



## Polarized Light Microscopical Features of Erosion and Remineralization Process by Two Different Mouthwashes

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### Abstract

**Aims:** The study aimed to estimate the in vitro effect of a cocoa bean husk extract (CBHE) mouth rinse in comparison with that of a fluoridated mouth rinse on microscopic features of enamel surface after subjecting the teeth to two types of energy drink challenge. **Materials and Methods:** Twenty sound maxillary first premolars were collected and randomly divided into four groups, the teeth in the first two groups were immersed in the tiger energy drink for 14 days, then the teeth in the first group were exposed to a CBHE mouthwash and the second group was exposed to a fluoride mouth wash for 7 days. While the remaining teeth in the second two groups were immersed in the red bull energy drink for 14 days, then one group in a CBHE mouth wash and the other in a fluoride mouth wash for 7 days. Enamel surface was evaluated by a polarized light microscope at a baseline, after erosive challenge and after remineralization with the different treatment materials. **Results:** Microscopic images confirmed that prolonged subjection of dental sample to energy drink could crack or erode the tooth surface and between the two energy drinks, tiger energy drink had a somewhat more destructive microscopic feature. Besides that, both mouthwashes were efficient in re-mineralizing the defected area, and CBHE mouth wash revealed somewhat a high amount of remineralization which appeared in the polarized light microscope images being more dark areas in comparison with fluoride groups. **Conclusion:** Within the limits of the current study, CBHE and fluoride mouth rinses had an effective re-mineralizing ability after erosive energy drinks which had a destructive effect on tooth enamel. CBHE mouth wash showed somewhat a high ability of remineralization when compared with fluoride groups.

### الخلاصة

**الأهداف:** تهدف هذه الدراسة الى تقدير ومقارنة تأثير غسول الفم المستخلص من قشور الكاكاو مع تأثير غسول الفم الفلورايد على السمات المجهرية لسطح المينا بعد تعريض الأسنان لنوعين من مشاريب الطاقة. **المواد وطرائق العمل:** تم جمع عشرين ضاحك أول من الفك العلوي قلع لغير تقويم وقسمت عشوائياً إلى أربع مجاميع، غمرت الأسنان في المجموعتين الأوليتين في مشروب الطاقة (التايرك) لمدة ١٤ يوماً ثم تم غمر إحدى المجاميع في مستخلص قشور الكاكاو والمجموعة الأخرى في غسول الفم الفلورايد لمدة ٧ أيام. أما باقي الأسنان في المجموعتين الأخرتين تم غمرها في مشروب الطاقة (الريديبول) لمدة ١٤ يوم ثم تم غمر إحدى المجاميع في مستخلص قشور الكاكاو والمجموعة الثانية في غسول الفم الفلورايد لمدة ٧ أيام. ثم تم تقييم سطح المينا بواسطة المجهر الضوئي المستقطب عند خط الأساس ، بعد التآكل وبعد إعادة التمدن بمواد المعالجة المختلفة. **النتائج:** أكدت الصور المجهرية أن التعرض المطول لعينة الأسنان لمشروب الطاقة يمكن أن يتسبب في تشقق سطح الأسنان وتآكله ، وبينت ان مشروب الطاقة (التايرك) نوعا ما لديه تأثير أكثر تدميراً على مينا الاسنان من مشروب الطاقة (الريديبول). إلى جانب ذلك ، أظهرت كلتا الغرغرتين إعادة تمدن للمناطق المتضررة كما بينت الصور المجهرية ان غسول الفم المستخلص من قشور الكاكاو أظهر نوعا ما مناطق إعادة تمدن أكثر من مجموعات الفلورايد التي تظهر في صور المجهر الضوئي المستقطب على شكل مناطق غامقة أكثر من باقي مناطق المينا. **الاستنتاجات:** في حدود الدراسة الحالية ، كان لمستخلص قشور الكاكاو وغسول الفم الفلورايد قدرة فعالة على إعادة التمدن بعد مشروبات الطاقة التي لها تأثير مدمر على مينا الأسنان. كما وأظهر غسول الفم المستخلص من قشور الكاكاو نوعا ما قدرة عالية على إعادة التمدن مقارنة بغسول الفم الفلورايد.

