	2.207	.094
	Within Groups	.924	76	.012		

No Statistically Significant Differences Exist

Comparison of the Variables Between Erosive Attack of the Two Energy Drink:

Table (5) displays the comparison of mean values of SR between the groups in each erosive challenge with tiger energy drink

or red bull energy drink variables. The results showed that there was no statistical significant difference in mean SR values between the two groups of teeth when they were exposed to the energy drinks.

Table (5): Comparison of Mean Values of SR After Exposure to the Two Energy Drinks.

Roughness	N	Mean	t-value	Sig.	Std. Deviation	Std. Error Mean
Tiger energy drink	40	1.950	.517	0.702	.223	.035
Red bull energy drink	40	1.925	.517		.221	.035

t-Test No Statistically Significant Differences Exist.

Comparison of the Variables Between Remineralization Groups:

One way analysis of variance (ANOVA) is presented in table (6) in the comparison of mean values of SR between the groups in remineralization phase. The analysis showed that there was no statistically significant difference in mean SR values between and within groups.

Table (7) displays that although the variation of the mean SR was small with a range between (1.25-1.32) a slight difference was observed as R-CBHE exhibited the largest roughness value followed by R-F in comparison with CBHE and F mouthwashes in the tiger energy drink.

Table (6): ANOVA Test of the Mean Values of SR For the Teeth Between the Remineralization Groups.

Roughness	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.081	3	.027	2.207	.094
Within Groups	.924	76	.012		

No Statistically Significant Differences Exist.

Table (7): Means and Standard Deviation of SR Mean Values of the Teeth Between the Fluoride and CBHE Groups

Groups Values	T-CBHE	TF	R-CBHE	R-F
Mean	1.329a	1.329a	1.252b	1.295ab
N	20	20	20	20
Std. Deviation	.132	.084	.117	.100

Duncan's Multiple Range Test, Means with Different Small Letters are Statistically Significant

DISCUSSION

Surface roughness measurements point to several significant findings evaluating independent variables of beverages, remineralization with fluoride or CBHE

mouthwashes, and exposure duration (baseline / erosive challenge / remineralization). Table (1) and (2) revealed a significant relation between the three independent variables. Highly significant differences were presented between exposure duration of the two energy drinks and the two mouth washes, with no significant differences exhibited between the two energy drinks and between the two mouth washes, as shown in tables (5) and (6) respectively, revealing that the significant impact factor was in the exposure duration between baseline, erosive challenge and remineralization stages and not beverage or mouthwash type.

The data reported in this research point that tiger and red bull energy beverages initiate significant long term enamel erosion. These outcomes are in agreement with studies accomplished by von Fraunhofer and Rogers (2004) and Wongkhantee *etal* (2006) ^(31,32) in which elevated levels of enamel destruction from carbonated beverages were testified. Increase acidic content of soft or energy drink cause erosion as reported by many studies ^(33,34). This might be attributed to that beverage which contain citric acid

have revealed a potential increase of hydroxyapatite dissolution as a result of the calcium binding (chelating action) of citric acid that take out calcium ions from the beverage and calcium citrate formation promoting increased levels of titratable acidity, bring about an increased dissolution predisposition because of losing the common ion effect.

Citric acid ($C_3H_5O(COOH)_3$) has (3) COOH groups per molecule, that can cause dental erosion by chelating and binding with calcium of the enamel ^(35,36) which correspondingly reinforces some of the findings in the present research, in that both the tiger and red bull drinks contain citric acid or citrates. Tiger energy drink also encompasses sodium citrate (sodium salt of citric acid), which is also sequestering agent that joins to calcium ^(23,37). Increased surface roughness has a bad consequence, as it leads to increased plaque biofilm, increase bacterial aggregation and increase in fracture rate due to loss of enamel. Tiger and Red Bull which revealed significant surface roughness values contain glucose and sucrose compounds that cause the production of acids, increase plaque biofilm, increase bacterial aggregation and a substantial lessening in plaque pH, and sequentially surface erosion of enamel hydroxyapatite ^(23,38).

Increased exposure duration has also been testified to raise the erosion and/or destruction of enamel surface structure ⁽²³⁾. These conclusions established the results

of the current study, whereby beverages comprising fruit-based sugar ingredients and/or citric acid (citrate) showed greater buffering capacity or titratable acidity and in turn, greater enamel dissolution. Although of that in tables (3) and (7) slight difference was observed between groups which exposed to a tiger energy drink and between that which exposed to red bull energy drink after remineralization and this led to minimum differences between the two mouth washes in which both mouth washes groups which exposed to red bull energy drink were superior than groups which exposed to tiger energy drink and this may be due to increased calcium concentration in red bull energy drink⁽⁵⁾.

A secondary experimental concern was the CBHE or fluoride mouth washes interaction or preventive effect of erosive challenge, although the remineralization effect of these two mouth was highly significant but the results of the mean surface roughness did not return to the base line values, and this in agreement with Farooq *etal.* (2020)⁽³⁹⁾ which assessed the effect of theobromine addition to the tooth paste on enamel hardness and roughness in comparison with different commercially available toothpaste.

Limitation of the study: this in vitro study had certain limitations as difficulty in simulating the oral environment, lack of bacteria in the artificial saliva solution used, lower level of salivary proteins,

control over the salivary flow rate and a more harsh and cruel acidogenic challenges used in a shorter period of time. Even though both mouth washes (CBHE and fluoride) were effective in reducing the destructive effect of the erosive challenge on the enamel of the teeth, long-term in vivo studies will be desirable to prove their beneficial outcome for the routine utilize in the prevention of dental erosion and caries.

CONCLUSION

Both energy drinks (Tiger and Red bull) increased the surface roughness of enamel resulting in erosion on the experimental teeth. While both mouth washes (CBHE and fluoride) were effective in reducing the destructive effect of the erosive challenge on the enamel of the teeth manifested in forms of reducing surface roughness, but definitely not to the baseline values for both parameters. CBHE shows remineralization effect as similar results as fluoride daily used mouth washes. Erosion unlike caries results in irreversible damage as part of the tooth enamel is being lost so primary prevention by reducing the consumption of the energy drinks is essential.

Conflicts of interest

The authors declare that they have no conflict of interest real or perceived, financial or non-financial in this article.

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