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## INTRODUCTION

Demineralization arises through the diffusion process that is the transfer of dissolved ions or molecules from the enamel to the saliva as a result of low salivary pH<sup>(1,2)</sup>. Throughout two primary ways, acidic attack leads to chemical demineralization of teeth which are microbial attack from bacteria present in the mouth and dietary acid consumed through food or drink<sup>(3,4)</sup>.

Erosion and carious lesions both are the chief consequences of demineralization. Community consciousness of dental erosion is yet not widespread, and among dental professionals its differential diagnosis has verified to be a challenge. The dietary consumption of fruit-based or soft drinks in the industrialized world is supposed to be above half of all of the liquids spent<sup>(1,5)</sup>. Currently there has been a dramatic increase in consumption of soft drinks and especially energy drinks<sup>(6-8)</sup>. Demineralization and the consequent loss of tooth surface and/or erosion against remineralization are dynamic progressions that are reliant on several modifying agents<sup>(1)</sup>. To observe and characterized the lesion depth, polarized light microscope was utilized since the histological structures of enamel and dentin can be imagined better owing to its birefringence characteristic, which is not satisfactory estimated in a transmitted light microscope<sup>(9,10)</sup>.

The current study aimed to compare and evaluate a CBHE mouth wash with that of a daily fluoride mouth wash after an erosive challenge for 14 days with the most popular and available energy drinks present in Iraq/ Mosul on microscopic features of enamel surface by polarized light microscope .

## 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. Twenty (20) sound maxillary first premolars were gathered from Al-Noor dental center and some private clinics in Mosul city from patients aged between (12-15) years old extracted for orthodontic intents, then the teeth were inspected under 10X magnifying lens to certify that the teeth are free from cracks, caries or any other defects<sup>(11)</sup>.

### Teeth Sample Preparation

The teeth were saved in a plastic bottle with 0.1% thymol solution at 4°C to prevent bacterial growth until their use<sup>(12-14)</sup>. Before employing the teeth, they were prepared by removing the soft tissue debris then polishing them with non-fluoridated pumice and a rubber prophylactic cup utilizing a low-speed hand piece, and they were rinsed in tap water. Then the crowns were separated from the roots by using a diamond disc bur in the high-speed hand

piece and cooled down with water then were mounted in an acrylic mold. Ground sections were prepared following the procedure described by Monalisa *etal* (2018) <sup>(15)</sup> in which sections were made bucco-lingually in each tooth by hard tissue microtome (LTD/UK) in the Department of Earth Science, University of Mosul. Each enamel longitudinal segment with a thickness of 0.3 mm was fixed on the middle of a glass slide. Then slides were examined with a polarized light microscope (OPTIKA/ Italy).

#### **Preparation of (CBHE):**

At the beginning, 5 g of cellulose were added to the outer covering of cocoa bean (1.0 kg) in 4.75 L of distilled water at 50°C for 4 hours. Then the addition of ethanol was up to 50% (v/v final concentration) and the mixture was refluxed for 1 hour then to the filtration. Afterward, the ethanol was eliminated by evaporation and the solution lyophilized to produce a powder. The powder was thawed in distilled water to get a solution with a final concentration of 1 mg/ml <sup>(16-18)</sup>.

The cocoa bean husk bag (Micacao/USA) that was employed in this study contained 113.36 gm and the treated ingredients were formulated as proportion and it yielded 13 gm.

#### **Group Design and Methods**

The specimens were divided randomly into four groups, five specimens per group with the treatment materials as following:

**First group:** n=5 immersed in the tiger energy drink (Free lines For General Trading Co. LLC/Jordan) for 14 days and then in a CBHE mouthwash for 7 days.

**Second group:** n=5 immersed in the tiger energy drink for 14 days and then in a fluoride mouth wash (Scitra Co. for Biofresh LLc /Dubai, U.A.E) for 7 days.

**Third group:** n=5 immersed in the red bull energy drink (Red bull GmbH/Austria) for 14 days and then in a CBHE mouthwash for 7 days.

**Fourth group:** n=5 immersed in the red bull energy drink for 14 days and then in a fluoride mouthwash for 7 days.

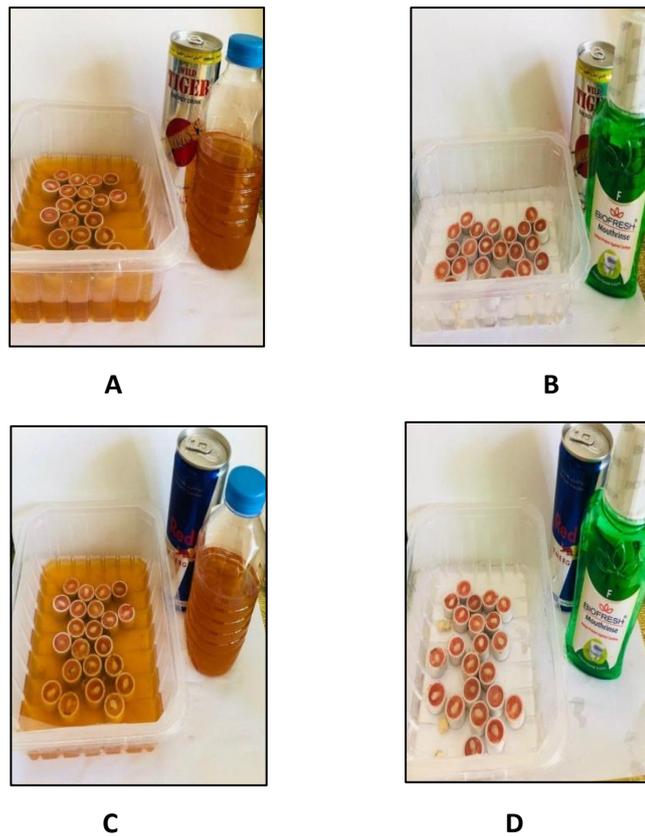
All samples were eroded daily - for 2 minutes each time – 4 times, along one single hour, by 2 drinks for athletes: Tiger or Red bull energy drinks for 14 days as seen in figure (1). For the immersion of each sample, 250 ml of each drink was utilized. Between the action interims of beverages, the samples were saved in artificial saliva <sup>(19)</sup>. Following the last immersion in acid drinks, the samples were rinsed with distilled water and dried, then reviewed.



**Figure (1):** Erosive Challenge With Tiger and Red bull Energy Drink.

Remineralization was conducted according to a modification of a method described by Puig-Silla *etal* (2009)<sup>(20)</sup>, every group was placed in 200 mL of the two mouth rinses twice a day for half a minute for 7 days as seen in figure (2), then washed with

distilled water perfectly and restored in the artificial saliva. The mouthwashes used were changed daily. After the final immersion, the samples were washed, dried then examined with the *microscope*.

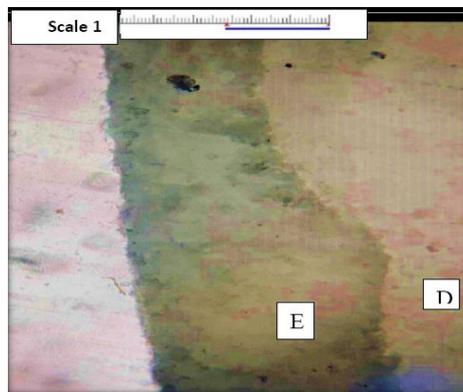


**Figure (2):** Remineralization Stage with Fluoride and Cocoa Bean Husk Extract Mouth Washes: **A-** CBHE Mouth Wash After Tiger Energy Drink. **B-** Fluoride Mouth Wash After Tiger Energy Drink. **C-** CBHE Mouth Wash After Red bull Energy Drink. **D-** Fluoride Mouth Wash After Red bull Energy Drink.

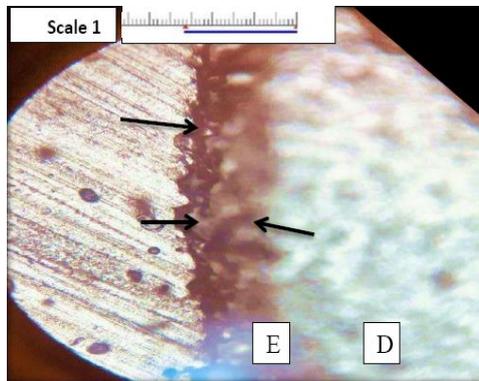
## RESULTS

Figure (3) shows the microscopic features of the sound enamel surface before being exposed to the erosive challenge, a highly mineral intact and continuous enamel surface is prominent and evident. After exposing the teeth to the two types of energy drinks, the enamel surface became eroded and appeared disrupted, and interrupted as seen in

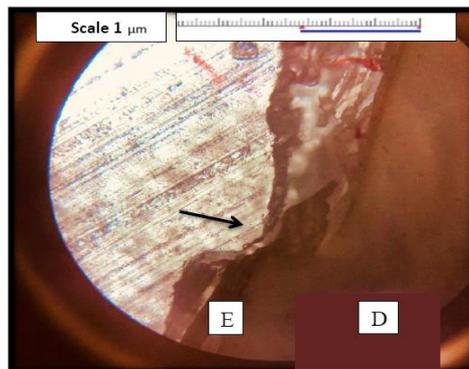
figures (4) and (5) for each energy drink. Figures (6) and (7) depicts the remineralization of the CBHE mouth wash which was clearer and more prominent on the surface of the eroded enamel in comparison with fluoride mouth wash that also made a layer on the surface of the eroded enamel as seen in figures (8), (9) filling in the irregularities that had been formed in the erosive attack.



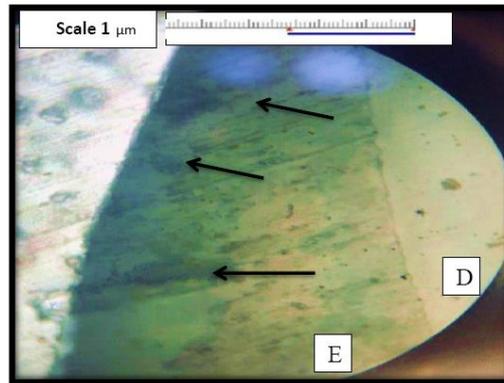
**Figure (3):** Clear Intact Enamel Surface at the Base Line; E: Enamel, D: Dentine.



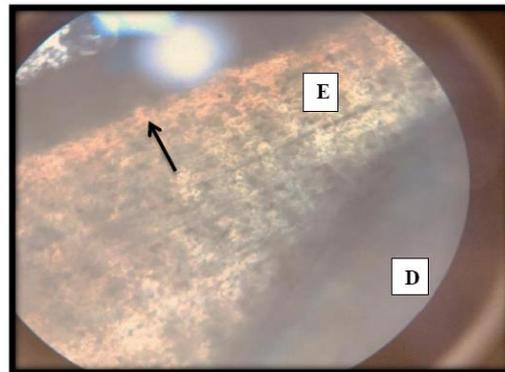
**Figure (4):** Interrupted Eroded Enamel Surface after Tiger Energy Drink.



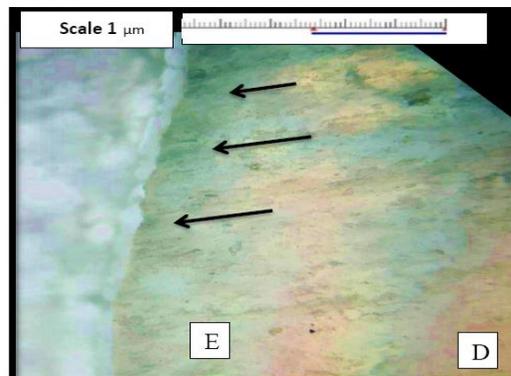
**Figure (5):** Interrupted Eroded Enamel Surface after Red bull Energy Drink.



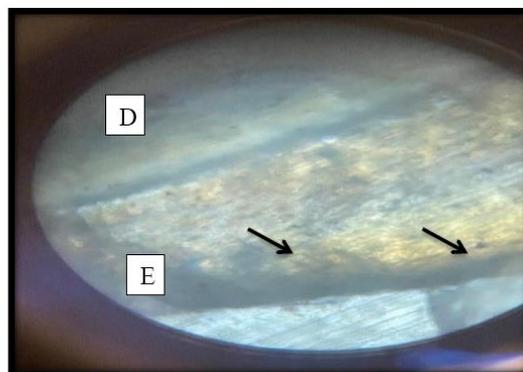
**Figure (6):** Enamel Remineralization after CBHE Mouth Wash Application.



**Figure (7):** Area of Remineralization after CBHE Mouth Wash.



**Figure (8):** Enamel Remineralization after Fluoride Mouth Wash.



**Figure (9):** Area of Remineralization after Fluoride Mouth Wash.

## DISCUSSION

Microscopic images confirmed that prolonged exposure of dental sample to energy drink could lead to crack and erode the tooth surface. The investigational results displayed that erosive tiger and red bull beverages could initiate the disbanding of phosphate and calcium substances of dental samples and cause alteration in the surface morphology when exposed to these beverages and this is in agreement with Enam *etal.* (2017) <sup>(21)</sup>, although the researchers utilized scanning electron microscopy for analysis. Polarized light microscope showed that the erosion depth in red bull energy groups is somewhat less than that of tiger groups, this may be due to the increasing calcium ion concentration in red bull energy drink <sup>(21)</sup>. This trend of diminishing erosion depth with raising calcium ion concentration corresponds to that in the previous studies <sup>(19,22-26)</sup>. The pH of the experimental solution and the erosion depth has a logarithmically inverse relationship <sup>(22,27)</sup> and the pH of red bull is more than a tiger and this may interpret why lesion depth in red bull groups is less than a tiger groups. In addition to that, the titratable acidity of tiger is more than a red bull and this increases the erosive effect of the drink. In the present study, CBHE mouth wash groups containing 1% theobromine, showed a high amount of remineralization which appeared in the polarized light microscope images being more dark areas when compared with the

fluoride groups. The observed remineralization in this group could be attributed to interstitial reaction as a replacement for the loss of hydroxyl apatite crystals <sup>(2,28)</sup>. A previous laboratory study showed that topical application of theobromine (which is present here in CBHE mouthwash) led to the formation of a precipitation on the enamel of humans, indicating a pronounced protection of the enamel <sup>(28)</sup> for this reason the remineralized layer in the CBHE mouthwash exhibited a more prominent feature on the teeth. Fluoride 0.025% daily mouthwash is transformed to more acid-resistant and extra stable fluorapatite on tooth surface, in consequence creating tooth more unaffected by acid attacks <sup>(10)</sup>. Thus, the eroded teeth can be cured non-invasively by CBHE and fluoride remineralization to repair esthetic appearance, strength, and to intensification the resistance to future acid challenge. Primary prevention is essential to prevent the erosive defects on the enamel and there is different preventive actions may be suggested, to lessen dental erosion, such as improving dental hygiene, improving drink pattern of acidic beverages or to consume modified drinks and reducing bad habits of consumption such as swishing the liquid through the mouth previous to swallowing, nipping from a bottle, or pulling from a straw, which leads to the extended period of an acidic-pH fluid in the oral cavity.

**Limitation of the study:** definite limitations were found in the current in

vitro research such as difficulty in mimicking the oral environs, minor level of salivary proteins, control over the salivary flow rate, absence of bacteria in the artificial saliva solution employed and a crueller erosive challenge employed in a shorter time. Even though both mouth rinses (CBHE and fluoride) were effective in lessening the destructive effect of the erosive effect on the enamel of the teeth, long-standing in vivo researchers will be desirable to verify their valuable outcome for the predictable employment in the prevention of dental erosion and caries.

**Conclusions:** Within the limitations of the current study, the erosive challenge by tiger and red bull energy drinks create a prominent erosive depth in enamel, CBHE and fluoride mouth wash showed promising remineralizing effect which was reflected by decreasing the erosive depth that created by the energy drinks on the enamel surface.

#### **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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